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MicroRNA expression following activated protein C treatment during septic shock

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ABSTRACT

Background: Sepsis induces systemic stress by augmenting inflammatory and procoagulant responses, resulting in microvascular dysfunction and end organ failure, events modulated by the protein C pathway. MicroRNAs (miRNAs) are small noncoding RNAs involved in post-transcriptional regulation of genes; yet, their role in sepsis is poorly defined. We hypothesized that activated protein C (aPC) selectively alters specific miRNA expression implicated in protection of hepatic function during septic shock.

Methods: Male Sprague-Dawley rats underwent sham or cecal ligation and puncture surgery; 24 h later, we randomized them to aPC (1 mg/kg) or vehicle (0.9% [w/v] saline) treatment via an indwelling venous catheter (12-h intervals for 24 h). We performed gene array and quantitative reverse transcriptase–polymerase chain reaction analysis on hepatic RNA to determine miRNA expression and determined predicted mRNA targets using a bioinformatics approach. We confirmed beneficial effects of aPC treatment in the cecal ligation and puncture model of sepsis by survival and blood chemistries, and histologically.

Results: Of 351 rat miRNAs examined, 17 were highly expressed during sepsis and restored to basal levels after aPC treatment. We confirmed expression of select miRNAs (miR-182, -199a-5p, -203, -211, -222, and -29b) using quantitative reverse transcriptase–polymerase chain reaction. *In silico* analysis identified nine miRNAs significantly regulating target genes of the focal adhesion pathway.

Conclusions: These data suggest that aPC treatment coordinates beneficial cytoprotective effects during sepsis by modulating miRNA expression. Whereas translational effects remain to be fully elucidated in a clinical setting, we demonstrate here the potential experimental and computational benefits of using of microRNA analysis in sepsis.

1. Introduction

Sepsis is a complex, often life-threatening, reaction to infection associated with coagulation and excessive stimulation of the immune system. Acute hepatic dysfunction emerges in the early hours after sepsis and is characterized by hypotension regardless of adequate fluid infusion. Despite the therapeutic success of supportive care strategies, patients who manifest systemic inflammatory response syndrome (SIRS) may still experience multiple organ dysfunction [1]. Progression to multiple organ failure can then ensue, with an associated mortality of 50%–70%. The cellular events

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culminating in SIRS and multiple organ dysfunction remain poorly understood. Recent reports from our group support the paradigm that patients develop multiple organ failure because of microcirculatory changes in response to endothelial dysfunction resulting from altered vasoregulation and inflammation [2]. Numerous therapeutic strategies have targeted proinflammatory mediators with promising effects when used in animal models; however, efficacy in clinical trials has failed [3].

In addition to "goal-directed" therapies in sepsis management, therapeutic strategies include low-dose glucocorticoid administration, intensive insulin therapy, and infusion of activated protein C (aPC), and have been used in critically ill patients [4–6]. Protein C is an endogenous molecule produced in the liver that promotes fibrinolysis while inhibiting thrombosis and reducing inflammation [7]. As such, the protein C pathway represents an important modulator of coagulation-inflammation associated with sepsis [8]. Depletion of endogenous aPC levels in septic patients may contribute to increased inflammatory congestion in the hepatic sinusoids [9,10]. We have previously reported that aPC treatment for 4 d improves mortality by modulating hepatic sinusoidal vasoregulation and restoring hepatic oxygenation in a severe animal model of sepsis [2]. Our cecal ligation and puncture (CLP) model resulted in 90% mortality, whereas treatment with activated aPC reduced mortality to 40%.

In addition, we observed that aPC treatment after septic insult decreased sinusoidal leukocyte-endothelial interactions and improved hepatic sinusoidal congestion [11]. A recent study reported that treatment with aPC alters leukocyte adhesion, migration, and apoptosis during sepsis [12]. However, the clinical efficacy of aPC treatment during sepsis has been the subject of controversy [13]. Recently, the Food and Drug Administration denounced the use of aPC in patients with severe sepsis because of the failure to show survival benefits (PROWESS-SHOCK trial). Nevertheless, multiple studies report anti-inflammatory effects of aPC [4,14]. These findings indicate an incomplete understanding of aPC at a mechanistic level and warrant further investigation.

A potential mechanism of action for aPC in the liver during sepsis may be via changes in microRNA (miRNA) expression and function. MicroRNAs are short, noncoding, endogenous RNAs that post-transcriptionally regulate gene expression by (predominantly) targeting the 3′-untranslated region of mRNAs [15]. Within the setting of sepsis or endotoxic shock, miR-146 and miR-155 are linked to inflammatory responses in human leukocytes [16]. Similarly, up-regulation of miR-150 expression in plasma of septic patients has raised the possibility of using this miRNA as a diagnostic tool [17]. In the liver, suppression of miR-142-3p in Kupffer cells attenuated proinflammatory cytokines levels after septic challenge [18]. In addition, endothelin-1, an important mediator of hepatic vascular tone under normal and pathological conditions, regulated miR-199a-5p expression in sinusoidal endothelial cells in an animal model of alcohol-induced liver damage [19]. Collectively, these data suggest that miRNAs act to modulate hepatic vasoregulators and/or inflammatory responses under pathological conditions, and that aPC may exert protective effects by altering miRNA expression. The overall objective of the current study was to examine the effect of aPC treatment on hepatic miRNA expression after sepsis in an animal model of CLP. Because the regulation of miRNA expression is dynamic, we strategically chose the earlier time point (48-h flowing CLP) based on previously published findings from our laboratory [11,20], to capture the initial molecular alterations in miRNA expression.

2. Materials and methods

2.1. Animals

We used male Sprague-Dawley rats (200–300 g; Charles River Laboratories, Wilmington, MA) for these studies. The Institutional Animal Care and Use Committee at Carolinas Medical Center approved all experiments, which we performed in accordance with the *National Institutes of Health Guidelines for the Care and Use of Laboratory Animals*.

2.2. Materials

We purchased recombinant human aPC (Eli Lilly, Indianapolis, IN) from the pharmacy at Carolinas Medical Center. We purchased miRNeasy from Qiagen (Valencia, CA), an RT^2 miRNA First Strand Kit, SYBR Green qPCR Mastermix, and qPCR assays from Qiagen (Valencia, CA).

2.3. Injury model

We randomized animals to one of two groups: sham and CLP. All animals received saline (0.9% [w/v]) resuscitation immediately after CLP surgery. We administered buprenorphine (0.03 mg/kg, subcutaneously) immediately after each procedure and at 8-h intervals thereafter for pain control.

2.4. Sham

We induced surgical anesthesia by isoflurane inhalation, performed a midline laparotomy, and closed it in two layers before placing an indwelling venous catheter in the right jugular vein, as previously reported [2].

2.5. CLP

We induced surgical anesthesia by isoflurane inhalation and performed CLP as previously reported [2,21]. Briefly, we exposed the cecum and ligated it just distal to the ileocecal valve with intestinal continuity maintained. We punctured the cecum twice (two holes) with a 22-gauge needle, closed the abdominal incision in two layers, and placed an indwelling venous catheter in the right jugular vein.

2.6. Experimental groups

At 24 h after surgery, we randomized animals to drug vehicle (V) or experimental (aPC) groups. Animals assigned to drug vehicle group received saline (0.9% [w/v]) via the indwelling venous catheter. We designed the dose of aPC to mimic that used in clinical trials [22]. Animals assigned to the experimental group received aPC (1 mg/kg) twice at 12-h intervals via the indwelling