Remote Ischemic Preconditioning and Protection of the Kidney—A Novel Therapeutic Option

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Objective: Acute kidney injury is a common complication in critically ill patients and is associated with increased morbidity and mortality. Sepsis, major surgery, and nephrotoxic drugs are the most common causes of acute kidney injury. There is currently no effective strategy available to prevent or treat acute kidney injury. Therefore, novel treatment regimens are required to decrease acute kidney injury prevalence and to improve clinical outcomes. Remote ischemic preconditioning, triggered by brief episodes of ischemia and reperfusion applied in distant tissues or organs before the injury of the target organ, attempts to invoke adaptive responses that protect against acute kidney injury. We sought to evaluate the clinical evidence for remote ischemic preconditioning as a potential strategy to protect the kidney and to review the underlying mechanisms in light of recent studies.

Data Sources: We searched PubMed for studies reporting the effect of remote ischemic preconditioning on kidney function in surgical patients (search terms: "remote ischemic preconditioning," "kidney function," and "surgery"). We also reviewed bibliographies of relevant articles to identify additional citations.

Study Selection: Published studies, consisting of randomized controlled trials, are reviewed.

Data Extraction: The authors used consensus to summarize the evidence behind the use of remote ischemic preconditioning.

Data Synthesis: In addition, the authors suggest patient populations and clinical scenarios in which remote ischemic preconditioning might be best applied.

Conclusions: Several experimental and clinical studies have shown tissue-protective effects of remote ischemic preconditioning in various target organs, including the kidneys. Remote ischemic preconditioning may offer a novel, noninvasive, and inexpensive treatment strategy for decreasing acute kidney injury prevalence in high-risk patients. Although many new studies have further advanced our knowledge in this area, the appropriate intensity of remote ischemic preconditioning, its mechanisms of action, and the role of biomarkers for patient selection and monitoring are still unknown. (*Crit Care Med* 2016; 44:607–616)

Key Words: acute kidney injury; biomarker; cell cycle arrest; remote ischemic preconditioning

Acute kidney injury (AKI) is a common complication in hospitalized patients and causes considerable harm (1, 2). AKI is associated with short-term morbidity, long-term risk of chronic kidney disease (CKD), and cardiovascular events, and it decreases survival (3–8). Leading causes of AKI are sepsis, major surgery, and nephrotoxic drugs (9). Hospitalized patients, particularly those with comorbidities and those undergoing complex procedures, are at high risk of developing AKI (7, 10). The prevalence of AKI after surgery has been reported to be as low as 1%, whereas the prevalence among critically ill patients can be as high as 70%, with an in-hospital mortality of 50% when AKI is part of the multiple organ dysfunction syndrome (11, 12). AKI is a syndrome comprising multiple clinical conditions, and outcomes are influenced by underlying disease; yet, AKI is an independent risk factor for death (13).

The introduction of a uniform classification system for AKI has enabled the collection of comparable data for the prevalence and epidemiology of AKI worldwide. By applying the Risk, Injury, Failure, Loss and End-stage kidney disease (RIFLE) criteria (2), it has been shown that AKI is associated with a significant increase in hospital mortality. In a large study including over 20,000 patients, Uchino et al (14) investigated the prevalence of AKI by using RIFLE criteria and demonstrated that the prevalence of "Risk", "Injury," and "Failure" was 10%, 5%, and 3.5%, respectively. Even patients with a mild AKI (Risk) had a three-fold increased mortality rate compared with control subjects without AKI. "Risk,"

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"Injury," and "Failure" were associated with odds ratios (ORs) for hospital mortality of 2.5, 5.4, and 10.1, respectively. A large multicentre trial included 120,123 critically ill patients at 57 ICUs (15). In this patient population, the overall prevalence of AKI was as high as 36%. As also previously noted, hospital mortality in patients with AKI significantly increased (OR, 3.29; 95% CI, 3.19–3.41; $p < 0.0001$).

The prevalence of AKI in critically ill patients was estimated to be as high as 60% (16). Interestingly, the RIFLE criteria were fulfilled in only 22% of patients at the time point of ICU admission. When the authors applied a multivariate analysis, AKI (hazard ratio [HR], 1.7; 95% CI, 1.28–2.13; $p < 0.001$) and maximum RIFLE classes "Injury" (HR, 1.4; 95% CI, 1.02–1.88; $p = 0.037$) and "Failure" (HR, 2.7; 95% CI, 2.03–3.55; $p < 0.001$) were shown to be strongly associated with hospital mortality. Despite increasing attention in recent years (1, 8, 9, 17), little improvement in outcomes for AKI has occurred.

Despite numerous clinical trials using various interventions (18), a reliable means to prevent AKI remains elusive. Importantly, treatments have largely focused on manipulating renal perfusion (e.g., dopamine and fenoldopam), diuretics (including natriuretic peptides), or sodium bicarbonate (17). None of these are currently recommended by international guidelines (17). Ischemic preconditioning, triggered by brief episodes of ischemia and reperfusion before a subsequent prolonged injury occurs, has been demonstrated to reduce the extent of organ damage. The concept of ischemic preconditioning was introduced by Murry et al (19), who first described the cardioprotective effect of several brief ischemic episodes before subsequent prolonged ischemic insult in animals with myocardial infarction. However, this protection not only operates locally but may also protect distant tissues, a phenomenon known as "remote ischemic preconditioning" (RIPC). RIPC was first shown in cardiac tissue in which brief episodes of myocardial ischemia and reperfusion decreased infarct size (20). In the following years, it has been demonstrated that brief episodes of ischemia and reperfusion induced in nontarget tissue confer protection at remotes sites, such as the lung, brain, intestine, kidney, or skeletal muscle (21–23).

The clinical application of RIPC is particularly interesting in renal medicine. By virtue of the nature of glomerular filtration and tubular reabsorption, the kidneys are uniquely situated to respond to remote insults (either tissue damage or pathogen) (24). Renal tubular epithelial cells possess a variety of pattern recognition receptors (PRRs), and these cells respond to a wide array of endogenous and exogenous stimuli. Importantly, the response of renal tubular epithelial cells to various stressors includes self-protective mechanisms, including down-regulation of ion transporter function, the primary energy user of these cells, and cell cycle arrest (24). Experimental and clinical evidence indicates that RIPC might be an effective tool to protect kidneys from injury. In this regard, we and others recently demonstrated that RIPC may offer a novel, noninvasive, and inexpensive strategy to reduce the occurrence of AKI in different clinical scenarios (23, 25). In this review, we summarize the current experimental and clinical evidence for RIPC as a potential renoprotective strategy and discuss the underlying mechanisms and recent clinical findings.

Evidence From Animal Studies

Although most studies investigating RIPC have focused on myocardial protection, a small number of studies examined whether RIPC may be able to protect the kidney. Previous studies suggest a beneficial effect of RIPC on renal function (26, 27). It has been shown that brief episodes of liver ischemia have a beneficial effect on renal ischemia as a remote organ even when liver ischemia is performed after the renal ischemia (28). Song et al (29) demonstrated that the application of brief small intestinal ischemia attenuates renal ischemia and subsequent reperfusion injury, as shown by decreases in the levels of plasma creatinine, blood urine nitrogen, and malondialdehyde, decreased renal morphologic change, and improved preservation of superoxide dismutase (SOD) and catalase activities. These results suggest that ischemia of the small intestine protects against renal ischemia-reperfusion injury by inhibition of lipid peroxidation and preservation of antioxidant enzyme activities. Wever et al (30) investigated whether brief hind limb occlusion can protect against renal ischemia-reperfusion injury and whether this protection is adenosine dependent. Rats underwent either unilateral RIPC, bilateral RIPC, or control (no RIPC) followed by bilateral ischemia-reperfusion. After 24 hours of reperfusion, renal function was improved by 30–60% in both bilateral RIPC groups and in the unilateral group, suggesting that brief hind limb ischemia induces protection against renal ischemia-reperfusion injury. However, bilateral RIPC was more effective than unilateral RIPC, and this protection occurs via an adenosine-independent mechanism. A recent metaanalysis of animal studies investigated three outcome measures: blood urea nitrogen (BUN), serum creatinine, and histologic renal damage after renal ischemia-reperfusion injury (31). Ischemic preconditioning reduced serum creatinine (standardized mean difference [SMD], 1.54 [95% CI, 1.16–1.93]), BUN (SMD, 1.42 [95% CI, 0.97–1.87]), and histologic renal damage (SMD, 1.12 [95% CI, 0.89–1.35]) when compared with controls. Factors influencing the efficacy were the window of protection (< 24 hr vs ≥ 24 hr) and animal species (rat vs mouse). No difference in efficacy between local and remote preconditioning was observed.

Clinical Evidence

In addition to experimental evidence, extensive progress has been made in translating RIPC from experimental models into clinical practice. Several clinical trials have been conducted thus far, and most suggest that RIPC may reduce kidney damage in humans (**Table 1**). However, some studies have found that RIPC has no effect on AKI. The reasons for the controversial results among different studies are manifold, including different patient populations, comorbidities, type of surgery, and RIPC protocols. **Table 2** shows the RIPC protocols used in various clinical studies and the patient populations that were included. We classified patients as "high risk" if they had several comorbidities associated with poor renal outcomes

TABLE 1. Clinical Trials on the Effect of Remote Ischemic Preconditioning on Acute Kidney Injury

References	Clinical Setting	Outcome Variables
Surgery patients		
Ali et al (41)	Abdominal aortic aneurysm repair ($n = 82$)	RIPC reduced the prevalence of AKI (30% vs 7%; $p = 0.01$)
Walsh et al (42)	Abdominal aortic aneurysm repair ($n = 51$)	No differences in renal outcome
Walsh et al (43)	Endovascular aneurysm repair ($n = 40$)	RIPC did not reduce the occurrence of AKI, but the release of biomarkers
Raman et al (40)	Cardiac surgery (CABG) ($n = 162$)	No differences in renal outcome
Venugopal et al (34)	Cardiac surgery (CABG) ($n = 78$)	RIPC decreased the prevalence of AKI
Huang et al (44)	Laparoscopic partial nephrectomy ($n = 82$)	RIPC reduced the rate of glomerular filtration rate reduction at 1 month
Zimmerman et al (32)	Cardiac surgery ($n = 120$)	RIPC reduced the occurrence of AKI compared with control (20% vs 47%; $p = 0.004$)
Thielmann et al (33)	Cardiac surgery ($n = 120$))	RIPC reduced peak creatinine serum concentration
Gallagher et al (38)	Cardiac surgery in patients with chronic kidney disease ($n = 86$)	RIPC did not reduce the rate of AKI
Zarbock et al (25)	Cardiac surgery in high-risk patients ($n = 240$)	RIPC significantly reduced the occurrence of AKI, need for renal replacement therapy, and length of hospital stay
Choi et al (36)	Cardiac surgery ($n = 76$)	No differences in AKI prevalence
Candilio et al (39)	Cardiac surgery ($n = 180$)	Secondary analysis: no differences in renal outcome
Young et al (37)	Cardiac surgery ($n = 96$)	Secondary analysis: no differences in renal outcome
Chen et al (46)	Renal transplantation ($n = 60$)	RIPC did not improve early renal function
Wu et al (47)	Renal transplantation ($n = 48$)	RIPC enhances the early recovery of renal function
Nonsurgery patients		
Er et al (23)	Coronary angiography ($n = 200$)	RIPC decreased contrast-induced AKI (40% in the control group vs 12% in the RIPC group)
Deftereos et al (35)	Patients with myocardial infarction undergoing percutaneous coronary intervention ($n = 225$)	RIPC significantly reduced the rate of AKI (12.4% vs 29.5%; $p = 0.02$)

RIPC = remote ischemic preconditioning, AKI = acute kidney injury, CABG = coronary artery bypass graft.

(e.g., preexisting CKD, low ejection fraction, diabetes, and chronic obstructive pulmonary disease). RIPC appears to be a safe procedure, as no adverse events related to RIPC application were reported in the clinical trials performed to date. However, a therapeutic index for RIPC has not been established, and as such, it is unclear what the minimally effective dose would be or at what dose toxicity might begin.

The effects of RIPC on the kidney have been extensively investigated in the setting of adult vascular and cardiac surgery. In a large multicenter, randomized double-blind clinical trial, we recently found that RIPC in high-risk patients before cardiac surgery was effective for reducing the occurrence of AKI (37.5% compared with 52.5% with sham; absolute risk reduction [ARR] 15%; 95% CI, 2.56–27.44; $p = 0.02$). Furthermore, fewer patients receiving RIPC received renal replacement therapy (RRT) (5.8% vs 15.8%; ARR, 10%; 95% CI, 2.25–17.75; $p = 0.01$) (25). Importantly, however, we found that the effectiveness of this intervention was strongly associated with the release of cell cycle arrest biomarkers into the urine. A singler center, randomized trial ($n = 120$) also demonstrated that RIPC reduces the rate of AKI after cardiac surgery. The primary outcome, occurrence of AKI, occurred in 12 patients treated with RIPC versus 28 control patients, reflecting an ARR of 27% and a significantly reduced relative risk (RR) with preconditioning of 43% (32). In line with these results, another small randomized study demonstrated that RIPC reduced the postoperative peak creatinine serum concentration compared with the control intervention (33). Furthermore, a retrospective study of nondiabetic patients undergoing elective coronary artery bypass graft (CABG) surgery showed that RIPC significantly reduced the prevalence of AKI (34). In addition, two randomized controlled trials showed that RIPC can also reduce contrast-induced AKI in high-risk patient populations (23, 35).

TABLE 2. Clinical Trials on the Effect of Remote Ischemic Preconditioning on Acute Kidney Injury: Patient Selection, Mature of Maneuver, and Definition of Acute Kidney Injury

References	Nature of the Maneuver	Definition of AKI	Patient Selection
Surgery patients			
Ali et al (41)	Location: iliac arteries, number of cycles: 2, duration of cycle: 10 min	Impaired renal function was defined as peak serum creatinine level of > 177 μmol/L (2.0 mg/dL)	Low-risk patients and procedures
Walsh et al (42)	Location: lower limb, number of cycles: 2, duration of cycle: 10 min	Serum creatinine concentrations	Low-risk patients and procedures
Walsh et al (43)	Location: iliac artery, number of cycles: 2, duration of cycle: 10 min	Serum creatinine concentrations and biomarkers	Low-risk patients and procedures
Rahman et al (40)	Location: upper limp, number of cycles: 3, duration of cycle: 5 min	Creatinine levels and Δcreatinine between days 0 to 4	Low-risk patients and procedures
Venugopal et al (34)	Location: upper limb, number of cycles: 3, duration of cycle: 5 min	AKIN within the first 72 hr after cardiac surgery	Low-risk patients and procedures
Huang et al (44)	Location: lower limb, number of cycles: 3, duration of cycle: 5 min	Serum creatinine concentrations and biomarkers	Low-risk patients and procedures
Zimmerman et al (32)	Location: lower limb, number of cycles: 3, duration of cycle: 5 min	AKIN	Low-risk patients and procedures
Thielmann et al (33)	Location: upper limb, number of cycles: 3, duration of cycle: 5 min	Serum creatinine concentration over 72 h after surgery	Low-risk patients and procedures
Gallagher et al (38)	Location: upper limb, number of cycles: 3, duration of cycle: 5 min	AKIN within 48 hr after cardiac surgery	High-risk patients: chronic kidney disease (< 60 mL/min per 1.73 m^2)
Zarbock et al (25)	Location: upper limb, number of cycles: 3, duration of cycle: 5 min	Kidney Disease: Improving Global Outcomes criteria within 72 hr after cardiac surgery	High-risk patients: several comorbidities and/or complex surgical procedure
Choi et al (36)	Location: lower limb, number of cycles: 3, duration of cycle: 10 min.	Acute kidney injury network within 48 hr after cardiac surgery	Low-risk patients and procedures
Candilio et al (39)	Location: upper and lower limbs, number of cycles: 2, duration of cycle: 5 min	RIFLE	Low-risk patients and procedures
Young et al (37)	Location: upper limb, number of cycles: 3, duration of cycle: 5 min	RIFLE	High-risk patients/ procedures: ejection fraction below 50%, reoperation, complex cardiac surgery
Chen et al (46)	Location: lower limb, number of cycles: 3, duration of cycle: 5 min	Serum creatinine concentrations	Low-risk patients and procedures
Wu et al (47)	Location: iliac artery, number of cycles: 3, duration of cycle: 5 min	Serum creatinine levels and biomarkers	Low-risk procedures
Nonsurgery patients			
Er et al (23)	Location: upper limb, number of cycles: 4, duration of cycle: 5 min	AKI: defined as an increment of serum creatinine ≥ 0.5 mg/dL or a relative increase of ≥ 25% over the baseline value within a period of 48 hr after contrast medium administration	Low-risk patients
Deftereos et al (35)	Location: coronary artery, number of cycles: 4, duration of cycle: 30 s	AKI: defined as an absolute increase in serum creatinine of ≥ 0.5 mg/dL or a relative increase of ≥ 25% compared with baseline within 96 h after percutaneous coronary intervention	Low-risk patients and procedures

AKI = acute kidney injury, RIFLE = Risk, Injury, Failure, Loss and End-stage kidney disease.

However, some studies have failed to demonstrate a beneficial effect of RIPC on kidney function. In a prospective randomized double-blind controlled trial of RIPC, Choi et al (36) used three 10-minute cycles of lower-limb ischemia and reperfusion in 76 patients undergoing complex valvular cardiac surgery. Primary outcomes were AKI (AKI network definition) and changes in two urinary biomarkers of kidney injury. There were no differences in the prevalence of AKI or in the concentrations of renal injury biomarkers between the two groups. Young et al (37) published another negative result arising from a small-scale prospective randomized controlled trial, which aimed to analyze the efficacy of RIPC in high-risk cardiac surgery. A total of 96 patients were randomized to receive either RIPC or to serve as control. Plasma concentrations of high-sensitivity troponin T at 6 and 12 hours after surgery and the postoperative AKI were used as primary study endpoints. Rates of AKI did not differ between the two groups. The authors concluded that RIPC provided neither myocardial nor renal protection. Gallagher et al (38) recently published a randomized controlled trial of RIPC to prevent AKI in 86 patients with CKD (estimated glomerular filtration rate (eGFR) under 60 mL/min per 1.73 m^2) undergoing CABG surgery. The primary endpoint was the development of AKI. Twelve patients in each group developed an AKI in within 48 hours of CABG. The authors concluded that RIPC had no effect on the frequency of AKI after CABG in patients with CKD (38). Although AKI was only a secondary outcome variable, two studies demonstrated that RIPC did not decrease the occurrence of AKI (39, 40). One possible explanation for the negative results is that these studies involved "low-risk" patients and/or low-risk procedures.

In a clinical trial, renoprotection and cardioprotection by RIPC were investigated in 82 adults undergoing abdominal aortic aneurysm repair (41). RIPC decreased the occurrence of AKI by 77% (30% vs 7%; $p = 0.01$). A similar study in the same clinical scenario but with fewer patients ($n = 51$) did not show a difference in renal outcomes (42). The same authors investigated in another clinical trial whether RIPC can reduce kidney injury after endovascular aneurysm repair (43). Although there was no difference in the occurrence of AKI, RIPC decreased kidney injury, as demonstrated by a reduction in urinary biomarker levels.

The effect of RIPC on renal function was also studied in patients undergoing laparoscopic partial nephrectomy (44). The primary outcome was the absolute change in GFR of the affected kidney by renal scintigraphy from baseline to 6 months. RIPC was associated with a lower rates of GFR reduction at 1 month after surgery (8.8% vs 15%; $p = 0.03$). However, there were no differences in the serum creatinine level or eGFR at 1 and 6 months between the two groups.

In transplantation medicine, strategies to reduce ischemia-reperfusion injury are particularly important, especially in the setting of kidney transplantation because of the high prevalence of dialysis-requiring renal dysfunction after surgery. Experimental evidence shows that RIPC reduces renal allograft injury and improves allograft function (45). However, only two studies have investigated the role of RIPC in human renal transplantation with controversial results. RIPC did not improve early renal function in patients receiving living-donor renal transplantation (46). The lack of effect of RIPC in this clinical scenario was unexpected and might be explained by the study design. RIPC was applied unilaterally in donors or recipients. In contrast, a recently published clinical trial investigating the effects of RIPC on renal function after kidney transplantation ($n = 48$) showed that RIPC enhances early recovery of renal function in recipients after kidney transplantation (47). More studies in this field are required to definitely answer the question whether RIPC can improve allograft function.

In summary, particularly based on the latest published reports, it seems that RIPC is especially beneficial in patients at intermediate or high risk, whereas no significant renoprotective effect is verifiable in patients at low risk. However, further large multicenter trials are still needed to establish the clinical benefits of RIPC and to understand the optimal dose and patient selection. The possible role of novel AKI biomarkers for these applications is now the subject of intense investigation.

RIPC: Mechanisms of Action

The underlying mechanisms of RIPC are complex and have not been fully elucidated. It has been hypothesized that RIPC involves humoral mediators, and experimental evidence suggests that protection is dialyzable, receptor mediated, and transferable from one individual to another (48, 49). The presence of a circulating cardioprotective factor after RIPC was first demonstrated in an animal model (50). RIPC in an acceptor pig provided potent cardioprotection to the subsequently transplanted and denervated donor heart. In another experimental setup, it was shown that plasma from remotely preconditioned animals is cardioprotective when perfused into an isolated naive heart (51). The plasma dialysate using a 15-kDa membrane was similarly cardioprotective, along with a protective kinase signature. Importantly, when dialysate from a preconditioned animal was given to isolated fresh cardiomyocytes, the resistance of cardiomyocytes to subsequent ischemia-reperfusion injury mimicked that of a local preconditioning stimulus.

RIPC is thought to activate several pathways, including systemic anti-inflammatory, neuronal, and humoral signaling pathways. The importance of the different signaling pathways may differ in response to the applied stimulus, and the pathways probably interact with each other. There is a growing body of evidence showing that RIPC reduces the release of injury biomarkers and maintains organ function (23, 25, 32, 52). We have hypothesized that renoprotection is mediated mainly through the release of damage-associated molecular patterns (DAMPs) that interact with PRRs on renal tubular epithelial cells (**Fig. 1**). However, a number of other mechanisms have been proposed, and it is possible that different organs are affected in different ways.

Neuronal and Humoral Effects

Blocking the autonomic ganglion reversed the cardioprotective effects of RIPC when RIPC is performed via intermittent mesenteric artery occlusion (53), indicating the potential

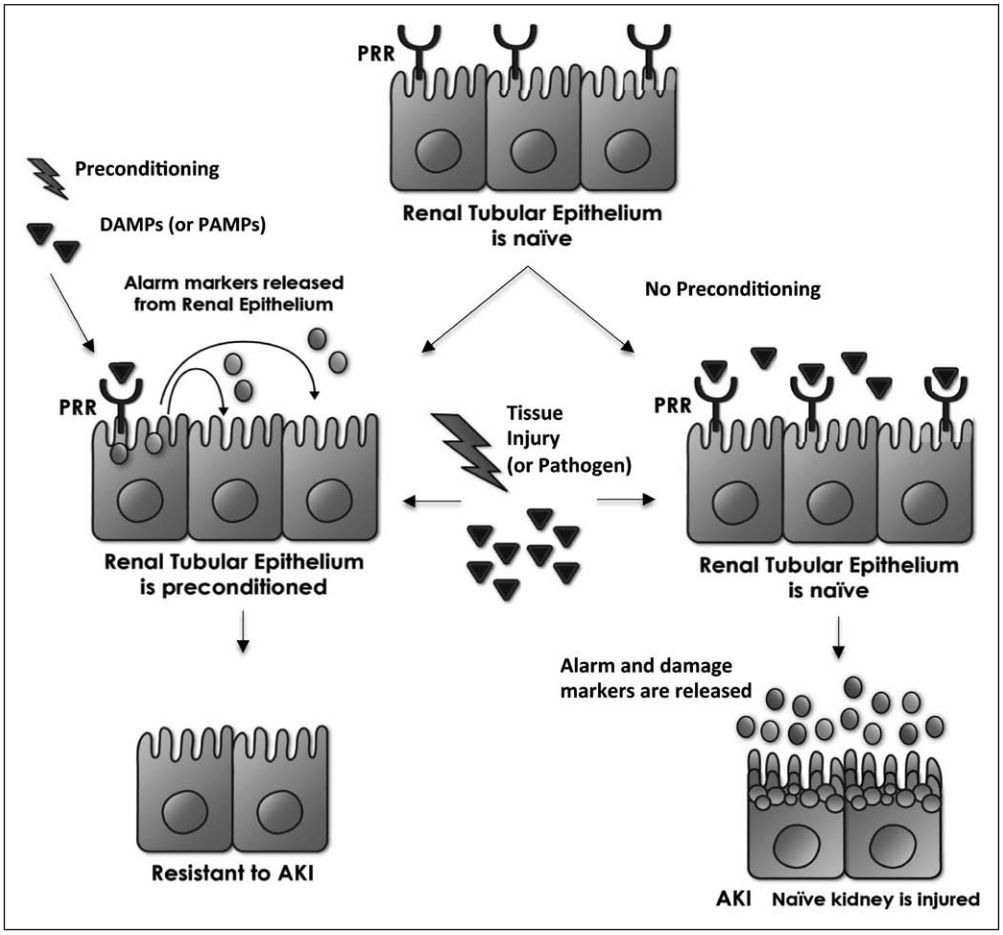

Figure 1. Remote ischemic preconditioning (RIPC) reduces the occurrence of acute kidney injury (AKI) by inducing the release of damage-associated molecular patterns (DAMPs) that bind to pattern recognition receptors (PRRs) on the surface of renal epithelial cells. Next, alarm markers, tissue inhibitor of metalloproteinases-2 and insulin-like growth factor–binding protein-7 (IGFBP7), are released from the epithelial cells signaling in autocrine and paracrine fashion to down-regulate cell function and energy utilization. Although subsequent surgical stress results in injury to renal epithelium with the release of both alarm biomarkers and damage markers, this injury is attenuated in the RIPC condition compared with control. PAMPs = pathogen-associated molecular pattern molecules.

involvement of neuronal pathways. Reducing the protective effect of RIPC with spinal cord transection at $T_{7-}T_{10}$ (54, 55) or intrathecal spinal opioid receptor blockade with naloxone (56) and infarct size decrease by spinal cord stimulation by C_8–T_2 (57) favor a spinal reflex response. Obviously, the efferent pathway involves the autonomous nervous system. The ganglionic blocker, hexamethonium, reduced protection by RIPC or local bradykinin administration in most (53, 54)) but not all studies (58). Another ganglionic blocker also reduced the protective effect of RIPC from ischemia/reperfusion-induced endothelial dysfunction in humans (59). Cardiac sympathetic nerves are involved in attenuation of the observed infarct size reduction upon spinal cord stimulation, and this effect can be attenuated by β- or α_1-blockers (57). Vagotomy ((55, 60) or atropine (55) mitigated protection induced by limb ischemic preconditioning (55, 60).

As recently demonstrated, this concept of RIPC-associated organ protection is obviously transferable to cerebral tissue (61), and adenosine receptors have been implicated in neuroprotection by RIPC. This effect is likely mediated through an increased production of NO and specific antioxidants (62, 63). Catecholamines may also be involved in the organ-protective effect of RIPC, as pretreatment with catecholamines can mimic the effect of preconditioning (64, 65). Additional mechanisms involved in organ protection by RIPC may include humoral factors released into the systemic circulation, such as bradykinin, adenosine, and other factors (66–70).

Signaling Pathways

As reviewed in detail by Hausenloy et al (71), protein kinases (PKs) are an important field of research because several signaling pathways converge on these molecules to exert downstream effects. Preconditioning triggers that elicit these effects include adenosine, bradykinin, and opioids. PK C (PKC) is one such mediator of ischemia-induced protection (72). Current evidence of downstream signaling in ischemic preconditioning suggests activation of the signaling pathways through different molecules, including phosphoinositide 3-kinase, Akt, endothelial nitric oxide (NO) synthase (NOS), cyclic guanosine monophosphate, and PK G (PKG). These signaling pathways may open the adenosine triphosphate (ATP)–dependent mitochondrial potassium (K_{ATP}) channel, which is a downstream target of PKC/PKG activation (71, 73, 74). The activated mitochondrial K_{ATP} channels are able to limit the opening of mitochondrial permeability transition pores, thereby reducing apoptosis and lengthening cell survival (75). New experimental evidence has demonstrated that blocking NOS isoforms by a nonselective NOS inhibitor abolishes the protective effects of ischemic preconditioning. This evidence suggests that NO is an important cytoprotective agent, which may act as an activator and mediator of RIPC (76). Ischemic preconditioning elevates NOS expression with subsequent increase of NO oxidation products, nitrate and nitrite (77, 78). Consequently, infusion of the NO blocker, N-nitro-L-arginine methyl ester, before RIPC reduced its protective effect against subsequent ischemia (79).

Several studies indicate that RIPC may exert organ protection by triggering antioxidant and anti-inflammatory effects,

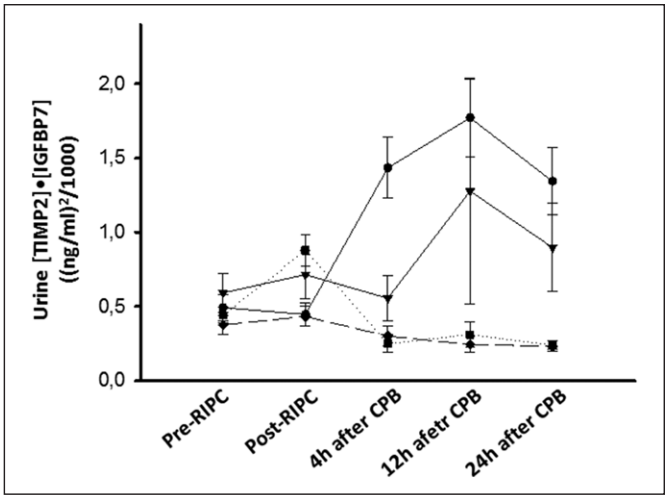

Figure 2. Mean (SEM) urinary [TIMP-2]•[IGFBP7] concentrations before and after remote ischemic preconditioning (RIPC) or control and start of cardiopulmonary bypass (CPB) stratified by the development of acute kidney injury (AKI) within 72 hr. Note that patients receiving RIPC but still developing AKI (*triangles/solid line*: 45 patients [38%]) had higher baseline biomarker concentrations and responded less to RIPC. *Circles with solid line* = sham with AKI; *triangles with solid line* = RIPC with AKI; *diamonds with long dashed line* = sham no AKI; *squares with dotted line*: RIPC no AKI. IGFBP7 = insulin-like growth factor–binding protein-7, TIMP-2 = tissue inhibitor of metalloproteinases-2.

including reduced extracellular levels of noxious metabolites (70, 80). This concept is further supported by evidence showing that RIPC abolishes neutrophil activation by reducing the formation of neutrophil-platelet aggregates and the expression of neutrophil CD11b (81). Furthermore, the activity of all key kinases involved in tumor necrosis factor (TNF) synthesis, mitogen-activated PK (MAPK)–activated PK 2, MAPK kinase kinase 2, and MAPK kinase kinase 8, was decreased. Ischemic preconditioning activated TNF receptor 1, which induces manganese SOD production, a strong protector against reactive oxygen species and antioxidants (81). In addition, RIPC reduced the expression of genes encoding key proteins involved in leukocyte chemotaxis, adhesion and migration, cytokine synthesis, exocytosis, innate immunity signaling pathways, and apoptosis (82, 83). Although the progress in identifying the precise molecular mechanisms has been slow, it is important that efforts to identify the molecular mechanisms continue—it may be possible to target these pathways pharmacologically.

Biomarkers Predict Protection and Damage–Two Sides of a Coin

We recently demonstrated that RIPC in high-risk patients undergoing cardiac surgery significantly reduced the occurrence of AKI, the need for RTT, and the length of ICU stay (25). Importantly, however, we found that the effectiveness of this intervention was strongly associated with the release of certain biomarkers into the urine. Tissue inhibitor of metalloproteinases-2 (TIMP-2) and insulin-like growth factor–binding protein-7 (IGFBP7) are recently discovered biomarkers for AKI (84, 85). In our study of RIPC in cardiac surgery (25), patients with urinary [TIMP-2]•[IGFBP7]) greater than or equal to 0.5 $(ng/mL)^2/1{,}000$ before surgery had a significantly reduced rate of AKI compared with patients with lower urinary [TIMP-2]•[IGFBP7] concentration (RR, 67%; 95% CI, 53–3; $p < 0.001$), whereas the biomarker concentrations after surgery predicted AKI as previously shown (25, 84–86).

The cell cycle consists of different phases, and each phase has a specific function that is required for appropriate cell proliferation. Quiescent cells are normally in G_0. Cells must enter and exit each phase of the cell cycle on schedule to divide and recover (87). If this tight schedule is disturbed, the normal repair and recovery process can become maladaptive (87). For instance, if epithelial cells remain arrested in G_1 or G_2, it favors a hypertrophic and fibrotic phenotype (88, 89). Conversely, exit from cell cycle in late G_1 leads to apoptosis (90). Each phase of the cell cycle is controlled by cyclins, cyclin-dependent kinases, and cyclin-dependent kinase inhibitors (87). Cells use cell cycle arrest as a protective mechanism to avoid cell division when potentially damaged (91). By initiating cell cycle arrest, cells can thus avoid cell division during stress and injury, which is protective.

Both IGFBP7 and TIMP-2 are inducers of G_1 cell cycle arrest, a mechanism involved in the early phase of AKI (92–94). Specifically, it has also been shown that renal tubular cells also go through this G_1 cell cycle arrest phase after stress because of a variety of insults (95). Induction of cell cycle arrest is not only associated with increased risk of AKI but also serves as a mechanistic link between AKI and CKD (89). Sustained cell cycle arrest will result in a senescent cell phenotype and lead to fibrosis. Intriguingly, the different TIMP proteins may have variable roles in the kidney. Wang et al (96) have demonstrated that TIMP-3 protects the cells from damage, whereas TIMP-2 seems to promote injury through matrix metalloproteinase activation.

We interpret our findings on the cell cycle arrest biomarkers TIMP-2 and IGFBP7 in RIPC as consistent with the known role of cell cycle arrest as part of the protective mechanisms endothelial cells use when exposed to stress (91, 97). Preemptively inducing these responses with RIPC should therefore reduce AKI (98). Importantly, only 56% of patients treated with RIPC achieved an increase in urine [TIMP-2]•[IGFBP7] to greater than or equal to 0.5, and only in this group was the intervention effective (25) (**Fig. 2**).

As discussed above, cell cycle arrest is a protective mechanism (98, 99), suggesting that temporary G_1 cell cycle arrest may reduce kidney damage. Using an animal model of septic AKI, we hypothesized that a pharmacologically induced early cell cycle arrest would be associated with less AKI (98, 100). We used cyclosporine A, a known inducer of cell cycle arrest and previously shown to attenuate kidney damage in the setting of folic acid–induced AKI (101), and found that a single dose given 18 hours after inducing sepsis and along with initial antibiotics was successful in reducing AKI (100). Consequently, manipulation of cell cycle may represent a new therapeutic strategy in the prevention and treatment of AKI.

In addition to or separate from the mechanisms described above, RIPC may attenuate renal injury by releasing various molecules, such as DAMPs from the remote tissue (Fig. 1). These

DAMPs are subsequently filtered by the kidney and signal through PRRs, such as toll-like receptors, in the proximal tubule epithelia (48, 102). This signaling may then induce natural defenses, such as bioenergetic down-regulation and temporary cell cycle arrest (97–99). These defenses, once engaged, can then protect the kidney during subsequent inflammatory or ischemic stress.

We have examined the mechanisms whereby RIPC induces renal protection. Our conceptual model for the proposed mechanism responsible for this effect in humans is shown in Figure 1. In our study (25), we measured high-mobility group box protein-1 (HMGB-1), a prototypical DAMP, at baseline and after RIPC (before cardiopulmonary bypass). Urinary HMGB-1 was similar in both groups at baseline. However, urinary HMGB-1 significantly increased immediately after RIPC. In multivariable logistic regression analysis, preoperative serum creatinine and previous heart surgery were associated with increased risk of AKI, whereas post-RIPC HMGB-1 (OR, 0.75; 95% CI, 0.61–0.91; $p = 0.005$) and [TIMP-2]•[IGFBP7] (OR, 0.57; 95% CI, 0.35–0.94; $p = 0.03$) were associated with lower risk of AKI. Furthermore, both HMGB-1 and RIPC were significant predictors of post-RIPC [TIMP-2]•[IGFBP7] greater than or equal to 0.5 $(ng/mL)^2/1{,}000$ (25).

These results are also important because they have implications for how we understand the pathogenesis of AKI. The available data suggest that cell cycle arrest signaling is a protective response, but when engaged by multiple cells such that increases in markers like TIMP-2 and IGFBP7 can be detected in the urine, it is often followed by AKI. Furthermore, if cell cycle arrest persists, the result can become maladaptive and lead to a fibrosis phenotype. Early protection of cells might be achievable by supporting the cell's own self-preservation mechanisms, including cell cycle arrest. Conversely, once the danger is past, it may be important to rapidly reverse this process; so, that the adverse consequences, including cell senescence and fibrosis, are avoided. Thus, cell cycle arrest activation and deactivation at critical clinical time points for a patient may prove to be targets of RIPC or other therapeutic intervention in the future.

However, there are still important unanswered questions. One question is whether HMGB-1 is the only or most important DAMP released after RIPC and to what extent released HMGB-1 is filtered at the glomerulus. Because many of the affected patients likely have a reduced GFR, it would be interesting to know whether the GFR influences the filtration of DAMPs released after RIPC and subsequently the effect of RIPC. Furthermore, the location and time course of the cell cycle arrest in the kidney need to be investigated in considerably greater detail.

CONCLUSIONS

Clearly, more research is needed to form a better evidentiary basis for the use of RIPC, to understand the therapeutic potential and potential risks, to determine when and in whom the intervention works, and to examine the role of biomarkers in sorting out these issues. However, to date, the existing data suggest that RIPC can greatly reduce the risk of AKI in high-risk patients undergoing cardiac surgery. High-risk patients seem to benefit most from this intervention, and indeed, the renoprotective effects may be limited to this patient population. The intervention may have a role not only in surgical patients but also in other clinical scenarios (e.g., contrast-induced nephropathy). Finally, novel molecular mechanisms for RIPC may exist in renoprotection and the role of cell cycle arrest biomarkers in monitoring this process, serving as "theranostics," is certainly intriguing.

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Is Remote Ischemic Preconditioning Really a Novel Renoprotective Option for Cardiac Surgery?

To the Editor:

The article published in a recent issue of *Critical Care Medicine* by Zarbock and Kellum (1) evaluating the clinical evidence for remote ischemic preconditioning (RIPC) as a potential strategy to protect the kidney was of great interest. We noted that a total of nine clinical studies containing 1,158 patients with cardiac surgery were included in this article. Of nine clinical studies, four trials showed a protective effect of RIPC on kidney function after cardiac surgery, as indicated by reduced postoperative peak creatinine serum level, improved glomerular filtration rate, and reduced incidence of acute kidney injury (AKI); however, other five trials failed to demonstrate a beneficial effect of RIPC on postoperative kidney function, as shown by no differences in AKI prevalence or renal outcome between the RIPC and control groups. According to these data, Zarbock and Kellum (1) conclude that RIPC can greatly reduce the risk of AKI in high-risk patients undergoing cardiac surgery.

Our aim of writing this letter to the editor is to remind the readers that the article by Zarbock and Kellum (1) does not include the recently published two large multicenter randomized controlled trials by Meybohm et al (2) and Hausenloy et al (3) in *New England Journal of Medicine*, which have failed to confirm protective effect of RIPC on kidney function of patients undergoing cardiac surgery.

A multicenter, randomized double-blind clinical trial by Meybohm et al (2) included a total of 1,385 patients (693 in the control group and 692 in the RIPC group) who were scheduled for elective cardiac surgery requiring cardiopulmonary bypass. RIPC was induced in anesthetized patients by four cycles of 5 minutes of ischemia of the arm interspersed with 5 minutes of reperfusion, a commonly used study regimen. They showed no significant between-group difference in prevalence of postoperative acute renal failure (5.1% in the control group vs 6 .1% in the RIPC group). Another multicenter randomized double-blind clinical trial by Hausenloy et al (3) enrolled a total of 1,612 patients (811 in the control group and 801 in the RIPC group) who were scheduled for elective on-pump coronary artery bypass grafting surgery (with or without valve surgery) at 30 cardiac surgery centers in the United Kingdom. RIPC procedure was same as the method described by Meybohm et al (2). Similarly, this trial did not show any significant between-group difference in the prevalence and severity of postoperative AKI (prevalences of grades 1, 2, and 3 AKI were 29.3%, 5.7%, and 3.0% in the control group, respectively, 30.7%, 5.1%, and 2.5% in the RIPC group). In addition, the two trials also showed that RIPC did not improve other clinical outcomes after cardiac surgery, such as death, myocardial infarction, and stroke. Thus, conclusions from both trials are definitive: RIPC is ineffective in improving clinical outcomes of patients undergoing cardiac surgery (4).

Given that the two studies by Meybohm et al (2) and Hausenloy et al (3) include number of patients, which are much larger than the total sample size of nine studies included in the study by Zarbock and Kellum (1), we suggest that they perform a meta-analysis based on the available 11 clinical trials to reevaluate protective effect of RIPC on kidney function after cardiac surgery. Perhaps, this would provide more robust evidence about whether RIPC is really a novel renoprotective option for cardiac surgery.

The authors have disclosed that they do not have any potential conflicts of interest.

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The authors reply:

We thank Xue et al (1) for their interest in our article (2) and their comments. Remote ischemic preconditioning (RIPC), defined as brief and transient episodes of ischemia at a remote site before a subsequent injury of the target organ, is believed to induce an adaptive response that protects against organ injury elicited by the new insult. In the past few years, studies investigating the effects of RIPC have been published with mixed results. In contrast to studies showing a positive effect of RIPC on the heart and kidney, some trials have demonstrated that RIPC does not affect organ function, complication rates, or mortality. We have not mentioned the two recently published multicenter trials on RIPC mentioned

by Xue et al (1) in our review because the studies had not been published by the time our work was accepted for publication. However, they are important studies and deserve mention.

These two large multicenter trials have not only studied the effects of RIPC for cardiac protection but also examined the effects on acute kidney injury (AKI). Hausenloy et al (3) randomly assigned 1,612 patients to RIPC (four 5-min cycles) or sham-RIPC at 30 cardiac surgery centers in the United Kingdom—the Effect of Remote Ischemic Preconditioning on Clinical Outcomes in Patients Undergoing Coronary Artery Bypass Graft Surgery (ERICCA) trial. The combined primary endpoint was death from cardiovascular causes, nonfatal myocardial infarction, coronary revascularization, or stroke, assessed 12 months after randomization. They found no significant difference in the cumulative prevalence of the primary endpoint: 212 RIPC patients (26.5%) versus 225 sham patients (27.7%) (hazard ratio, 0.95; 0.79–1.15; $p = 0.58$). Furthermore, there were no significant between-group differences in either adverse events or the secondary endpoints including stage 2–3 AKI 57 of 749 (7.6%) versus 67 of 772 (8.7%). A very similar trial (the RIP Heart Study) was performed by Meybohm et al (4). This trial compared the same RIPC protocol with sham in 1,403 patients in 14 centers in Germany. The primary endpoint was a composite of death, myocardial infarction, stroke, or stage 2–3 AKI. No significant differences were seen in rates of the composite primary endpoint: 99 RIPC patients (14.3%) versus 101 sham patients (14.6%) ($p = 0.89$) or in any of the individual components including AKI: 42 (6.1%) versus 35 (5.1%) ($p = 0.45$).

Differences in these results compared with those discussed in our review (1) might be explained by the differences in study design, patient selection, or cointerventions. As for all therapies, treating patients with little or no risk biases the overall treatment effect toward the null. Applying RIPC to low-risk patients may dilute any possible signal. Conversely, efficacy may be easier to demonstrate (and might only be important in) high-risk patients. Another aspect of RIPC could be that marked heterogeneity of treatment effect occurs such that some patients have significant benefit, whereas others have no benefit at all. We demonstrated in our Renal RIPC trial that biomarkers can identify patients who benefit from this intervention (5). Finally, greater than 90% of patients in ERICCA and 100% of patients in RIP Heart were treated with propofol for anesthesia—a drug know to inhibit the effects of RIPC (6).

Performing a meta-analysis as suggested by Xue et al (1) is very difficult because of the different AKI definitions and treatment strategies used in the different trials and the heterogeneous patient populations investigated in the trials. Clearly, more research is needed to form a better evidence for the use of RIPC. However, there is currently evidence suggesting that RIPC can reduce the risk of AKI in high-risk patients undergoing cardiac surgery.

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Hemostasis and Extracorporeal Membrane Oxygenation: Bleeding Cannot Be Seen in Aliquots

To the Editor:

Malfertheiner et al (1) are congratulated on their comparison of three different veno-venous extracorporeal membrane oxygenation (vv-ECMO) systems in a recent issue of *Critical Care Medicine*. However, from my point of view, I have some concern regarding whether an actual statement about "hemostatic changes" can be made. My response about the above concern is as follows:

Bleeding is a major complication in at least every one-third of ECMO patient (2) with important clinical relevance. In the 2012 ELSO registry report, Paden et al (3) states that bleeding from cannula and surgical sites is a significant remaining problem of vv-ECMO, which is associated with worse survival. Although external bleeding usually is controllable, a prevalence up to 19% for intracranial hemorrhage (4) with a survival rate of only 20% is reported (3).

The contact with the artificial surface and the shear stress in oxygenators and pumps activate the coagulation system and lead to an inflammatory response. Clot formation with consecutive activation of physiological fibrinolysis leads to a waste of coagulation factors. Hemolysis as well is common during extracorporeal therapy. These facts are known for many years. Annich in 2011 (5) conceded that "we have learned to circulate, oxygenate, and ventilate blood outside of the body but we still do not command the blood-surface interface" and recently highlighted the "precarious balance of hemostasis during ECMO therapy" (6).