Deficiency of cold-inducible ribonucleic acid-binding protein reduces renal injury after ischemia-reperfusion

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Background. *Renal ischemia-reperfusion injury, commonly caused by major operation and shock, leads to acute kidney injury and is associated with high morbidity and mortality. Cold-inducible ribonucleic acid-binding protein, a cold shock protein, has recently been identified as a damage-associated molecular pattern. We hypothesized that cold-inducible ribonucleic acid-binding protein exacerbates severity of injury in renal ischemia-reperfusion.*

Methods. *Renal ischemia was induced in an 8-week-old male C57BL/6 wild-type mice and* $Cirp^{-/-}$ *mice via bilateral clamping of renal pedicles for 30 minutes, followed by reperfusion for 5 or 24 hours and harvest of blood and renal tissue for analysis. Anti–cold-inducible ribonucleic acid-binding protein antibody or non-immunized immunoglobulin G (IgG) was injected intravenously (10 mg/kg body weight) at time of reperfusion.*

Results. *After renal ischemia-reperfusion,* $Cirp^{-/-}$ *mice demonstrated a reduction of blood urea nitrogen and creatinine of 53% and 60%, respectively, compared with wild-type mice. Serum IL-6 levels were reduced significantly: 70% in* $Cirp^{-/-}$ *mice compared with wild-type mice after renal ischemia-reperfusion. Levels of nitrotyrosine, an oxidatively modified protein marker, and cyclooxygenase-2, an inflammatory mediator, also were significantly decreased in the kidneys of the* $Cirp^{-/-}$ *mice compared with wild-type mice after renal ischemia-reperfusion. Renal caspase-3 activity was decreased in* $Cirp^{-/-}$ *mice compared with wild-type mice after renal ischemia-reperfusion, which corresponded to the reduction of apoptotic cells determined by terminal deoxynucleotidyl transferase dUTP nick-end labeling assay. Injection of neutralizing anti-cold-inducible ribonucleic acid-binding protein antibody into wild-type mice led to an 82% reduction in blood urea nitrogen compared with the vehicle after renal ischemia-reperfusion.*

Conclusion. *Deficiency of cold-inducible ribonucleic acid-binding protein results in less renal injury after renal ischemia-reperfusion by attenuating inflammation and oxidative stress. Furthermore, blockade of cold-inducible ribonucleic acid-binding protein shows a protective effect, indicating cold-inducible ribonucleic acid-binding protein as a target in the treatment of renal ischemia-reperfusion. (Surgery 2016;160:473-83.)*

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Presented at the American College of Surgeons 101st Annual Clinical Congress, Scientific Forum, Chicago, IL, October 2015.

Accepted for publication April 13, 2016.

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0039-6060/$ - see front matter

http://dx.doi.org/10.1016/j.surg.2016.04.014

Renal ischemia-reperfusion (RIR) injury, commonly caused by major operation and shock, leads to acute kidney injury (AKI), and is associated with high morbidity and mortality. Critically ill patients with AKI are 4 times more likely to die in the hospital, with an estimated mortality rate of 30–70%, and these patients have a particularly greater risk of mortality when presenting with stage 2 or 3 AKI.[1-5] Many patients who survive AKI progress to chronic kidney disease, and some to end-stage renal disease. These patients are also twice as likely to be discharged to a short- or long-term care

facility instead of to their homes, adding to their health care costs.[1,3,6] Current treatment is limited to prevention and supportive care, although advancements in understanding the pathophysiology of ischemic AKI have allowed for experimental research and the exploration of novel therapies.

In ischemia, deprivation of oxygen and nutrient delivery to cells lead to accumulation of metabolic waste products in the tissue.[1,7] In the kidneys, anoxic cell injury develops in the tubular epithelium and can lead to either apoptosis or necrosis (acute tubular necrosis [ATN]).[1] This leads to the release of damage-associated molecules patterns (DAMPs) recognized by pathogen recognition receptors causing a sterile inflammatory response despite the absence of pathogens.[8,9] Innate immune cells, such as neutrophils and macrophages, are activated, which in turn causes additional cellular injury of the renal epithelium through the release of cytokines and reactive oxygen species.[10,11]

Cold-inducible ribonucleic acid- (RNA) binding protein (CIRP), a 172-amino-acid protein, has recently been identified as a DAMP.[12] It is expressed constitutively at low levels in various tissues and normally functions as an RNA chaperone protein during translation.[12,13] CIRP is upregulated and secreted from the cell under conditions of stress, such as hypothermia, hypoxia, and ultraviolet radiation.[14-16] Administration of recombinant murine CIRP rmCIRP to healthy animals results in an increase of serum organ injury markers and proinflammatory cytokines.[12]

In our study, we used a mouse model of RIR and showed that CIRP levels rise in renal tissue after ischemic and reperfusion injury. This was associated with increased levels of organ injury, inflammation, apoptosis, and oxidative stress. Administration of a neutralizing anti-CIRP antibody led to reduction of organ injury. Together, the data support our hypothesis that CIRP exacerbates severity of injury in RIR.

MATERIALS AND METHODS

Animal model of RIR. Adult male C57BL/6 wild-type (WT) mice and CIRP knockout ($Cirp^{-/-}$) mice (20–25 g) were selected for either a sham or RIR group, totaling 4 groups. The animals were induced with 2.5% inhalational isoflurane, then their abdomens prepped with 10% povidone-iodine wash. A midline incision was performed, and the bowel was displaced to reveal the bilateral renal hila. Microvascular clips were applied to each renal pedicle for 30 minutes; after removal, the abdomen was closed with a running 6-0 nylon suture, and a 500-μL bolus of normal saline was given subcutaneously. Reperfusion was allowed for 5 or 24 hours; animals were then harvested for blood and renal tissue. Sham animals underwent laparotomy without renal ischemia. All experiments were performed in accordance with the guidelines for the use of experimental animals by the National Institutes of Health (Bethesda, MD) and were approved by the Institutional Animal Care and Use Committee of the Feinstein Institute for Medical Research.

Western blot analysis. Renal tissue was homogenized in lysis buffer (10 mM Tris-HCl, pH 7.5; 120 mM NaCl; 1% NP-40; 1% sodium deoxycholate; and 0.1% sodium dodecyl sulfate) containing protease inhibitor (Roche Diagnostics, Indianapolis, IN). Protein concentrations were determined by Bio-Rad protein assay reagent (Bio-Rad laboratories, Hercules, CA). Total lysate was electrophoresed on sodium dodecyl sulfate-polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were blocked with 0.1% casein in 0.2× phosphate-buffered saline and then incubated with anti-CIRP, nitrotyrosine, or β-actin (Sigma-Aldrich, St. Louis, MO) primary antibodies. After washing, the membranes were incubated with a fluorescently labeled secondary antibody (LI-COR, Lincoln, NE). The Odyssey image system (LI-COR) was used to scan the membranes, and the Odyssey densitometric software used to measure band intensities.

Analysis of serum organ injury markers and interleukin 6. Blood samples were centrifuged at 2,000g for 15 minutes to collect serum and then either analyzed for injury parameters immediately or stored at −80°C. Blood urea nitrogen (BUN) and creatinine were measured using commercial assay kits according to the manufacturer's instructions (Pointe Scientific, Lincoln Park, MI). IL-6 levels were determined with an enzyme-linked immunosorbent assay (ELISA) kit, specific to mouse IL-6 (BD Biosciences, San Diego, CA).

Quantitative real-time PCR analysis. Total RNA was extracted from renal tissue using TRIzol (Invitrogen, Carlsbad, CA) and reverse-transcribed into complementary deoxyribonucleic acid (cDNA) using murine leukemia virus reverse transcriptase (Applied Biosystems, Foster City, CA). The PCR reaction was performed in 25 μL of final volume containing 0.08 μmol of forward and reverse primer, cDNA, and 12.5 μL SYBR Green PCR Master Mix (Applied Biosystems). The thermal profile used by the Applied Biosystems 7300 real-time PCR machine was 50°C for 2 minutes, 95°C for 10 minutes, 45 cycles of 95°C for 15 seconds, and 60°C for 1 minute. Mouse