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Adenosine monophosphate-activated protein kinase activation protects against sepsis-induced organ injury and inflammation

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ABSTRACT

Background: Mortality in sepsis is most often attributed to the development of multiple organ failure. In sepsis, inflammation-mediated endothelial activation, defined as a proinflammatory and procoagulant state of the endothelial cells, has been associated with severity of disease. Thus, the objective of this study was to test the hypothesis that adenosine monophosphate-activated protein kinase (AMPK) activation limits inflammation and endothelium activation to protect against organ injury in sepsis. 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), which is an adenosine monophosphate analog, has been used to upregulate activity of AMPK. Compound C is a cell-permeable pyrrazolopyrimidine compound that inhibits AMPK activity.

Methods: Wild-type mice underwent cecal ligation and puncture (CLP) or sham surgery. Mice were randomized to vehicle, AICAR, or compound C. Mouse kidney endothelial cells were used for *in vitro* experiments. Renal and liver function were determined by serum cystatin C, blood urea nitrogen (BUN), creatinine, and alanine aminotransferase. Serum cytokines were measured by enzyme-linked immunosorbent assay. Microvascular injury was determined using Evans blue dye and electron microscopy. Immunohistochemistry was used to measure protein levels of phospho-AMPK (p-AMPK), microtubule-associated protein 1A/1B-light chain 3 (LC3), and intracellular adhesion molecule. LC3 levels were used as a measure of autophagosome formation.

Results: AICAR decreased liver and kidney injury induced by CLP and minimized cytokine elevation *in vivo* and *in vitro*. CLP increased renal and hepatic phosphorylation of AMPK and autophagic signaling as determined by LC3. Inhibition of AMPK with compound C prevented CLP-induced autophagy and exacerbated tissue injury. Additionally, CLP led to endothelial injury as determined by electron microscopy and Evans blue dye extravasation,

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and AICAR limited this injury. Furthermore, AICAR limited CLP and lipopolysaccharide (LPS)-induced upregulation of intracellular adhesion molecule *in vivo* and *in vitro* and decreased LPS-induced neutrophil adhesion *in vitro*.

Conclusions: In this model, activation of AMPK was protective, and AICAR minimized organ injury by decreasing inflammatory cytokines and endothelial activation. These data suggest that AMPK signaling influences sepsis or LPS-induced endothelial activation and organ injury.

1. Introduction

Sepsis is the leading cause of death in the critically ill patient population [1]. Despite important efforts to understand the syndrome and multiple trials to test promising therapies, death rates have remained relatively stable for decades. Mortality by sepsis is directly related to the development of organ dysfunction [2], a process that remains incompletely understood. The pathogenesis of organ dysfunction is multifactorial and includes direct cellular activation from circulating bacterial products, elaborated cytokines, and subsequent tissue hypoperfusion. Recent data have demonstrated that the cellular response to sepsis includes significant bioenergetic and metabolic regulation, including significant changes in mitochondrial responses [3–6].

Under normal physiologic conditions, cells maintain energy homeostasis through highly coordinated systems. Mitochondria have been shown to be central to these processes, not only in regards to production of adenosine triphosphate (ATP) but also as a critical signaling organelle that can sense changes in the metabolic environment and then signal to initiate adaptive responses. Adenosine monophosphate-activated protein kinase (AMPK) is one of the most important energy regulators in the cell [7,8]. AMPK is a heterotrimeric kinase that fulfills a dual role. First, it is a very fine sensor of alterations in energy homeostasis as it monitors adenosine monophosphate (AMP) to ATP ratio. Others and we have demonstrated increased AMP levels in the setting of sepsis [4,5], suggesting an increment in ATP turnover and perhaps a decrease in cellular energy charge. Second, its activation by relative increments of AMP to ATP modulates the activity and expression of key rate-limiting enzymes that control energy-consuming and energy-generating pathways [9,10]. In essence, AMPK regulates energy utilization and promotes energy homeostasis in the cell.

More recently, AMPK has been shown to regulate several additional important cellular pathways and processes, including transcription and protein synthesis, a number of membrane transport proteins in the kidney and other tissues [11] and autophagy [3,12]. These pleiotropic effects of AMPK are consonant with its role as a guardian of cellular energy homeostasis [7,13].

Based on the fact that AMPK activation is part of the cellular response to stress [14] and based on the suggestion that such activation can protect against organ injury by decreasing inflammation in multiple animal models including hemorrhagic shock, ischemic preconditioning, and ischemia–reperfusion [3,15–21], these experiments were designed to test the hypothesis that AMPK protects against sepsis-induced endothelial activation and injury, and that AMPK agonists would limit organ injury and inflammation.

2. Materials and methods

2.1. Cecal ligation and puncture

Animal protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Experiments were performed in adherence to the National Institutes of Health Guidelines on the Use of Laboratory Animals. Cecal ligation and puncture (CLP) was performed on male C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME; aged 8–10 wk). These animals were anesthetized with pentobarbital (70 mg/kg, intraperitoneal [IP]). A 1- to 2-cm midline laparotomy was performed, and the cecum was identified. Stool was expressed to the tip of the cecum, and then the cecum was ligated at the level of the second cecal artery with 2-0 silk. The cecum was then perforated twice with a 22-gauge needle and returned into the abdomen. The muscle and skin were closed with a running 4-0 vicryl suture (Ethicon, Scottland, No SC 132162). Control animals underwent laparotomy and bowel manipulation without ligation or perforation. Animals were resuscitated with 1.0 mL of 0.9% normal saline, immediately after surgery via a subcutaneous injection. Tissue and blood collection occurred at 8 or 24 h after CLP. No antibiotics were used, and animals had free access to food and water preoperatively and postoperatively. In some experiments, mice were randomized to receive the AMPK agonist 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR [Biovision, San Francisco, CA]; 100 mg/kg; IP), or the AMPK inhibitor compound C (Biovision) (30 mg/kg; IP). Control mice received saline as vehicle only at the same volume (500 mL). Doses were selected based on previous reports from the literature [20,22–24].

2.2. Cell culture

Primary mouse peritoneal macrophages were harvested from male C57BL/6 mice by lavage of the peritoneal cavity with phosphate-buffered saline (PBS) [25]. Macrophages were then plated on 6-well plates in RPMI 1640 medium supplemented with 10% fetal bovine serum, 50 IU/mL penicillin, 50 μg/mL streptomycin, and 2 mM L-glutamine. A total of 4 h after plating, cells were washed three times with PBS to remove the nonadherent cells. Adherent cells were then incubated for an additional 24 h at 37°C before treatment. Primary male C57BL/6 mouse kidney glomerular endothelial cells (MKGECs) were purchased from Cell Biologics (Chicago, IL). They were cultured in cell culture medium (Cell Biologics) supplemented with minimum essential medium nonessential amino acids solution (5.0 mL), L-glutamine (5.0 mL), penicillin-