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AMPK 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 AMPK activation limits inflammation and endothelium activation to protect against organ injury in sepsis. 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR), which is an AMP analogue, 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 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, BUN, creatinine, and ALT. Serum cytokines were measured by ELISA. Microvascular injury was determined using Evan's blue dye and electron microscopy. Immunohistochemistry was used to measure protein levels of p-AMPK, LC3, and ICAM. 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

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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 Evan's blue dye extravasation, and AICAR limited this injury. Furthermore, AICAR limited CLP and LPS induced upregulation of ICAM *in vivo* and *in vitro*, and decreased LPS induced neutrophil adhesion *in vitro*.

Conclusion—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.

Keywords

Sepsis; AMPK; energy; inflammation; endothelium; organ injury

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, as well as subsequent tissue hypoperfusion. Recent data has 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 ATP, but also as a critical signaling organelle that can sense changes in the metabolic environment and then signal to initiate adaptive responses. AMP-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 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.

Materials and methods

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; 8–10 weeks of age). 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. 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 subcutaneous injection. Tissue and blood collection occurred at 8 or 24 hours post-CLP. No antibiotics were used, and animals had free access to food and water pre and postoperatively. In some experiments, mice were randomized to receive the AMPK agonist 5-Aminoimidazole-4-carboxamide ribonucleotide [AICAR (Biovision, San Francisco, CA); 100mg/kg; IP], or the AMPK inhibitor Compound C (Biovision) (30mg/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)

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% FBS, 50 IU/ml penicillin, 50 μg/ml streptomycin and 2 mM L-glutamine. Four hours after plating, cells were washed 3 times with PBS to remove the non-adherent 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 MEM Non-essential amino acids solution (5.0 mL), L-Glutamine (5.0 mL), Penicillin-Streptomycin (5.0 mL) and 5% fetal bovine serum on coverslips for immunohistochemistry. Cells were used on day 2 of harvest. For in vitro experiments some cells were exposed to lipopolysaccharide (LPS 10–100ng/mL), AICAR (1mM), and/or Compound C (10μM).

Neutrophil adhesion assay

Mouse bone marrow neutrophils were prepared as described with some modifications (26). Briefly, PMNs were isolated from femurs and tibias flushed with Ca2+/Mg2+-free Hanks' balanced salt solution (HBSS)-BSA. The obtained marrow was centrifuged at 300 g, 4°C for

10 min, and resuspended in 3 ml of HBSS. The suspension was subjected to a Percoll step gradient, the gradient was then centrifuged, and cells were removed from the neutrophil-enriched fraction. This procedure yielded >95% PMN purity and >95% viability, assessed by Trypan blue exclusion. Cells were washed with Ca2+/Mg2+-free HBSS (for calcein AM labeling). The assay for PMN adhesion to endothelial cells was performed as previously described (27). MKGECs were isolated and grown to confluence in 96-well gelatin-coated plates. Bone marrow PMNs loaded with calcein AM (Molecular Probes) at 2 μg/ml for 30 min at room temperature were added to MKGECs pretreated with AICAR (1 mM) for 1 h at 37°C. Cells were then treated with LPS (100 ng/mL and 1ug/mL) for 6 h. PMN adhesion was evaluated after treatment of PMNs with anti-CD11b mAb (M1/70) or anti-CD11a mAb (M17/4), each at a concentration of 10 μg/ml (BD Biosciences, San Diego, CA). The fluorescence readings were obtained with the Vactor II spectrofluorometer (Photon Technology International, Monmouth Junction, NJ) with detection at 485 and 535 nm, respectively. The percentage of adherent PMNs was calculated, and all assays were performed in duplicate.

Vascular leak assay

Eight hours post control or CLP surgery, animals were injected with 200 μL of 0.5% Evans blue dye (Sigma-Aldrich, St Louis, MO) via tail vein. The dye was allowed to percolate to the subendothelial spaces for 30 minutes, and then mice were sacrificed. Animals were perfused with cold PBS to wash away extra dye; whole kidneys were weighed and then dissociated with formamide (Sigma-Aldrich) for 48 h at 37° C. After 2 days, supernatants were spun down and read on a spectrophotometer at 620 nm.

Immunocyto/histochemistry

Cells were fixed on coverslips with paraformaldehyde for 15 minutes and then rinsed with cold PBS. Slides were then stained for intracellular adhesion molecule-1 (ICAM-1) (Santa Cruz Biotechnology, Dallas, TX) or vascular cell adhesion molecule-1 (VCAM-1) (Santa Cruz) to monitor endothelium activation. For immunohistochemistry, tissues were harvested, washed in cold PBS and then placed in paraformaldehyde (2%) for 1 hour, and then switched to 30% sucrose solution for 12 hours. The tissue was then slowly frozen in 2-methylbutane. Sections were stained against p-AMPK (Cell signaling, Beverly, MA), LC-3 (Cell signaling), or ICAM-1. Images were taken with an Olympus Provis Fluorescence microscope. Autophagy was determined as elevated LC3 levels in immunohistochemistry.

Electron microscopy

Mice were perfused with cold PBS, then with 2% paraformaldehyde and 2% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4) and processed for transmission electron microscopy (TEM) as described before (28). After dehydration, thin sections were stained with uranyl acetate and lead citrate for observation under a JEM 1011CX electron microscope (JEOL, Peabody, MA). Images were acquired digitally from a randomly selected pool of 10–15 fields under each condition.

Organ Injury measurement

Blood samples were obtained from cardiac puncture at 8 and 24 hours post-CLP. Cystatin C was determined from serum using a mouse Cystatin C kit according to the manufacturer's instructions (R&D systems, Minneapolis, MN). Serum concentration of BUN, Creatinine, ALT and AST was determined with a HESKA DRI-CHEM 4000 chemistry analyzer (Loveland, CO).

Data analysis

Data is presented as mean ± standard error. One-way analysis of variance was used to determine differences between treatment groups. Statistical significance was determined as $p<0.05$.

Results

AMPK activation by AICAR minimizes sepsis-induced organ injury

The influence of AICAR on tissue injury was investigated in animals with CLP-induced sepsis. Sepsis-induced kidney failure was measured by serum Cystatin C (pg/mL), BUN, and Creatinine (mg/dL). As expected, CLP animals had a significant elevation of all Acute Kidney Injury (AKI) markers, compared to control animals (Cystatin C: 103.93 ng/mL ± 6.85 vs 29.18 ng/mL ± 2.42, p=0.0004; BUN: 62.18 mg/dL ± 1.2 vs 18 mg/dL ± 1.6, p<0.001; Creatinine 0.43 mg/dL ± 0.02 vs 0.16 mg/dL ± 0.01, p>0.001; Figure 1A–C). Administration of AICAR 24 hours before CLP protected against renal injury, with lower levels of AKI markers when compared to CLP mice (Cystatin C: 49.32 ng/mL ± 2.6, p=0.01; BUN: 43.71 ± 1.9, p=0.01; Creatinine: 0.20mg/dL ± 0.002, p=0.0003). There was a trend towards increased liver injury in CLP mice at the 8-hour time point (96.7 IU/L ± 9.93 vs 53.8 IU/L ± 11.8, p=0.3) and AICAR attenuated this injury (65 IU/L ± 3.6, p=0.3 versus CLP alone; Figure 1D).

AMPK activation by AICAR decreased sepsis-induced cytokines

The activation of an inflammatory cascade with increased secretion of proinflammatory cytokines has been previously shown to contribute to the sepsis-induced organ dysfunction (29, 30). CLP induced increases in the serum level of multiple cytokines, including IL-1β, IL-6, IL-10, IL-17, RANTES, and TNF-α (*P<0.05 compared to control mice; Figure 2A–F). AICAR pretreatment limited these sepsis-induced changes (#P<0.05 compared to vehicle treated, CLP mice). The influence of AMPK activation on LPS-induced cytokines in cultured primary peritoneal macrophages was also determined. In vitro, AICAR limited LPS-induced increases in cell culture media TNF-α and IL-6 levels, and interestingly increased levels of the anti-inflammatory cytokine IL-10 (*P<0.05 compared to unstimulated controls; #P<0.05 compared to LPS alone; Figure 3A–C).

Inhibition of AMPK prevents autophagy and increases tissue injury

The activation of AMPK has been shown to be associated with increased adaptive cell signaling responses, including autophagy (3, 12, 31, 32). Additionally, autophagic signaling has been shown to be a protective response in the setting of sepsis (4, 12, 33–35). The

influence of CLP on phosphorylation of AMPK was confirmed (Figure 4A demonstrates increases in hepatic phospho-AMPK, which is inhibited by Compound C). Compound C also inhibited CLP-induced autophagic signaling as determined by hepatic and renal LC3 staining (Figures 4B, C). Inhibition of AMPK activation with compound C also led to increased CLP-induced cell death as determined by TUNEL staining at 24 hours (Figure 4D). Moreover, inhibition of AMPK by compound C led to exacerbated IL-6 levels, liver, and kidney injury at a 24 hour time point after CLP compared to vehicle treated, CLP controls (Figures 4E–H).

AICAR limits CLP-induced endothelial activation and injury

One of the hallmarks of sepsis is the up regulation of adhesion molecules and endothelium activation. This is a normal defense mechanism that allows neutrophils to transmigrate from the circulation to the affected region and fight infection. However, this effect is also partly responsible of the capillary leak and microvascular disruption that characterizes the septic clinical syndrome and likely one of the pathways to explain organ dysfunction. The potential protective effect of AMPK signaling in sepsis on endothelial activation and injury was investigated. CLP increased both renal and hepatic up regulation of the adhesion molecule ICAM-1 (Figure 5A, B). AICAR pretreatment limited this increase in ICAM-1 protein levels. Furthermore, microvascular endothelial injury was also determined by transmission electron microscopy ultra structural changes. CLP leads to renal microvascular injury demonstrated by increased endothelial fenestrations. AICAR pretreatment prevented these changes (Figure 6A). Moreover, AICAR limited CLP-induced vascular leakage as determined by Evan’s blue renal tissue extravasation (Figure 6B).

AMPK activation by AICAR minimizes in vitro endothelium activation, and leucocyte adhesion

The influence of AICAR on LPS-induced endothelial activation was determined. Primary mouse kidney glomerular endothelial cells (MKGECs) were utilized for these experiments and were treated with or without LPS for 4 hours, and with or without AICAR. Similar to that seen in vivo, LPS increased protein levels of ICAM-1 and VCAM-1 (Figure 7A, B). These changes were prevented by AICAR treatment. As a functional marker of endothelial activation, neutrophil adhesion to MKGECs was determined. LPS treatment of MKGEC increased neutrophil adhesion and this was limited by AICAR (*$P<0.05$ compared to control; #$P<0.05$ compared to vehicle treated, LPS activated cells; Figure 7C).

Discussion

The release of PAMPs and DAMPs locally and systemically leads to the activation of endothelial cells, circulating and tissue based immune cells, as well as parenchymal cells. Many inflammatory pathways are activated by such signaling, and subsequent expression of proinflammatory cytokines such as interleukin 1β (IL-1β) and TNF-α (36) follows. This enhanced pro-inflammatory state has been associated with several effects that characterize the clinical phenotype of sepsis, including oxidative stress in parenchymal cells of diverse organs, as well as endothelial activation with increased production of reactive oxygen and nitrogen intermediates, such as superoxide and nitric oxide (NO). This excess in NO

production is a major contributor to the vasodilatation and vascular hyporeactivity seen in septic shock. Inflammation has been shown to cause an alteration to endothelial lining of organs, and there is recent evidence of TNF induced glomerular damage in the kidney with TLR 4 activation by LPS (37).

The results of this study suggest that activation of the AMPK signaling pathway serves to limit inflammation, endothelial activation and organ dysfunction in experimental sepsis. Importantly, these results illustrate that this signaling pathway can be activated exogenously, suggesting that the system can be harnessed to limit injury. Although causality is not hereby established, these data suggest that the protective effects of AICAR may be at least in part explained by modulation of the inflammatory response and protection of the endothelium. Furthermore, inhibition of AMPK activation by Compund C exacerbated injury supporting the hypothesis that the AMPK pathway may be part of the adaptive response to sepsis. These findings are in agreement with previous studies suggesting a link between metabolic homeostasis regulation and the immune response (19, 38). As Sag et al. has suggested (38), our data suggest that AMPK activation through AICAR produces a significant decline in cytokine release and an association with maintenance of renal function early after the septic insult. This is important, because decreasing inflammation in the vicinity of the tubular epithelium may help decrease overall cellular injury, allowing the tubular epithelial cells to maintain important processes for the organ as a whole (i.e. tubular transport) and thus sustain renal function. Our experiments showed a discrepancy between the in vivo and in vitro release of IL-10 to inflammatory stimuli. However, our data agrees with that of other groups that have found similar elevations of IL-10 after stimulation of peritoneal macrophages in vitro.(39) The difference with the results in the in vivo experiments may have several explanations. First, the inflammatory injury caused by LPS vs. CLP is not the same, with LPS exerting a more specific TLR-4 mediated response, whereas CLP with multi-organism peritonitis exerts a more diffuse compromise in terms of cellular signaling; second, the cytokine release in the whole animal may be very different to that in cell culture, as there are many other mechanisms in play that can modify the response to inflammation and treatment; third, AICAR and AMPK activation are known to improve neutrophil chemotaxis, and bacterial clearance, suggesting that pre-treated animals with AICAR could have less inflammatory response (and this is inflammatory and anti-inflammatory), possibly due to better control of the infectious source. However, our data does not provide enough information as to describe the mechanism by which AICAR exerts its protective effects on renal function. An important question to answer is what is the contribution of AMPK activation to energy regulatory processes in parenchymal cells, such as the tubular epithelial cell, and what is the effect of this on organ function not only early after the septic insult, but also during convalescence.

We have also demonstrated that pre-emptive AMPK activation decreases global endothelial activation as measured by a decrease in endothelial expression of adhesion molecules (specifically ICAM-1), a decrease in vascular leak (Evan’s blue) and a decrease in neutrophil adhesion, as well as minimizing morphological changes induced by sepsis (TEM). We acknowledge that these effects may be secondary to the effects of AICAR on the inflammatory response described above. In the same way, these results are in agreement with data by other groups that have shown that AMPK activation in the vascular

endothelium may confer protection via decreased cytokine-induced NF-kB activation (40). Other studies have demonstrated suppression of oxidative stress by induction of MnSOD and PGC-1α-dependent mitochondrial biogenesis (41, 42), as well as decreased mitochondrial production of radical oxygen species (ROS) (41) and inhibition of apoptosis (43), as pathways to enhance organ protection via AMPK upregulation. In addition, the data cannot clarify whether this endothelial protection actually preceded the decrease in cytokine outflow, and thus whether protection came from the action of AICAR on the endothelium, or instead on circulating activated leukocytes.

In conclusion, this study demonstrated that AICAR-induced AMPK activation has a measurable effect on cytokine release, on disease-relevant markers of endothelial activation, and ultimately on renal function. This data suggest that AMPK activation protects against sepsis-induced renal dysfunction, and that this protection is associated with AMPK-induced decrease in circulating cytokines and endothelial activation. Whether re-prioritization of energy utilization within the renal tubular cell has a role in such a protective signal remains unknown. Finally, these data suggest that this protection may not be organ-specific, given that we also found a signal suggesting liver protection.

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List of Abbreviations

AKI	Acute Kidney Injury
AMPK	AMP activated protein kinase
CLP	Cecal Ligation and Puncture
DAMPs	Damage Associated Molecular Patterns
ICAM-1	Intracellular adhesion molecule-1
LPS	Lipopolysaccharide
MKGECs	Mouse Kidney Glomerular Endothelial Cells
mTOR	mammalian Target of Rapamycin
NO	Nitric Oxide
PAMPs	Pathogen Associated Molecular Patterns
PBS	Phosphate-buffered Saline
PMNs	Polymorphonuclear lymphocytes
ROS	Reactive Oxygen Species
TEM	Transmission Electron Microscopy

VCAM-1 Vascular adhesion molecule-1

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Highlights

- AICAR induced AMPK activation was associated with a decrease in sepsis-induced acute kidney and hepatic injury markers early after induction of CLP.
- AMPK activation limited sepsis-induced inflammatory response as measured by plasma cytokines.
- Activation of AMPK decreased endothelial activation and vascular leak in vivo, and neutrophil adhesion in vitro.

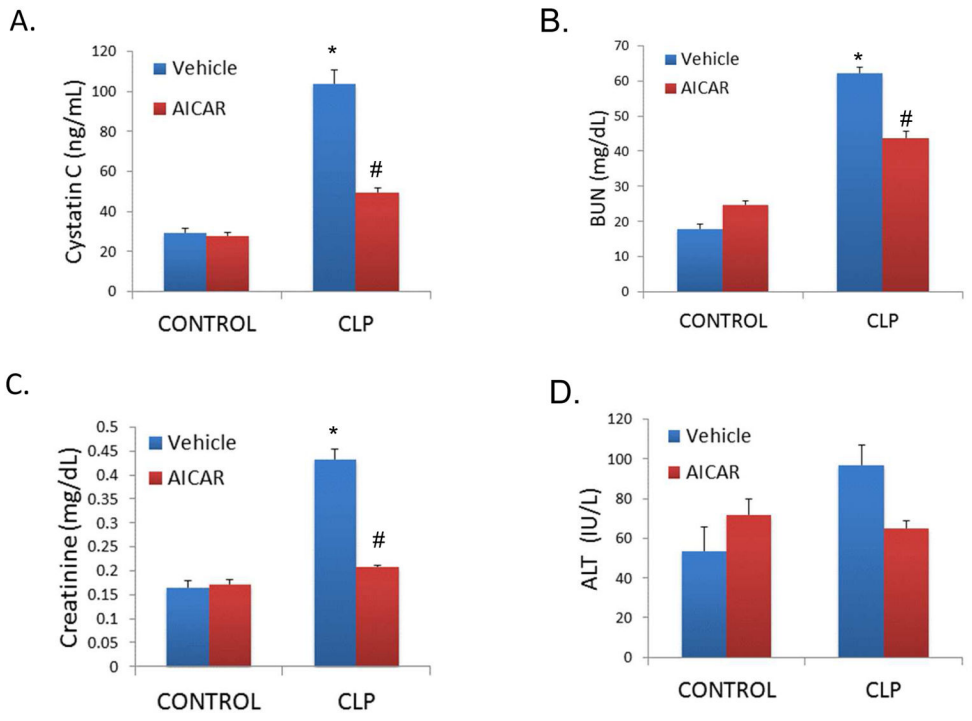

Figure 1.

AICAR limited CLP-induced tissue injury. CLP resulted in acute kidney injury as determined by serum Cystatin C (**A**.), BUN (**B**.), and Creatinine (**C.**). CLP also resulted in liver injury as determined by serum ALT (**D**.). AICAR pretreatment protected against both acute kidney and liver injury. (*$p<0.05$ vs control; #$p<0.05$ vs CLP). Tissue was harvested 8h after CLP.

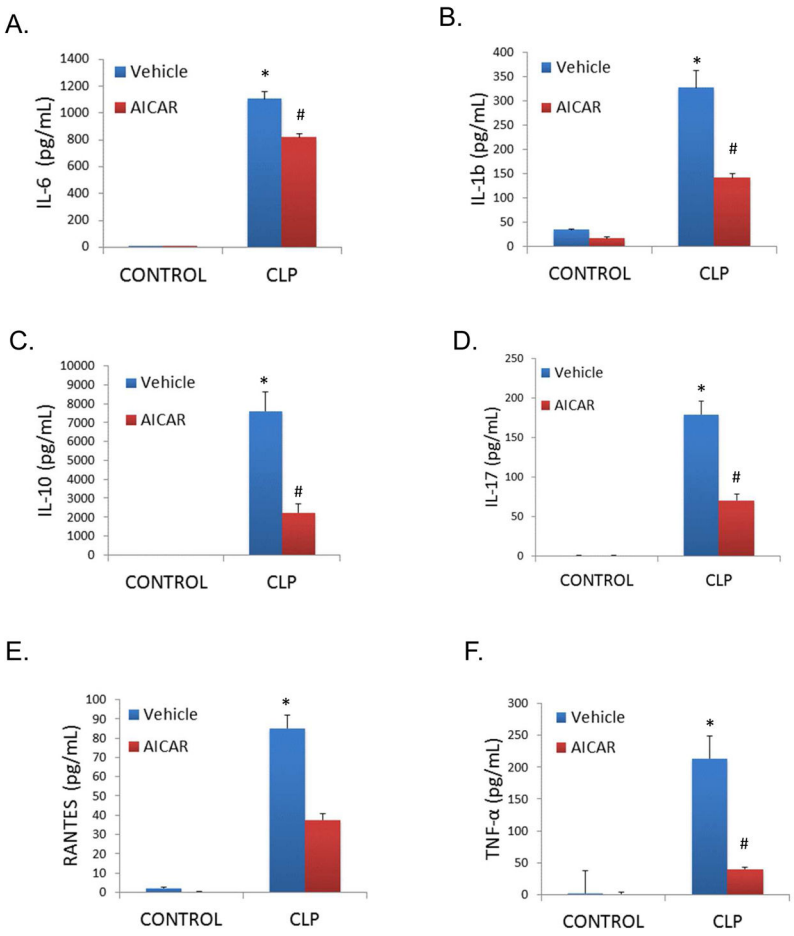

Figure 2.

AICAR pretreatment limited serum cytokine levels induced by CLP. Cytokine and chemokine levels (**A.** IL-6, **B.** IL-1b, **C.** IL-10, **D.** IL-17, **E.** RANTES, **F.** TNF-α) were measured in plasma by Luminex magpix assay 8 hours following CLP. *p<0.05 vs Control; #p<0.05 vs CLP.

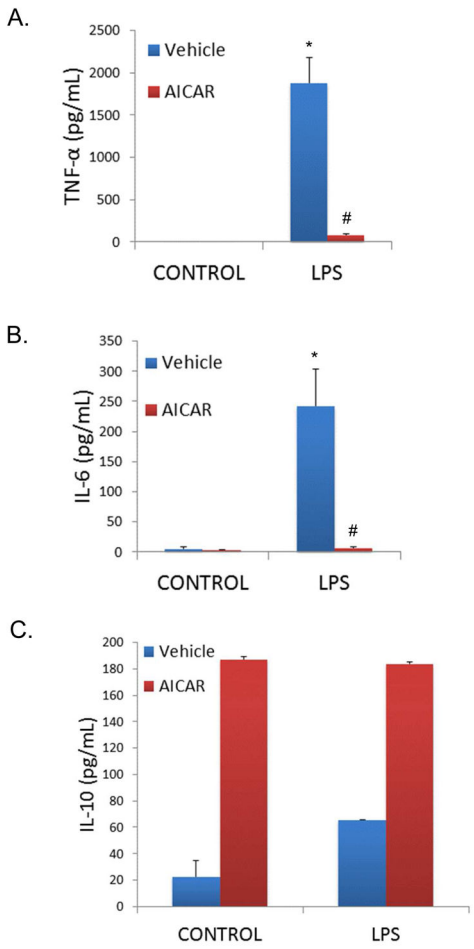

Figure 3.

AICAR pretreatment minimized cytokine level expression in LPS stimulated macrophages (**A.** TNF-α, **B.** IL-6, **C.** IL-10). Peritoneal macrophages were pretreated with or without AICAR for 1 hour, and LPS (10ng/mL) was added to the cell culture. Cytokine levels were measured in cell culture media after 4 hours by ELISA. *p<0.05 vs Control; #p<0.05 vs LPS.

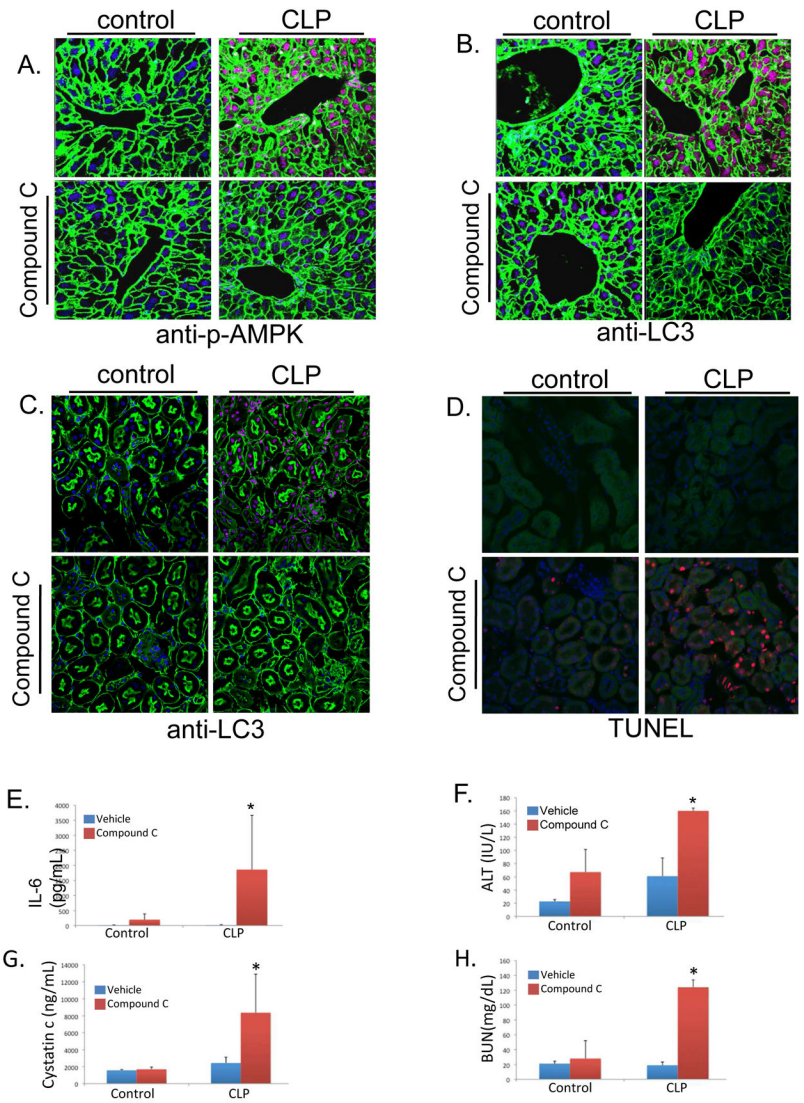

Figure 4.

Inhibition of AMPK signaling exacerbated injury and inflammation**. A.** CLP increased phosphorylation of AMPK as demonstrated by immunohistochemistry in liver tissue. Compound C prevented CLP-induced AMPK phosphorylation [Green=actin; blue=dapi; red=p-AMPK]. **B, C**. Autophagy as determined by LC3 protein levels was increased in liver (**B**.) and Kidney (**C**.) following CLP [Green=actin; blue=dapi; red=LC3]. Compound C limited these increases in LC3 levels. **D.** Minimal apoptotic cell death is seen following CLP, however, there is increased apoptotic cell death with inhibition of AMPK signaling as determined by TUNEL staining in kidney tissue [Green=actin; blue=dapi; red=TUNEL]. **E–H**. Inhibition of AMPK signaling resulted in worse inflammation and tissue injury at 24 hours. Serum IL-6 levels normalized by 24 hours following CLP, however, Compound C treated animals continued to show elevated levels at this time point (*P<0.05 compared to vehicle, CLP mice; **E**.). Furthermore, there was no significant difference in organ injury in vehicle treated CLP mice compared to control mice at 24 hours following CLP. Compound

C pretreatment led to exacerbated injury at this timepoint (*P<0.05 compared to vehicle, CLP mice; **F–H**.).

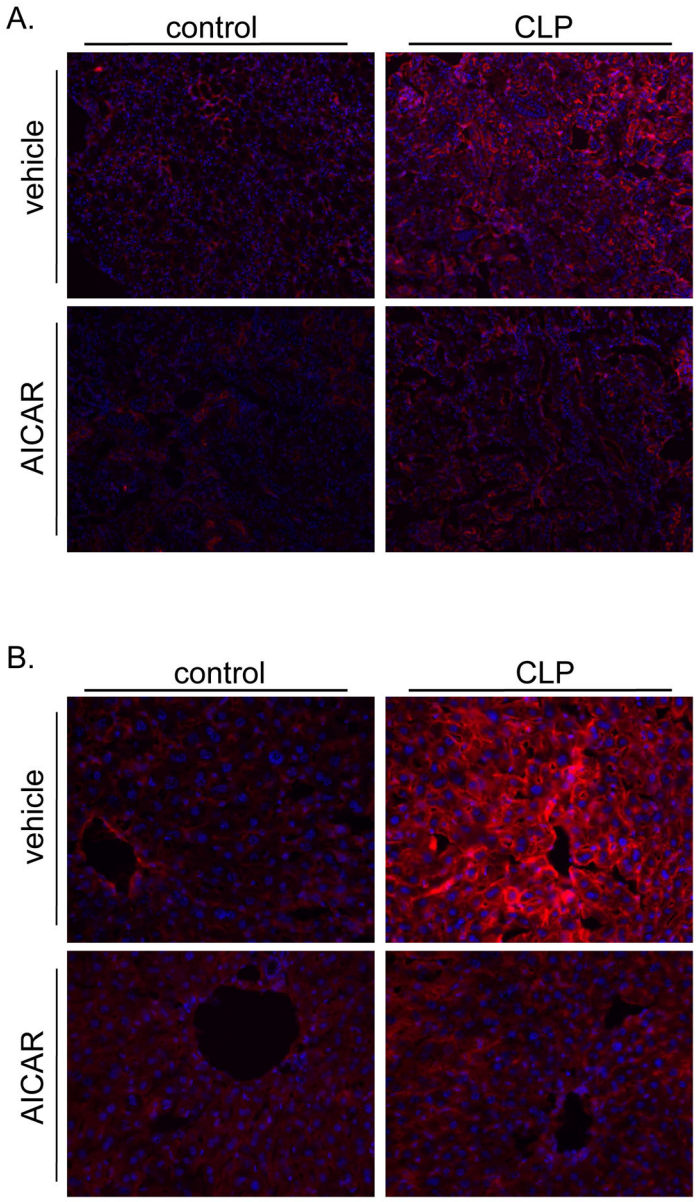

Figure 5.

AICAR pretreatment minimized endothelial cell activation induced by CLP. **A.** CLP increased endothelial activation in kidney as demonstrated by immunohistochemistry staining of ICAM-1 (red) in kidney tissue, at 8 hours following CLP. Pharmacological activation of AMPK by AICAR given 24 hours before CLP minimized this upregulation. **B.** CLP also caused an increase in endothelial activation in liver, measured by ICAM-1 (red), which was minimized by AICAR pretreatment.

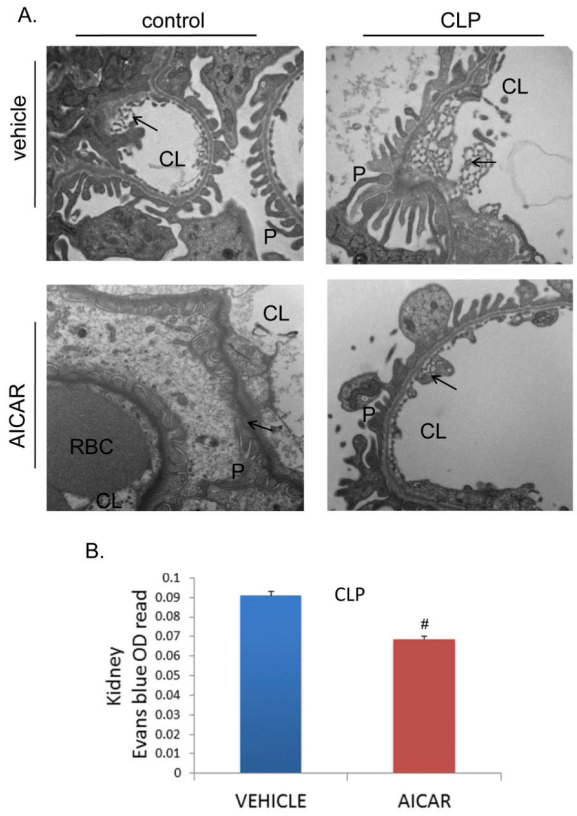

Figure 6.

AICAR minimized morphological changes and microvascular injury induced by CLP. **A.** Transmission Electron Microscopy of kidney tissue 8h following CLP, demonstrates an increase in the diameter and number of fenestrations induced by CLP. AICAR pretreatment minimized these morphological changes. Podocyte structure and number were not affected by CLP. Arrow demonstrates endothelial fenestrations in kidney tissue. CL= capillary lumen, P= Podocytes, RBC= Red blood cell. **B.** Microvascular injury was measured by Evan's blue dye extravasation in kidney injury at 8h following CLP with or without AICAR pretreatment. AICAR pretreatment minimized microvascular injury as measured by Evan's blue dye extravasation in kidney (#$p<0.05$ compared to vehicle treated CLP animals).

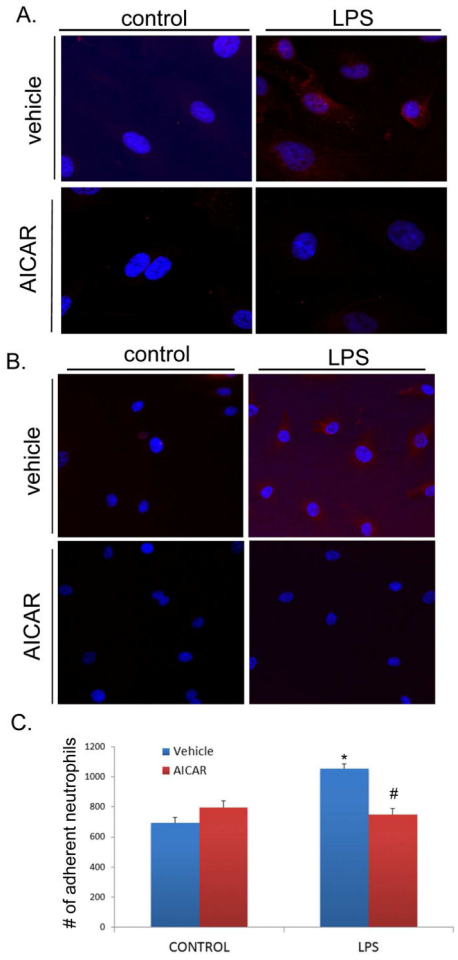

Figure 7.

AICAR minimized endothelial cell activation, and neutrophil adhesion. **A, B.** Mouse Kidney Glomerular Endothelial Cells were pretreated with or without AICAR for 1 h, and LPS (10ng/mL) was added to the cell culture. LPS induced upregulated expression of ICAM-1(**A.**), and VCAM-1 (**B.**). AICAR pretreatment minimized the upregulation of ICAM-1 and VCAM-1. (Nucleus=blue, ICAM-1, VCAM-1=red). **C.** Bone marrow derived neutrophils were added to Mouse Kidney Glomerular Endothelial Cell culture with or without AICAR pretreatment, and stimulated with LPS for 6 hours. Neutrophil adhesion was increased in vehicle treated cells (*P<0.05 vs control). AICAR pretreatment minimized the number of adhered neutrophils when compared to LPS stimulated cells (#p<0.05 vs LPS alone).