ORIGINAL ARTICLE

Use of activated protein C has no avail in the early phase of acute pancreatitis

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Abstract

Objectives. Sepsis and acute pancreatitis have similar pathogenetic mechanisms that have been implicated in the progression of multiple organ failure. Drotrecogin alfa, an analogue of endogenous protein C, reduces mortality in clinical sepsis. Our objective was to evaluate the early therapeutic effects of activated protein C (APC) in a rat model of acute necrotizing pancreatitis. *Subjects and method.* Acute necrotizing pancreatitis was induced by intraductal injection of 5% Na taurocholate. Hourly bolus injections of saline or recombinant human APC (drotrecogin alfa) was commenced via femoral venous catheter four hours after the induction of acute pancreatitis. The experiment was terminated nine hours after pancratitis induction. Animals in group one ($n=20$) had a sham operation while animals in group two ($n=20$) received saline and animals in group three ($n=20$) received drotrecogin alfa boluses after acute pancreatitis induction. Pancreatic tissue for histopathologic scores and myeloperoxidase, glutathione reductase, glutathione peroxidase, and catalase activites were collected, and blood for serum amylase, urea, creatinine, and inleukin-6 measurements was withdrawn. *Results.* Serum amylase activity was significantly lower in the APC treated group than the untreated group ($17,435\pm432$ U/L vs. $27,426\pm118$ U/L, respectively). While the serum interleukin-6 concentration in the APC untreated group was significantly lower than the treated group (970 ± 323 pg/mL vs. 330 ± 368 pg/mL, respectively). *Conclusion.* In the early phase of acute pancreatitis, drotrecogin alfa treatment did not result in a significant improvement in oxidative and inflammatory parameters or renal functions.

Key Words: drotrecogin alfa; acute pancreatitis; oxidative stress; activated protein C; glutathione reductase; glutathione peroxidase; myeloperoxidase; catalase

Introduction

Acute pancreatitis includes a spectrum of diseases, from sterile inflammation at one end, to multisystem organ failure at the other. In severe acute pancreatitis, the local pancreatic inflammation extends to a systemic inflammatory response that often leads to multiple organ failure. It is now clear that certain cytokines released from inflammatory cells play an important role in causing the systemic inflammatory response in acute pancreatitis [1].

A synergy between pro-inflammatory cytokines and oxidative stress has been reported in the development of the inflammatory response in acute pancreatitis. Pro-inflammatory cytokines and oxidative stress induce common signal transduction pathways in the augmentation of the inflammatory cascade, mainly through activation of mitogen-activated protein kinases and nuclear factor kappaβ. Pro-inflammatory cytokines and oxidative stress contribute to the formation of a vicious circle in acute pancreatitis [2].

Inflammatory cytokines interact with the microcirculation, inducing increased vascular permeability, thrombosis, and hemorrhage, which results in tissue necrosis. Activated protein C (APC) inhibits thrombin formation, and thus, the clotting cascade; along with likely inhibiting the inflammatory cascade and endothelial cell apoptosis [3]. Rabbits with acute pancreatitis had lower protein C activity than the sham operated animals [4], and lower plasma levels of protein C have been measured in non-surviving patients with acute pancreatitis compared with the survivors [5].

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Recombinant human APC is indicated in patients with a high risk of death from sepsis with no absolute contraindication related to bleeding risk or relative contraindication that outweights potential benefits [6]. Severe acute pancreatitis has many similarities to sepsis syndrome and septic shock [7].

In acute pancreatitis, there may be a place for APC as the infection dominates the clinical picture in later stages of the disease. However, in the early stages of acute pancreatitis, when there is no evidence of infection, the role of APC is questionable. We aimed to evaluate the effect of APC in early acute necrotizing pancreatitis in a sodium taurocholate-induced rat model in different aspects of acute pancreatitis.

Materials and methods

This randomized controlled experimental study was carried out in 60 male Wistar albino rats (250–300 g) purchased from Ege University Faculty of Medicine Research Laboratory. The room was maintained on a 12 hour light–dark cycle and at a temperature of 24°C. Food was withdrawn 12 hours before the experiment.

Experimental model of acute pancreatitis

Acute necrotizing pancreatitis was induced by intraductal infusion of 5% Na taurocholate as described by Schwarz et al. [8]. Rats were anesthetized by intramuscular injection of xylazine (10 mg/kg) plus ketamine hydrochloride (50 mg/kg), and smaller doses were repeated when necessary in order to keep the rats under anesthesia throughout the experiment. A midline laparotomy was performed under sterile conditions. The biliopancreatic duct was cannulated with a 27-gauge needle. The distal biliopancreatic duct was ligated to avoid reflux of taurocholate into the duodenum. To prevent leakage of bile salt into the liver, the bile duct was clamped temporarily near the hepatic hilum. Sterile 5% sodium taurocholate (0.1 mL per 100 g body weight) was infused slowly under 25 cm H_2O pressure.

Venous line

Access to the right femoral vein was accomplished through an inguinal incision. A 24G cannula was then inserted into the femoral vein.

Study design

Sixty rats were randomly divided into three groups. Rats in group 1 ($n = 20$) underwent laparotomy and manipulation of the pancreas (sham procedure), and intravenous cannula insertion. Four hours post-abdominal incision, hourly infusion of 0.3 mL saline was commenced and perpetuated until the termination of the experiment. Groups 2 and 3 underwent laparotomy with induction of acute necrotizing pancreatitis and intravenous cannula insertion. Rats in group 2 (positive control; $n = 20$) received hourly boluses of 0.3 mL saline, which were commenced four hours post-abdominal incision and perpetuated until the termination of the experiment. Rats in group 3 (treatment group; $n = 20$) received 24 µg/kg/h of recombinant human APC (6 µg in 0.3 mL serum physiologic/hourly boluses) (Xigris; Lilly, Istanbul, Turkey). The boluses were commenced four hours post-acute pancreatitis induction and perpetuated until the termination of the experiment. The experiments were terminated nine hours after the induction of acute pancreatitis. Blood samples were taken from the heart before the animals were sacrificed by cervical dislocation.

Grading of morphologic severity of acute necrotizing pancreatitis

Pancreatic tissue was excised and one piece was fixed in 5% formaldehyde for histologic examination and another was stored at $-70°C$ for myeloperoxidase (MPO), glutathione reductase, glutathione peroxidase and catalase activity measurements. Histologic examination was done under light microscopy after staining with hematoxylin and eosin by an experienced pathologist. The severity of necrotizing pancreatitis was determined by the scoring system described by Spormann et al. [9]. This score includes the graded assessment of pancreatic edema (0–3), inflammatory infiltration (0–3), pancreatic necrosis (0, 3, 5, 7), pancreatic hemorrhage (0, 3, 5, 7), and extrapancreatic fat necrosis (0, 3, 5, 7) for a maximum possible score of 27.

The rat serum interleukin-6 immunoassey kit was purchased from Biosource Europa S.A. Belgium. Serum urea, creatinine, and amylase kits were purchased from Roche Diagnostics. For MPO determination, the method of Suzuki et al. was used with a slight modification [10]. Glutathione reductase, glutathione peroxidase, and catalase activities were determined according to the methods described by Beutler [11], Paglia [12], and Aebi [13], respectively. Protein determinations were performed by the method described by Lowry et al. [14]

All experiments were conducted with approval of the Animal Research Committee at Ege University Medical Center, Izmir, Turkey.

Statistics

The results were given as mean $\pm$ SD. The SPSS statistical package (SPSS software, PC version 10.01; SPSS, Chicago IL) was used for statistical analyses. The Mann–Whitney U and Kruskal Wallis tests were used when appropriate. $P < 0.05$ was considered statistically significant.

Results

Rats with acute necrotizing pancreatitis (ANP) had extensive parenchyma and fat necrosis, and edema on histologic examination. The mean histopathologic scores were 9.1 ± 2.1 and 9.9 ± 2.3 in the treatment group and in the untreated group, respectively. The difference between the histopathologic scores of the two groups was not statistically significant.

Mean pancreatic MPO activity was 1916 ± 2672 U/g of wet weight tissue in the treatment group and 1814 ± 1900 U/g of wet weight tissue in the untreated group. The difference between the pancreatic MPO activities of the two groups was not statistically significant. The mean pancreatic MPO activity in the sham group was 189.9 ± 67.5 U/g of wet weight tissue.

The mean serum interleukin-6 concentration of the APC treated group (970 ± 323 pg/mL) was significantly higher than the untreated group (330 ± 368 pg/mL). The mean serum interleukin-6 concentration in the sham group was 78 ± 7 pg/mL ($p < 0.001$) (Figure 1).

Mean serum amylase activity of the treatment group was significantly lower ($17,435 \pm 4326$ U/L) than that of the APC untreated group ($27,426 \pm 11,864$ U/L), which is shown in Figure 2 ($p = 0.02$).

The mean values of the other parameters are shown in Table I.

Discussion

Our results show that APC decreases amylase levels and increases serum IL-6 concentrations in acute pancreatitis in rats. However, no amelioration in pancreas histology, pancreas MPO level, pancreas oxidative stress, or renal functions was achieved.
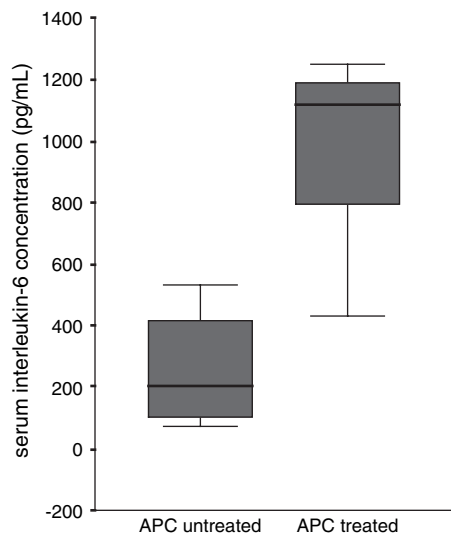
Figure 1. Mean serum interleukin-6 concentrations in APC treated and untreated rats with acute pancreatitis.
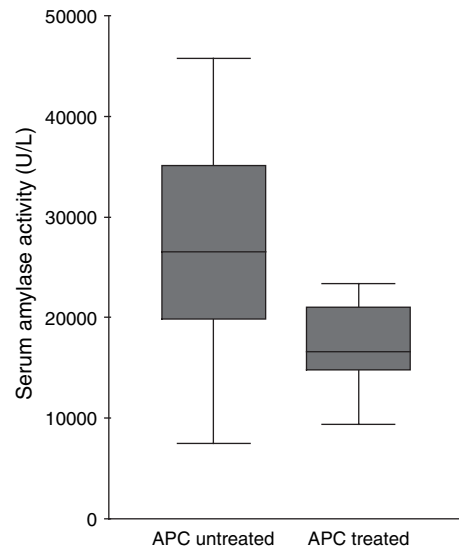
Figure 2. Mean serum amylase activity in APC treated and untreated rats with acute pancreatitis.

Acute pancreatitis is similar to severe septic responses in many aspects. The hemodynamic features of cardiovascular instability are indistinguishable in each of these conditions. In addition, there are many striking similarities in the cytokine and inflammatory mediator profile, suggesting that the hemodynamic abnormalities may result from the same pathogenetic mechanism, albeit as a result of different inflammatory stimuli. Although septic complications of severe acute pancreatitis do arise, these are usually late features, and in the early phase of a severe attack, sterile pancreatic necrosis is found. Evidence suggests that the important cytokines in the development of complications and multiple organ failure in severe acute pancreatitis are tumor necrosis factor alfa, interleukin-1, interleukin-6, and interleukin-8 [7].

It has been shown that acinar cells produce large amounts of reactive oxygen species (ROS) during the early stages of acute pancreatitis in rats [15]. Under normal conditions, a natural system of scavengers and antioxidants counteracts the cytotoxicity of ROS produced from molecular oxygen in the mitochondria [16]. The reductions in antioxidant levels are concominant with overt signs of pancreatitis, such as increases in amylase levels and pancreatic edema [17]. The antioxidant enzyme levels represent the severity in the early phase of acute pancreatitis, and in our study, the decreases in the antioxidant levels did not show any difference between the treated and untreated groups. In other words, drotrecogin alfa showed no effect on oxidative stress in the early phase of acute pancreatitis.

APC has anti-inflammatory properties related, in part, to inhibition of the production of monocyte tumour necrosis factor alfa and the modulation of E-selectin expression by endothelial cells [18]. However, pancreas MPO activity did not show any difference between the treated and untreated groups.

Table I. Laboratory values and histopathologic scores.

	Treated (n = 20)	Untreated (n = 20)	Sham (n = 20)	P-value
Mean histopathologic score	9.1 ± 2.1	9.9 ± 2.3		$p > 0.05$
Mean pMPO activity (U/g of wet weight tissue)	1916 ± 2672	1814 ± 1900	189 ± 67	$p > 0.05$
Mean serum amylase activity (U/L)	17,435 ± 4326	27,426 ± 11,864	1844 ± 427	$P = 0.02$
Mean serum urea concentration (mg/dL)	100 ± 13	100 ± 14	56 ± 0.8	$p > 0.05$
Mean serum creatinine concentration (mg/dL)	0.73 ± 0.1	0.89 ± 0.11	0.38 ± 0.03	$p > 0.05$
Mean serum interleukin-6 concentration (pg/mL)	970 ± 323	330 ± 368	78 ± 7	$p < 0.001$
Mean tissue catalase activity (U/mg protein)	592 ± 261	703 ± 439	2928 ± 243	$p > 0.05$
Mean tissue glutathione reductase activity (mU/mg protein)	3.6 ± 1.8	3.1 ± 1.4	5.4 ± 0.7	$p > 0.05$
Mean tissue glutathione peroxisade activity (mU/mg protein activity)	23.6 ± 4.7	28.2 ± 11.9	36.8 ± 0.4	$p > 0.05$

In addition, inflammatory infiltrate in pancreas histology and the overall histology scores of both groups were not statistically different.

During the first 24 hours of acute pancreatitis, IL-6 has 70% sensitivity in predicting the disease severity, which is higher than C-reactive protein. In acute pancreatitis and sepsis, higher IL-6 concentrations correlate with poor outcomes [19]. In this study, rats in the untreated group had lower IL-6 concentrations than the drotrecogin treated group, which may indicate that drotrecogin might indeed be deleterious in the early phase of acute pancreatitis. However, Li et al. [20] showed that on human umblical vein endothelial cells stimulated with lipopolysaccharide, APC increased interleukin-6 levels. Therefore, the higher interleukin-6 levels in the APC group in our study may be the direct result of APC administration. The only parameter improved with APC treatment was serum amylase, but serum amylase levels do not have prognostic significance or indicate the severity of the disease in the rat model or in human acute pancreatitisis.

With this model of acute pancreatitis combined with continuous ketamine anesthesia, a high rate of mortality after the ninth hour of the experiment was experienced in our preliminary studies. Thus, we terminated the experiment nine hours post-induction of ANP.

The search for treatment options for acute pancreatitis led to many experimental drug studies with positive results, but none of the molecules or drugs have come into clinical use. Yamanel et al. reported that APC improved the pancreas histology, superinfection rates, and serum markers of inflammation in the taurocholate acute necrotizing pancreatitis model [21]. In a similar model, Chen et al. showed that APC ameliorated acute pancreatitis via regulation of mitogen-activated protein kinases [22]. However, in both studies, a single injection of APC was performed. According to the provided human data, more than 70% of the administered APC is eliminated within 30 minutes of administration and it is recommended to be given as a continuous intravenous infusion [23]. We tried to imitate the human use of APC in sepsis, in dose and route of administration, because there is no other reasonable reference for APC administration in a rat model. Even the use of APC in severe sepsis is questionable because there is a recently published metaanalysis of clinical trials that does not support the use of APC in severe sepsis [24].

In conclusion, despite the benefits reported by others, our results do not provide experimental evidence to promote APC use in early phase of ANP.

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