ORIGINAL ARTICLE

Decreased Inflammation and Improved Survival With Recombinant Human Activated Protein C Treatment in Experimental Acute Pancreatitis

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Hypothesis: Drotrecogin alfa (activated), the pharmacologic form of activated protein C and the first Food and Drug Administration–approved drug for treatment of severe sepsis, is beneficial in experimental acute pancreatitis (AP).

Design: Animal study.

Setting: Laboratory.

Subjects: Male Sprague-Dawley rats.

Interventions: Mild (intravenous cerulein) or severe (intravenous cerulein plus intraductal glycodeoxycholic acid) AP was induced in 72 rats, and coagulation evaluated. Rats with severe AP were randomized to treatment with drotrecogin alfa (activated), 100 µg/kg per hour, or isotonic sodium chloride.

Main Outcome Measures: Histologic scoring of pancreatic necrosis, inflammation of the pancreas and lung (measured by myeloperoxidase concentration), coagulation measures, and 24-hour survival.

Results: Severe consumptive coagulopathy, hemoconcentration, and leukocytosis were observed 6 hours after induction of severe AP, but not in mild AP. Treatment of AP with drotrecogin did not worsen coagulation measures. Although the degree of pancreatic necrosis was comparable in treated and untreated animals with severe AP, drotrecogin significantly reduced myeloperoxidase levels in the pancreas ($P=.009$) and lungs ($P=.03$). The 24-hour survival in severe AP was markedly improved in animals treated with drotrecogin (86% vs 38%; $P=.05$).

Conclusions: Animals with severe AP have severe consumptive coagulopathy, but administration of drotrecogin alfa (activated), 100 µg/kg per hour, does not worsen coagulation abnormalities. Drotrecogin treatment reduces inflammation in the pancreas and lungs and significantly improves survival. These results encourage clinical investigation of drotrecogin in the treatment of severe AP.

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ACTIVATED PROTEIN C (APC) is a plasma glycoprotein that regulates coagulation after activation by thrombin.[1] It inhibits the coagulation process by degrading factors Va and VIIIa[2] and indirectly enhances fibrinolysis by promoting fibrin degradation by plasmin.[3,4] Experimental studies with APC have demonstrated anti-inflammatory effects[5,6] and anti-ischemic properties[7,8] and have improved survival and reduction of organ damage after experimental sepsis.[9] The improved organ function and decreased mortality in a large clinical study of septic patients treated with APC[10,11] led to its approval for use in severe sepsis.

Distant organ failure is the most important determinant of severity in acute pancreatitis (AP).[12,13] Although results of studies evaluating changes in the coagulation system in AP are conflicting,[14,15] intravascular coagulation and thromboembolism are believed to play an important role in the pathogenesis of its complications,[16] and hypercoagulability has been reported.[17]

In this study, we evaluated changes in the coagulation system during experimental pancreatitis of graded severity in rodents and investigated the possible therapeutic effects of human recombinant APC (drotrecogin alfa [activated]) in this setting.

See Invited Critique and Invited Response at end of article

METHODS

ANIMALS

Male Sprague-Dawley rats (mean ± SEM weight, 300 ± 50 g) were obtained from Charles River Laboratories (Wilmington, Mass). Care was provided in accordance with the procedures outlined in the *Guide for the Care and Use of Laboratory Animals*.[18] The study was approved by the subcommittee on animal research at our institution. Rats were housed individually in hanging cages under standard

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conditions (12-hour light-dark cycle and temperature of 21°C ± 1°C) and fed regular rat chow. The animals were not fed overnight before the experiment with free access to water.

Laboratory-grade drotrecogin alfa (activated) was provided by the manufacturer (Eli Lilly & Co, Indianapolis, Ind). All other reagents were obtained from Sigma-Aldrich Corp (St Louis, Mo) unless otherwise specified.

EXPERIMENTAL DESIGN

Time Points

In this study, several time points were chosen for comparison. The 6-hour time point represents the time of maximum changes or effects in this model of severe AP. The 24-hour time point was used to assess mortality. To evaluate changes in an early phase, the 2-hour time point was chosen.

Part 1: Assessment of Coagulation Changes in AP With Graded Severity

A total of 114 animals were divided into 4 groups: group 1, healthy animals (n=6); group 2, controls that underwent sham operation (n=36); group 3, rats with mild pancreatitis (n=36); and group 4, rats with severe AP (n=36). At 0.5, 1, 2, 6, 12, and 24 hours after induction of pancreatitis, 6 animals from groups 2 to 4 were humanely killed, and partial thromboplastin time (PTT), prothrombin time (PT), fibrinogen, white blood cell (WBC) count, platelet count, and hematocrit were measured.

Part 2: Safety Assessment of Drotrecogin

Fifteen healthy animals received a 6-hour infusion of 5 different concentrations of drotrecogin alfa (activated). Each concentration (12.5, 17, 21, 50, and 100 µg/kg per hour) was infused into 3 healthy animals. Before the start of the infusion and every 2 hours thereafter, hematocrit was measured with a centrifuge (Readacrit; Clay Adams, Parsippany, NJ). Blood was drawn from the ventral tail artery by nicking it superficially and collected in microhematocrit tubes (75 × 0.56 mm; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). After 6 hours, the animals were anesthetized and blood samples were collected for measurement of PTT, PT, fibrinogen, WBC count, platelet count, and hematocrit. Autopsy was performed to evaluate for hematomas or abnormal bleeding.

Part 3: Effect of Treatment of Severe AP With Drotrecogin on Hematologic Disturbances and Tissue Injury

Twenty-four animals were divided into 2 groups: group 5, severe AP; and group 6, severe AP and treatment with drotrecogin alfa (activated), 100 µg/kg per hour. Six animals from each group were killed at 2 and 6 hours after induction of pancreatitis. Blood was drawn and pancreas and lungs were harvested. For both time points, PTT, PT, fibrinogen, WBC count, platelet count, and hematocrit were measured.

In both groups, necrosis and edema of pancreas were evaluated by a pathologist (K.B.L.) who was blinded to the treatment group and used a previously described and validated scoring system.[19] A score of 0 describes absence of that variable, while a score of 4 describes maximum development of the variable (ie, complete necrosis of organ).

Myeloperoxidase (MPO), a quantitative indicator of leukocyte infiltration, was determined in the pancreas and lungs 6 hours after induction of pancreatitis in both groups with severe AP (untreated and treated).

Part 4: Effect of Drotrecogin on 24-Hour Survival in Severe AP

Sixteen animals were divided into 2 groups: group 7, severe AP; and group 8, severe AP and treatment with drotrecogin alfa (activated), 100 µg/kg per hour. Animals were monitored for 24 hours and time of death was recorded. Surviving animals were humanely killed 24 hours after induction of pancreatitis by overdose of pentobarbital sodium.

Anesthesia and Catheter Placement

Surgical anesthesia was induced by intraperitoneal pentobarbital sodium (Nembutal, 50 mg/kg; Abbott Laboratories, North Chicago, Ill) and intramuscular ketamine hydrochloride (Ketalar, 10 mg/kg; Monarch Pharmaceuticals Inc, Bristol, Tenn). The right and/or left internal jugular vein was cannulated with a soft polyethylene catheter (Intramedic; internal diameter, 0.023 in; Clay Adams) for infusion of the study drugs. These catheters were tunneled subcutaneously to the neck and brought out via a flow-through tether, which permitted free movement. In groups 5 through 8, 2 catheters were placed into the right and left external jugular vein to provide a separate line for the infusion of drotrecogin (to exclude any potential direct interaction of drotrecogin with the cerulein infusion).

Induction of Pancreatitis and Treatment With Drotrecogin

Sham-operation controls received only a median laparotomy. Well-established and studied models of mild and severe experimental pancreatitis were induced as previously described.[20] Briefly, the biliopancreatic duct was cannulated with a 24-gauge intravenous catheter (Angiocath; Becton Dickinson, Sandy, Utah) and bile and pancreatic juice were drained by gravity in a 60° reverse Trendelenburg position for 5 minutes. During the last 2 minutes of this procedure, the main duct was clamped below the liver to allow complete emptying of the biliary and pancreatic ductal system and to facilitate the subsequent intraductal infusion. For induction of mild pancreatitis, isotonic sodium chloride solution was infused; for severe AP, freshly prepared glycodeoxycholic acid glycylglycine–sodium hydroxide–buffered solution (pH 8.0 at room temperature) at a concentration of 10mM in a pressure-controlled (30 mm Hg) and volume-controlled (0.12 mL/100 g) manner were infused in 10 minutes. After completion of the intraductal infusion, all animals received continuous secretory hyperstimulation for 6 hours with intravenous cerulein (5 µg/kg per hour) at 8 mL/kg per hour as baseline hydration. Sodium bicarbonate (0.2 mL/100 g) and ketamine hydrochloride (0.2 mL) were added to this infusion. Animals in the treatment and survival experiments (parts 3 and 4) received a second simultaneous infusion at a rate of 0.6 mL/kg per hour with drotrecogin alfa (activated), 100 µg/kg per hour (groups 6 and 8), or isotonic sodium chloride groups 5 and 7). Sham-operation controls received an infusion of isotonic sodium chloride solution at 8 mL/kg per hour for 6 hours. After completion of the cerulein infusion, a second saline infusion for 6 hours at 8 mL/kg per hour was administered in sham-operation controls and those with mild and severe AP, whereas animals in the treatment groups (groups 6 and 8) received a 6-hour infusion of drotrecogin alfa (activated) (100 µg/kg per hour), also at a rate of 8 mL/kg per hour.

BLOOD COLLECTION AND MEASUREMENTS

Blood was drawn by cardiac puncture, collected in citrate (citrate-blood ratio, 1:9), put on ice, and immediately spun at 4°C, 2500*g* for 15 minutes. Plasma was aliquoted and frozen at –80°C.

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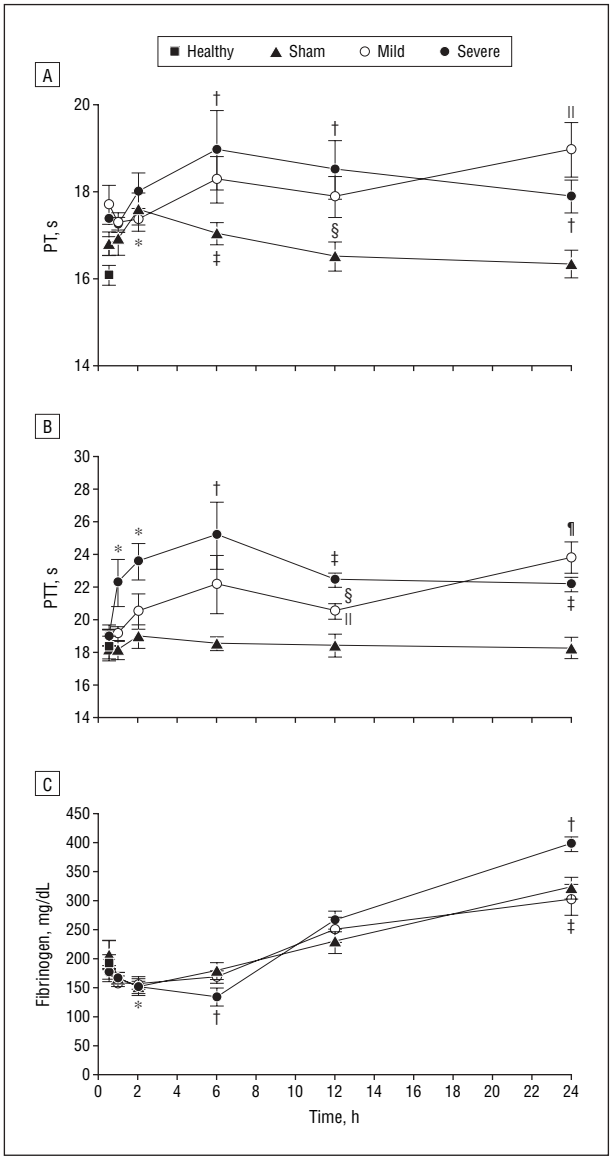

Figure 1. Coagulation abnormalities in acute pancreatitis of graded severity. In the early phase of severe pancreatitis there is consumptive coagulopathy compared with sham-operated controls. PT indicates prothrombin time; PTT, partial thromboplastin time. To convert fibrinogen to micromoles per liter, multiply by 0.0294. A, *$P<.005$, sham vs healthy; †$P<.05$, severe vs sham; ‡$P<.05$, sham vs healthy; §$P<.05$, mild vs sham; and ‖$P<.005$, mild vs sham. B, *$P<.05$, severe vs sham; †$P<.0005$, severe vs sham; ‡$P<.005$, severe vs sham; §$P<.05$, mild vs severe; ‖$P<.05$, mild vs sham; and ¶$P<.005$, mild vs sham. C, *$P<.05$, sham vs healthy; †$P<.05$, severe vs sham; and ‡$P<.005$, mild vs severe.

The PTT, PT, and fibrinogen were measured with an automated analyzer (MDA 180; Organon Teknika Corp, Durham, NC) according to the instructions of the manufacturer. In principle, coagulation was activated with specific substrates, and changes in absorption were measured at 35 wavelengths from 405 to 710 nm.

Cells were counted with a blood analyzer (ADVIA 120 Hematology System; Bayer, Leverkusen, Germany), which is equipped with special software that supports Sprague-Dawley rats.

Tissue was homogenized with a homogenizer (Brinkmann Instruments Inc, Westbury, NY) with 50mM potassium phosphate monobasic buffer (pH 6.0) for 1 minute. Samples were spun at 20 000*g* for 20 minutes and the supernatant was discarded. Pellets then were resuspended with 50mM potassium phosphate monobasic–0.5% hexadeyltrimethyl ammonium bromide buffer (pH 6.0) and sonicated with an ultrasonic cell disruptor-homogenizer (Sonifier 450; Ultrasonics Corp, Danbury, Conn) for 30 seconds followed by 3 freeze-thaw cycles. Then samples were sonicated for 30 seconds and incubated at 60°C for 2 hours. With this preparation method, it is possible to decrease background by eliminating nonspecific peroxidase activity. After spinning at 15 000*g* for 15 minutes, supernatant was transferred to a 96-well plate, and hexadeyltrimethyl ammonium bromide buffer dissolved in dimethyl sulfoxide was added. After reaction buffer consisting of 80mM potassium phosphate monobasic (pH 5.4) plus hydrogen peroxide was added, absorbance was read at 620 nm every 50 seconds for 5 minutes.

STATISTICAL ANALYSIS

Data are presented as mean ± SEM. Differences between groups were compared by *t* test or Mann-Whitney test. Survival was analyzed by the Kaplan-Meier method. Data were analyzed with GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, Calif; http://www.graphpad.com).

RESULTS

PART 1: ASSESSMENT OF COAGULATION CHANGES IN AP

PT, PTT, and Fibrinogen

Results for PT, PTT, and fibrinogen in healthy animals, sham-operation controls, and rats with mild and severe AP are shown in **Figure 1**. Prolongation of PT and decrease in fibrinogen level were observed with sham surgery alone. Animals with severe AP had further worsening of PT and fibrinogen levels and a significantly prolonged PTT. Mild pancreatitis produced intermediate changes.

Hematocrit, WBC Counts, and Platelets

Figure 2 shows the results for these measures. With the exception of a slight decrease in WBC count, there were no changes in sham-operation animals compared with healthy controls. The only prominent change in rats with mild pancreatitis was leukocytosis at 24 hours. By contrast, rats with severe AP had significant hemoconcentration that began shortly after induction, more marked leukocytosis, and marked thrombocytopenia that became apparent at 24 hours.

PART 2: SAFETY ASSESSMENT OF DROTRECOGIN

During the 6-hour infusion of drotrecogin, hematocrit remained constant with every dose given (data not shown). After the infusion, there was no difference in PTT, PT, fibrinogen, WBC count, or hematocrit between sham-operation controls and animals with any concentration of drotrecogin alfa (activated) (data not shown). However, platelet count was significantly lower with 12.5 µg/kg per hour (699 000 ± 34 000/µL; $P<.005$), 21 µg/kg per hour (763 000 ± 19 000/µL; $P<.005$), 50 µg/kg per hour (712 000 ± 33 000/µL; $P<.005$), and 100 µg/kg per hour (756 000 ± 41 000/µL; $P<.005$) compared with sham-operation controls (1 093 000 ± 49 000/µL). None of the animals that received drotrecogin displayed any abnormal bleeding or hematomas on autopsy.

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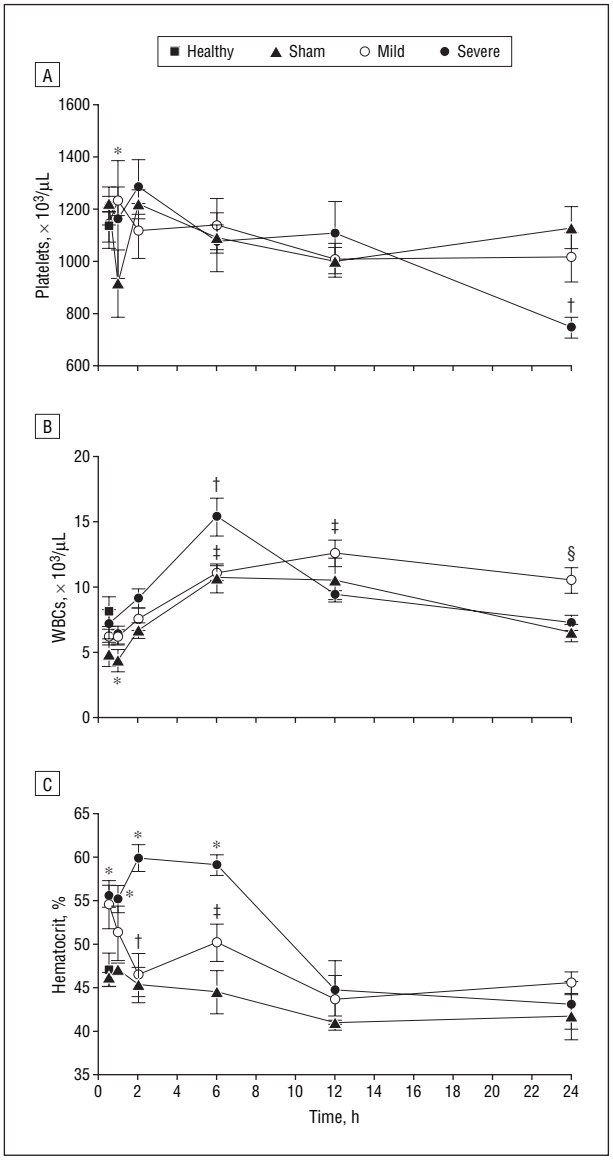

Figure 2. Changes in the platelet count, white blood cell (WBC) count, and hematocrit in acute pancreatitis of graded severity. Of note are leukocytosis at 6 hours in severe pancreatitis and hemoconcentration in severe pancreatitis compared with sham-operation controls. A, *$P<.05$, mild vs sham; †$P<.005$, severe vs sham. B, *$P<.05$, sham vs healthy; †$P<.005$, severe vs sham; ‡$P<.05$, mild vs severe; and §$P<.05$, mild vs sham. C, *$P<.005$, severe vs sham; †$P<.05$, mild vs severe; and ‡$P<.005$, mild vs severe.

PART 3: TREATMENT OF SEVERE AP WITH DROTRECOGIN

At 2 and 6 hours after induction of pancreatitis, there was no difference in PTT, PT, fibrinogen, hematocrit, or WBC count between animals with severe AP and animals with severe AP treated with drotrecogin alfa (activated), 100 µg/kg per hour (**Figure 3**). With the exception of a lower platelet count at 2 hours ($P=.03$), there was no difference between control and treatment groups.

Histologic Assessment of Severity of Pancreatitis

Mean histologic scores of necrosis for animals with severe AP and drotrecogin-treated animals were 2.8±0.3 and 2.8±0.5 at 2 hours and 3.3±0.3 and 3.2±1.7 at 6 hours, respectively. Scores of edema for controls and drotrecogin-treated animals were 1.8±0.1 and 2.0±0.0 at 2 hours and 2.2±0.2 and 2.0±0.1 at 6 hours, respectively. None of these differences was significant.

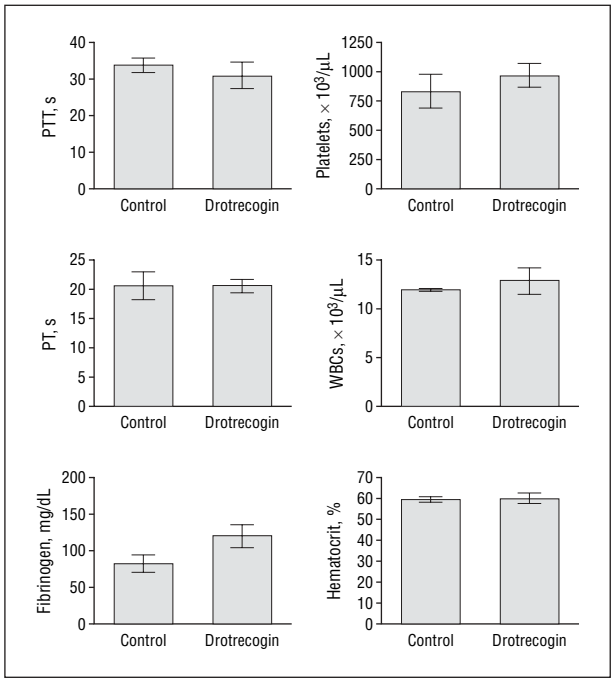

Figure 3. Coagulation abnormalities and changes in the platelet count, white blood cell (WBC) count, and hematocrit 6 hours after induction of severe pancreatitis in controls and animals treated with drotrecogin alfa (activated). There was no difference between the 2 groups. PT indicates prothrombin time; PTT, partial thromboplastin time. To convert fibrinogen to micromoles per liter, multiply by 0.0294.

MPO Activity in Pancreas and Lung 6 Hours After Induction of Pancreatitis

Myeloperoxidase was detectable in healthy pancreas at levels of 1.5±0.5 mU/mg of protein in tissue, which was not different from the value in sham-operation controls (2.4±0.1 mU/mg of protein). Pancreatic MPO concentration was significantly increased in severe AP compared with sham-operation controls (22.0±6.9 mU/mg of protein; $P=.009$). In severe AP treated with drotrecogin alfa (activated), 100 µg/kg per hour, MPO concentration (3.3±1.2 mU/mg of protein; $P=.009$) was markedly lower and in fact no different from that of sham-operation controls or healthy animals (**Figure 4**).

In lungs of healthy animals, MPO concentration was 0.2±0.1 mU/mg of protein, but sham operation alone caused a significant increase compared with healthy lungs (2.7±0.3 mU/mg of protein; $P=.001$). The MPO concentration in lungs of animals with severe AP was similar to that of sham-operation controls (2.8±0.6 mU/mg of protein). Treatment with drotrecogin alfa (activated), 100 µg/kg per hour, decreased MPO levels significantly to the level found in healthy lungs (0.4±0.02 mU/mg of protein; $P=.03$) (**Figure 5**).

PART 4: STUDY OF 24-HOUR SURVIVAL

The 24-hour survival was significantly improved in animals treated with drotrecogin (86% [6/7] vs 38% [3/8];

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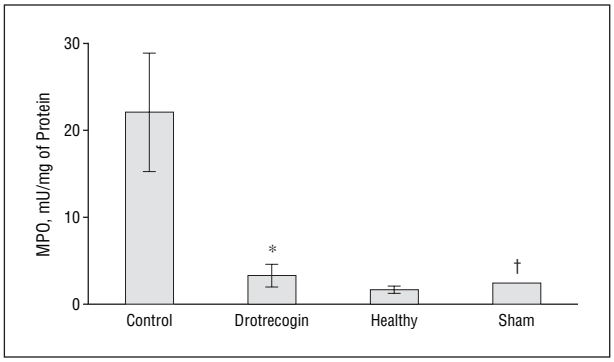

Figure 4. Myeloperoxidase (MPO) concentrations in the pancreas 6 hours after induction of pancreatitis in controls and animals treated with drotrecogin alfa (activated) compared with sham-operation controls and healthy animals. *P=.009, control vs drotrecogin; †P<.001, control vs sham. There were no significant differences among the drotrecogin, healthy, and sham groups.

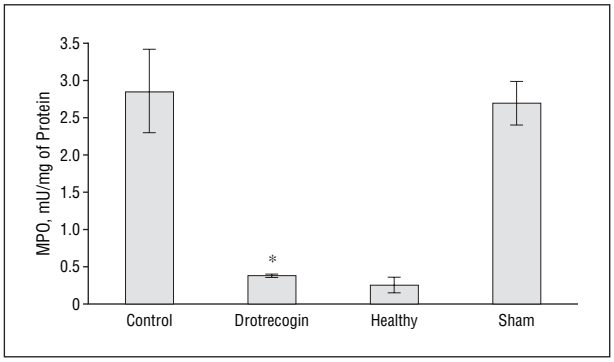

Figure 5. Myeloperoxidase (MPO) concentrations in the lung 6 hours after induction of pancreatitis in controls and animals treated with drotrecogin alfa (activated) compared with sham-operation controls and healthy animals. *P=.03, drotrecogin vs control and drotrecogin vs sham. There were no significant differences between the drotrecogin and healthy groups.

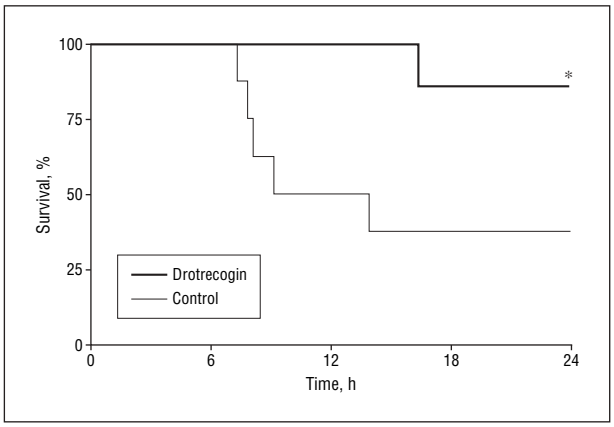

Figure 6. The 24-hour survival of animals with acute severe pancreatitis compared with animals with acute severe pancreatitis that were treated with drotrecogin alfa (activated). Median survival was 11.6 hours in controls. Median survival had not been reached by 24 hours in drotrecogin-treated animals. *P<.05.

P=.05) (**Figure 6**). Median survival in animals with severe AP was 11.6 hours, whereas median survival in animals treated with drotrecogin alfa (activated), 100 µg/kg per hour, was not reached by the end of the 24-hour observation period.

COMMENT

Protein C is a 60-kDa multidomain protein that circulates in plasma as a zymogen and is activated by thrombin bound to thrombomodulin at the surface of endothelial cells.[2] Activated protein C was first described in 1979 and is a regulator of coagulation with high substrate specificity to coagulation factors Va and VIIIa.[21,22] Activated protein C degrades those factors and therefore inhibits the coagulation cascade.[2] It also forms complexes with plasminogen-activator-inhibitor 1 and, by this mechanism, prevents the inhibition of plasmin generation and acts as a profibrinolytic agent.[3,4] In 1991, Esmon et al[23] proposed a link between coagulation and inflammation through a common pathway mediated by protein C. This hypothesis was based on earlier observations in which the infusion of APC blocked the lethal effect of *Escherichia coli* infusion in the baboon, and administration of a monoclonal antibody against protein C turned a sublethal dose of *E coli* into a lethal response in the same model.[9] Subsequent studies showed marked anti-inflammatory effects of APC in various animal models.[7,8,24,25] Joyce and coworkers[6] identified 10 genes with broad transcriptional profiling in human umbilical vein endothelial cells that were directly modulated by APC and resulted in anti-inflammatory and antiapoptotic effects. In a randomized, double-blind, placebo-controlled, multicenter trial[10] evaluating the effect of drotrecogin alfa (activated) in 1690 patients with severe sepsis (Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis [PROWESS]), administration of drotrecogin significantly decreased mortality and reduced the absolute risk of death by 6.1%. In that study, patients with pancreatitis were excluded, unless they had an established source of infection.

Distant organ failure is the most important determinant of severity in AP,[12,13] and coagulation abnormalities contribute to systemic complications.[16] Ranson et al,[16] prospectively evaluating coagulation changes and complications in 35 patients with AP, found that disseminated intravascular coagulation and thromboembolism were related to overall morbidity. Lasson and Ohlsson[26] described consumptive coagulopathy and increased fibrinolysis in 27 patients with pancreatitis. These results were confirmed in experimental studies in dogs in which pancreatitis induced by intraductal bile infusion resulted in elevated PT and PTT, decreased fibrinogen, and thrombocytopenia.[27] Other studies found fibrinolysis early in canine AP[15] and low fibrinolytic activity in taurocholate pancreatitis in rats.[14] Ottesen et al[28] described a decrease in levels of protein C in animals with AP. Thus, the combination of coagulation abnormalities and inflammation in pancreatitis provide a setting in which activated protein C could play a therapeutic role.

In the PROWESS trial, there was a nonsignificant increased incidence of bleeding in patients treated with drotrecogin (3.5% vs 2%). This potential risk of bleeding coupled with a background of coagulopathy from the pancreatitis could have detrimental effects. To assess this, we determined the baseline coagulation changes in our model of pancreatitis and found consumptive coagulopathy in the early phase of severe AP, similar to what has

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been described in other models,[16,27] although significant thrombocytopenia was not found until 24 hours after induction. Platelet counts in human and experimental AP differ between studies and have been reported as both elevated[16] and decreased.[26,27,29]

We also performed a safety assessment by infusing drotrecogin into healthy rats and found no increase in PTT or any bleeding events. Other experimental studies with APC also have not found an increased bleeding tendency.[2,27,30] We did observe a decrease in platelet count with administration of drotrecogin in healthy animals. Activated protein C has been reported to inhibit thrombocytopenia in lipopolysaccharide-induced disseminated intravascular coagulation in rats,[31] but, to our knowledge, there is no report of any effect on platelet count in healthy individuals. We considered all tested doses of drotrecogin as safe and chose the highest dose for the treatment experiments. The same dose (100 μg/kg) has been used in numerous studies in rats and rabbits[2,5,8,32] and is 4 times higher than the dose administered in the PROWESS trial, which was 24 μg/kg per hour. However, requirement for higher doses of human recombinant APC in animal studies has been described with species specificity.[23] Because of the short half-life of APC of about 25 minutes (31 minutes in citrated blood and about 18 minutes in whole blood[33]), we decided to use a continuous infusion, as was done in the PROWESS trial.[10]

In the present study, severe AP was confirmed by histologic examination, elevated hematocrit, and findings on autopsy. There was no difference in any coagulation measures nor any increased bleeding between control or drotrecogin-treated animals with severe AP. Although PT and PTT were prolonged in severe AP, as described in the "Results" section, drotrecogin neither worsened nor improved coagulation abnormalities. A significant finding was the decrease in MPO levels in pancreas and lung in the treated animals. Even the increase in MPO in lungs that resulted from sham operation was not observed with drotrecogin, demonstrating its anti-inflammatory effect, which has also been shown in other experimental models of inflammation.[10,24,34] The reduction of activated leukocytes in the tissue could be explained by recent findings of APC receptors on neutrophils. Activated protein C and protein C were found to inhibit neutrophil migration, which was probably mediated by this receptor.[34,35] Interestingly, despite a decrease in MPO levels, there was no difference in the degree of pancreatic damage between groups. Although the scoring system in our study has been shown to be capable of demonstrating gradations in mild, moderate, and severe AP, the intensity of pancreatic necrosis in these experiments may overwhelm the ability to detect differences that may be present. Alternatively, the pathogenesis of pancreatic necrosis may not be related to the inflammation process, but rather may be a consequence of inappropriate interstitial protease activation in which APC would not be expected to be relevant.

Despite the absence of difference in the pancreatic injury scores, administration of drotrecogin dramatically improved survival in our study. Survival benefit with APC has also been observed in a stroke model in mice[7] and in a sepsis model in the baboon.[9] In the PROWESS trial, mortality was decreased in nearly every subgroup of patients.[36] The mortality rate of patients with pancreatitis in the PROWESS trial was 13.79% for the drotrecogin-treated group and 24.24% for the placebo arm (data on file, Eli Lilly & Co). Since drotrecogin did not reverse the coagulopathy associated with AP in our study, the beneficial effects on survival appear to be independent of the coagulation system. Other experimental studies also show that improvement in survival is unrelated to improvement of coagulation changes.[23,37]

In summary, treatment with drotrecogin markedly improved survival in this model of rodent pancreatitis without concomitant reversal of coagulopathy or protection against pancreatic tissue damage. The basis for this benefit is unclear. Since pulmonary and other organ failure are determinants of mortality in AP, it is possible that the suppression of inflammation by drotrecogin, documented in this study in the lung and pancreas but also likely present in other organs, may also be of importance in this disease process. This study, which we believe to be the first to propose a therapeutic role for APC in pancreatitis, provides a basis for a clinical trial of drotrecogin alfa (activated) in patients with severe AP.

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REFERENCES

1. Grinnell BW, Hermann RB, Yan SB. Human protein C inhibits selectin-mediated cell adhesion: role of unique fucosylated oligosaccharide. *Glycobiology*. 1994; 4:221-225.
2. Arnljots B, Dahlback B. Antithrombotic effects of activated protein C and protein S in a rabbit model of microarterial thrombosis. *Arterioscler Thromb Vasc Biol*. 1995;15:937-941.
3. Esmon CT. Protein C anticoagulant pathway and its role in controlling microvascular thrombosis and inflammation. *Crit Care Med*. 2001;29(suppl):S48-S51.
4. Sakata Y, Loskutoff DJ, Gladson CL, Hekman CM, Griffin JH. Mechanism of protein C–dependent clot lysis: role of plasminogen activator inhibitor. *Blood*. 1986; 68:1218-1223.
5. Murakami K, Okajima K, Uchiba M, et al. Activated protein C prevents LPS-induced pulmonary vascular injury by inhibiting cytokine production. *Am J Physiol*. 1997;272:L197-L202.
6. Joyce DE, Gelbert L, Ciaccia A, DeHoff B, Grinnell BW. Gene expression profile of antithrombotic protein C defines new mechanisms modulating inflammation and apoptosis. *J Biol Chem*. 2001;276:11199-11203.
7. Shibata M, Kumar SR, Amar A, et al. Anti-inflammatory, antithrombotic, and neuroprotective effects of activated protein C in a murine model of focal ischemic stroke. *Circulation*. 2001;103:1799-1805.
8. Mizutani A, Okajima K, Uchiba M, Noguchi T. Activated protein C reduces ischemia/reperfusion–induced renal injury in rats by inhibiting leukocyte activation. *Blood*. 2000;95:3781-3787.
9. Taylor FB Jr, Chang A, Esmon CT, D'Angelo A, Vigano-D'Angelo S, Blick KE. Protein C prevents the coagulopathic and lethal effects of *Escherichia coli* infusion in the baboon. *J Clin Invest*. 1987;79:918-925.
10. 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.

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11. Vincent JL, Angus DC, Artigas A, et al. Effects of drotrecogin alfa (activated) on organ dysfunction in the PROWESS trial. *Crit Care Med*. 2003;31:834-840.
12. Perez A, Whang EE, Brooks DC, et al. Is severity of necrotizing pancreatitis increased in extended necrosis and infected necrosis? *Pancreas*. 2002;25:229-233.
13. Tenner S, Sica G, Hughes M, et al. Relationship of necrosis to organ failure in severe acute pancreatitis. *Gastroenterology*. 1997;113:899-903.
14. Rydzewska G, Kosidlo S, Gabryelewicz A, Rydzewski A. Tissue plasminogen activator, plasminogen activator inhibitor, and other parameters of fibrinolysis in the early stages of taurocholate acute pancreatitis in rats. *Int J Pancreatol*. 1992;11:161-168.
15. Izquierdo R, Sandberg L, Squillaci G, et al. Effects of protease inhibitors on coagulation abnormalities in acute canine pancreatitis. *Am Surg*. 1984;50:317-323.
16. Ranson JH, Lackner H, Berman IR, Schinella R. The relationship of coagulation factors to clinical complications of acute pancreatitis. *Surgery*. 1977;81:502-511.
17. Shinowara GY, Stutman L, Walters MI, Ruth ME, Walker EJ. Hypercoagulability in acute pancreatitis. *Am J Surg*. 1963;105:714-719.
18. *Guide for the Care and Use of Laboratory Animals*. Bethesda, Md: Dept of Health and Human Services, Office of Science and Health Reports; 1985. DHHS publication (NIH) 85-23.
19. Schmidt J, Lewandrowski K, Fernández-del Castillo C, et al. Histopathologic correlates of serum amylase activity in acute experimental pancreatitis. *Dig Dis Sci*. 1992;37:1426-1433.
20. Schmidt J, Rattner DW, Lewandrowski K, et al. A better model of acute pancreatitis for evaluating therapy. *Ann Surg*. 1992;215:44-56.
21. Comp PC, Esmon CT. Activated protein C inhibits platelet prothrombin-converting activity. *Blood*. 1979;54:1272-1281.
22. Esmon CT, Owen WG. Identification of an endothelial cell cofactor for thrombin-catalyzed activation of protein C. *Proc Natl Acad Sci U S A*. 1981;78:2249-2252.
23. Esmon CT, Taylor FB Jr, Snow TR. Inflammation and coagulation: linked processes potentially regulated through a common pathway mediated by protein C. *Thromb Haemost*. 1991;66:160-165.
24. Murakami K, Okajima K, Uchiba M, et al. Activated protein C attenuates endotoxin-induced pulmonary vascular injury by inhibiting activated leukocytes in rats. *Blood*. 1996;87:642-647.
25. Grey ST, Tsuchida A, Hau H, Orthner CL, Salem HH, Hancock WW. Selective inhibitory effects of the anticoagulant activated protein C on the responses of human mononuclear phagocytes to LPS, IFN-gamma, or phorbol ester. *J Immunol*. 1994;153:3664-3672.
26. Lasson A, Ohlsson K. Consumptive coagulopathy, fibrinolysis and protease-antiprotease interactions during acute human pancreatitis. *Thromb Res*. 1986;41:167-183.
27. Satake K, Uchima K, Umeyama K, Appert HE, Howard JM. The effects upon blood coagulation in dogs of experimentally induced pancreatitis and the infusion of pancreatic juice. *Surg Gynecol Obstet*. 1981;153:341-345.
28. Ottesen LH, Bladbjerg EM, Osman M, et al. Protein C activation during the initial phase of experimental acute pancreatitis in the rabbit. *Dig Surg*. 1999;16:486-495.
29. Kwaan HC, Anderson MC, Gramatica L. A study of pancreatic enzymes as a factor in the pathogenesis of disseminated intravascular coagulation during acute pancreatitis. *Surgery*. 1971;69:663-672.
30. Arnljots B, Bergqvist D, Dahlback B. Inhibition of microarterial thrombosis by activated protein C in a rabbit model. *Thromb Haemost*. 1994;72:415-420.
31. Aoki Y, Ota M, Katsuura Y, Komoriya K, Nakagaki T. Effect of activated human protein C on disseminated intravascular coagulation induced by lipopolysaccharide in rats. *Arzneimittelforschung*. 2000;50:809-815.
32. Isobe H, Okajima K, Uchiba M, et al. Activated protein C prevents endotoxin-induced hypotension in rats by inhibiting excessive production of nitric oxide. *Circulation*. 2001;104:1171-1175.
33. Heeb MJ, Gruber A, Griffin JH. Identification of divalent metal ion-dependent inhibition of activated protein C by α_2–macroglobulin and α_2-antiplasmin in blood and comparisons to inhibition of factor Xa, thrombin, and plasmin. *J Biol Chem*. 1991;266:17606-17612.
34. Sturn DH, Kaneider NC, Feistritzer C, Djanani A, Fukudome K, Wiedermann CJ. Expression and function of the endothelial protein C receptor in human neutrophils. *Blood*. 2003;102:1499-1505.
35. Nick JA, Coldren CD, Geraci MW, et al. Recombinant human activated protein C reduces human endotoxin-induced pulmonary inflammation via inhibition of neutrophil chemotaxis. *Blood*. 2004;104:3878-3885.
36. Ely EW, Laterre PF, Angus DC, et al. Drotrecogin alfa (activated) administration across clinically important subgroups of patients with severe sepsis. *Crit Care Med*. 2003;31:12-19.
37. Taylor FB Jr, Chang AC, Peer GT, et al. DEGR-factor Xa blocks disseminated intravascular coagulation initiated by *Escherichia coli* without preventing shock or organ damage. *Blood*. 1991;78:364-368.

Invited Critique

The study by Alsfasser and his colleagues[1] suggests that drotrecogin (or activated protein C) decreases the 24-hour mortality in a modified cerulein-injection model of acute pancreatitis from 5 of 8 control rats to 1 of 8 treated rats. Interestingly, the data show that this survival advantage occurred despite the *same degree of tissue injury* in the pancreas of treated and untreated animals. No histologic evaluation of lung tissue was performed, but the tissue myeloperoxidase level, which is said to be "a quantitative indicator of leukocyte infiltration," was reduced in the pancreas and lungs of treated animals, and the authors conclude that "treatment reduces inflammation in the pancreas and lungs." Unfortunately, no assays of mediators of inflammation such as proinflammatory or anti-inflammatory cytokines were performed, no other autopsy findings were described, and hematologic and coagulation measures such as white blood cell count, hematocrit, platelet count, prothrombin time, and partial thromboplastin time were also not affected, so the mechanism of this survival benefit remains unclear from this study.

The research was funded by Eli Lilly & Co, and the bulk of the study (114 animals' worth) was devoted to demonstrating that the coagulopathy that accompanies acute pancreatitis in this model is not made worse by administration of activated protein C, a natural inhibitor of coagulation. This is an important marketing issue for Eli Lilly, as the risk of bleeding is taken as a relative contraindication to the administration of their product in patients with sepsis (their primary market). As Eli Lilly & Co seeks to expand the indications for their product to include acute pancreatitis, it is necessary to establish the hematologic safety of the drug in an animal model before full-scale clinical trials can get under way; the Food and Drug Administration requires it.

Is this an example of "partnership" that benefits both the company that paid for the study and the clinician-investigators who performed it? Absolutely. Is it unbiased research? Absolutely not. Is it a big step forward in our understanding of the pathophysiology of acute pancreatitis? The jury remains out.

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1. Alsfasser G, Warshaw AL, Thayer SP, et al. Decreased inflammation and improved survival with recombinant human activated protein C treatment in experimental acute pancreatitis. *Arch Surg*. 2006;141:670-676.

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