Postconditioning Attenuates Renal Ischemia-Reperfusion Injury by Preventing DAF Down-Regulation

Wei Wang,* Tielong Tang,* Peng Zhang and Hong Bu†

From the Department of Pathology (WW), West China Second University Hospital, and Departments of Pathology (HB) and Urology (PZ), West China Hospital, Sichuan University, Chengdu and Department of Urology, Affiliated Hospital of North Sichuan Medical College (TT), Nanchong, Sichuan, People's Republic of China

Abbreviations and Acronyms

BUN = blood urea nitrogen

DAF = decay-accelerating factor

HRP = horseradish peroxidase

IP = ischemic preconditioning

IRI = ischemia-reperfusion injury

MAC = membrane attack complex

Postcon = postconditioning

R = receptor

RT-PCR = reverse transcriptase-polymerase chain reaction

Scr = serum creatinine

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* Equal study contribution.

† Correspondence: Department of Pathology, West China Hospital, Sichuan University, Chengdu, 610041 People's Republic of China (telephone: 86-28-85164030; FAX: 86-28-85164034; e-mail: hongbu@scu.edu.cn).

Purpose: There is increasing evidence that ischemic postconditioning may noticeably attenuate renal ischemic-reperfusion injury, although the specific mechanisms are not fully clear. We examined the role of the complement system, especially membrane bound complement regulatory proteins, in postconditioning after renal ischemic-reperfusion injury in a right nephrectomy rat model.

Materials and Methods: After right nephrectomy the left renal pedicles were occluded for 60 minutes, followed by 24-hour reperfusion. Postconditioning was induced by 6 cycles of 10-second ischemia and 10-second reperfusion before reperfusion. After 24-hour reperfusion without a control blood samples were obtained via the vena cava. Renal samples were also obtained. DAF, CD46, CD59, C3aR and C5aR mRNA and protein expression was examined by reverse transcriptase-polymerase chain reaction, Western blot and immunohistochemistry. C3/C9 deposition in tissue was detected by immunofluorescence. Renal function, histology and cellular apoptosis were also observed.

Results: In renal tissue postconditioning prevents DAF down-regulation, which is induced by ischemic-reperfusion injury. It results in the decreased renal necrosis caused by ischemic-reperfusion injury mediated complement activation. However, in all experimental groups renal CD46/CD59 expression was not altered. Increased DAF expression due to postconditioning may decrease C5aR expression in renal tissues compared with ischemic-reperfusion injury, which can decrease apoptosis. C3aR expression did not differ among the experimental groups.

Conclusions: These findings provide new evidence that postconditioning protects kidneys from ischemic-reperfusion injury, at least in part, by preventing DAF down-regulation.

Key Words: kidney; antigens, CD55; reperfusion injury; renal circulation; Wistar rats

ISCHEMIA-REPERFUSION injury occurs after blood flow is restored after a significant ischemic period. It has significant clinical importance since it is the predominant cause of tissue/organ damage, and associated with high morbidity and mortality. Renal IRI is a consequence of arterial occlusion, shock and organ transplantation, and a common cause of acute renal failure, delayed graft function and renal graft rejection.[1,2] Ischemia can result in epithelial cell necrosis and apoptosis when blood flow is interrupted, but more severe injury is often due to blood reperfusion. Thus, it is important to improve the ability of organs to tolerate ischemic injury.

IP and ischemic Postcon are 2 important surgical methods to improve

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the ability of organs to tolerate ischemic injury.[3,4] IP has powerful protective effects on IRI but its clinical application is largely limited since IP must be started before the ischemic event. The onset of reperfusion is more predictable. Postcon is defined as rapid, intermittent interruptions of blood flow in the early reperfusion phase that mechanically alters reperfusion hydrodynamics. Unlike IP, Postcon theoretically allows unrestricted application in clinical settings and, thus, it has attracted much attention in regard to renal IRI.[5,6] Currently the specific mechanisms promoting the protective function of Postcon are not fully clear.

Increasing evidence shows that complement activation has a predominant role in IRI in various organs, including the heart, brain and kidney.[7–10] In the kidney ischemia and subsequent reperfusion lead to complement activation via the alternative and mannose-binding lectin pathways.[11,12] After activation the MAC dependent and anaphylatoxin dependent C3a and C5a pathways were proposed as mechanisms by which the complement cascade induces tubular apoptosis and necrosis in renal IRI animal models.[13,14] Recent studies further showed that the membrane bound complement regulatory proteins DAF and CD59 can effectively decrease renal IRI severity.[15,16] These findings led us to consider the roles of the membrane complement regulators CD46, DAF and CD59 in Postcon.

To our knowledge this study is the first to suggest that Postcon protects the kidney from IRI induced complement mediated injury by inhibiting the downregulation of DAF rather than CD46 or CD59. We provide new evidence that Postcon protects the kidney from IRI mediated injury, at least in part, through complement mediated mechanisms.

MATERIALS AND METHODS

Surgical Procedure

Adult male Wistar rats weighing 200 to 250 gm were anesthetized with intraperitoneally administered 5% hydrated chloroacetaldehyde (6 mg/kg). Body temperature was maintained at 37C using a homeothermic pallet unit and monitored by a rectal probe. After a stabilization period midline laparotomy and right nephrectomy were done, and the left renal vessels were widely dissected. All animals were randomized into 3 groups, including group 1—10 with IRI in which the left renal pedicle was occluded for 60 minutes, followed by 24-hour reperfusion, group 2—10 with ischemic Postcon, in which after 60 minutes of ischemia and immediately before the onset of reperfusion reflow was initiated for 10 seconds of full renal vascular flow, followed by 10-second re-occlusion with this repeated for 5 cycles for a total of 6 cycles (total 2 minutes) and group 3—10 sham operated, normal controls, in which the left renal pedicle was subjected to isolation only. After these treatment protocols the laparotomy was closed with 2-zero nylon. At 24 hours postoperatively a plasma sample was obtained for BUN and Scr analysis, and the left kidney was isolated for further examination.

DAF, CD59, CD46, C3aR and C5aR mRNA Analysis

Total cell RNA was isolated from the stripped rat thoracic aorta using an RNeasy™ Mini Kit. RT-PCR was done using the SuperScript™ One-Step RT-PCR System. Glyceraldehyde-3-phosphate dehydrogenase cDNA served as an internal control.

The synthesized primers were rat DAF, 5′-GCC TTG AGG AAT TAg TAT GG-3′ (sense) and 5′-TGC ACT TGG GTG GTG CAC TA-3′ (antisense); rat CD46, 5′-CTA TGT TAC AGG ACC CTT CG-3′ (sense) and 5′- CTG GGT TAg GAT CAC AAC TG-3′ (antisense); rat CD59, 5′-GAG GGG ATT CAT CTT ACT CC-3′ (sense) and 5′-ACG CTG TCT TCC CCA ATA GG-3′ (antisense); rat C3aR, 5′- ATC CCA CCT CAG TGC TCT TG-3′ (sense) and 5′- TGT GTT CAC GGT CCT CTT CA-3′ (antisense); rat C5aR, 5′- TGC TAC ACC TTC CTC CTG CT-3′ (sense) and 5′-TAT GAT GCT GGG GAG AGA CC-3′ (antisense); and rat glyceraldehyde-3-phosphate dehydrogenase, 5′-GGT CGG TGT GAA CGG ATT TG-3′ (sense) and 5′-GCC TTC TCC ATG GTG GTG AA-3′ (antisense).

Immunohistochemistry

Sections were dewaxed in xylene, dehydrated through serial dilutions of ethanol to water and pretreated with microwave antigen retrieval in 10 mM citrate buffer (pH 6.0) under pressure. Endogenous peroxidase activity was blocked with methanol containing 0.3% hydrogen peroxide for 10 minutes and nonspecific protein binding with 5% bovine serum albumin in tris buffered saline for 10 minutes at 37C. After extensive washing with phosphate buffered saline sections were incubated with goat anti-rat DAF polyclonal antibody (1:200), goat anti-rat CD46 polyclonal antibody (1:200), goat anti-rat CD59 monoclonal antibody (1:100), goat anti-rat C3aR polyclonal antibody (1:200) and goat anti-rat C5aR polyclonal antibody (1:200) for 1 hour at 37C and overnight at 4C. Sections were washed and incubated with HRP conjugated goat anti-rat IgG (1:500) and HRP-rabbit anti-rat IgG (1:400) for 1 hour at 37C. Peroxidase activity was visualized using diaminobenzidine substrate consisting of 0.3% diaminobenzidine in 0.1 M phosphate buffered saline (pH 7.0) and 0.003% H_2O_2 for 5 minutes. Sections were washed and counterstained with hematoxylin. Results were quantified with Image Pro® Plus.

Western Blot

Western blot analysis was done according to a standard protocol[17] using anti-rat DAF, CD46, CD59, C3aR and C5aR polyclonal antibodies, and HRP conjugated rabbit anti-goat IgG. The signal of each band was quantified by Image Pro Plus 4.5. All results were normalized to β-actin.

C3 and C9 Deposition Immunofluorescence

Cryostat sections (6 μm) of renal tissue were fixed with methanol/acetone and stained for complement factors C3 and C9 using fluorescein isothiocyanate conjugated anti-rat C3 antibody (1:500), fluorescein isothiocyanate conjugated secondary anti-rabbit IgG antibody (1:200) (DakoCytomation, Carpinteria, California) and rabbit anti-rat

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