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Remote ischemic preconditioning and protection of the kidney - a novel therapeutic option

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Abstract

Objective—Acute kidney injury (AKI) 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 AKI. There is currently no effective strategy available to prevent or treat AKI. Therefore, novel treatment regimens are required to decrease AKI incidence and to improve clinical outcomes. Remote ischemic preconditioning (RIPC), triggered by brief episodes of ischemia and reperfusion applied in distant tissues or organs before injury of the target organ, attempts to invoke adaptive responses that protect against AKI. 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 RIPC on kidney function in surgical patients (search terms: “remote ischemic preconditioning” and “kidney function” and “surgery”). We also reviewed bibliographies of relevant papers 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 RIPC might be best applied.

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Conclusions—Several experimental and clinical studies have shown tissue-protective effects of RIPC in various target organs, including the kidneys. RIPC may offer a novel, non-invasive and inexpensive treatment strategy for decreasing AKI incidence in high risk patients. Although many new studies have further advanced our knowledge in this area, the appropriate intensity of RIPC, its mechanisms of action, and the role of biomarkers for patient selection and monitoring are still unknown.

Keywords

Remote ischemic preconditioning; Acute kidney injury; biomarker; cell cycle arrest

Introduction

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 for AKI are sepsis, major surgery, and nephrotoxic drugs (9). Hospitalized patients, particularly those with comorbidities and those undergoing complex procedures are at high risk for developing AKI (7, 10). The incidence of AKI after surgery has been reported to be as low as 1%, whereas the incidence 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 incidence and epidemiology of AKI worldwide. By applying the RIFLE (Risk, Injury, Failure, Loss and End-stage kidney disease) 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. investigated the incidence of AKI by using RIFLE criteria (14) and demonstrated that the incidence of “Risk”, “Injury” and “Failure” was 10%, 5% and 3.5%, respectively. Even patients with a mild AKI (Risk) had a 3-fold increased mortality rate compared to control subjects without AKI. “Risk”, “Injury” and “Failure” was associated with an odds ratio for hospital mortality of 2.5, 5.4 and 10.1, respectively. A large multicentre trial included 120,123 critically ill patients at 57 intensive care units (15). In this patient population, the overall incidence of AKI was as high as 36%. As also previously noted, hospital mortality in patients with AKI was significantly increased (OR ratio 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 (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, 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 and colleagues, who first described the cardioprotective effect of several brief ischemic episodes before subsequent prolonged ischemic insult in animals with myocardial infarction (19). 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 non-target tissue confer protection at remote site 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 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 and these cells respond to 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 (for a recent review see 78). 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 non-invasive 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. Older 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 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 and colleagues investigated whether brief hind limb occlusion can protect against renal ischemia-reperfusion injury and whether this protection is adenosine dependent (30). Rats underwent either no RIPC, unilateral RIPC or bilateral RIPC. After 24 h 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 meta-analysis of animal studies investigated three outcome measures: 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]), blood urea nitrogen (SMD 1.42 [95% CI 0.97, 1.87]) and histological renal damage (SMD 1.12 [95% CI 0.89, 1.35]) as compared to controls. Factors influencing efficacy were the window of protection (<24 h vs. ≥24 h) 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 (e.g., pre-existing chronic kidney disease, low ejection fraction, diabetes, COPD). RIPC is a safe procedure, as no relevant 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 prior to cardiac surgery was effective for reducing the occurrence of AKI (37.5% compared to 52.5% with sham; ARR, 15%; 95% CI, 2.56% to 27.44%; $P=0.02$). Furthermore, fewer patients receiving RIPC received RRT (5.8% versus 15.8%; ARR, 10%; 95% CI, 2.25% to 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 monocenter, 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 absolute risk reduction of 0.27 and a significantly reduced relative risk due to preconditioning of 0.43 (32). In line with these results, another small randomized study demonstrated that RIPC reduced the postoperative peak creatinine serum concentration compared to the control intervention (33). Furthermore, a retrospective study of nondiabetic patients undergoing elective CABG surgery showed that RIPC significantly reduced the incidence of AKI (34). In addition, two randomized controlled trials showed that RIPC can also reduce contrast-induced AKI in a high-risk patient populations (23, 35).

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 and colleagues (36) used three 10-min cycles of lower-limb ischemia and reperfusion in 76 patients undergoing complex valvular cardiac surgery. Primary outcomes were AKI incidence (AKIN definition) and changes in two urinary biomarkers of kidney injury. There were no differences in the incidence 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 receive either RIPC or to serve as control. Plasma concentrations of high-sensitive troponin T at 6 and 12 h after surgery and the postoperative incidence of AKI were used as primary study endpoints. The incidence of AKI did not differ between the two groups. The authors concluded that RIPC provided neither myocardial nor renal protection. Gallagher and colleagues (38) recently published a randomized controlled trial of RIPC to prevent AKI in 86 patients with chronic kidney disease (estimated GFR under 60 ml/min per 1.73 m²) undergoing coronary artery bypass graft surgery. The primary end point was the development of AKI. 12 patients in each group developed an AKI in within 48 h of CABG. The authors concluded that RIPC had no effect on the frequency of AKI after CABG in patients with CKD (38). Although the incidence of AKI was only a secondary outcome parameter, two studies demonstrated that RIPC did not decrease the incidence 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, reno- and cardioprotection by RIPC were investigated in 82 adults undergoing abdominal aortic aneurysm repair (41). RIPC decreased the incidence of AKI by 77% (30% versus 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 incidence of GFR reduction at 1 month after surgery (8.8% versus 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 due to the high incidence 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 inefficiency 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 appears 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 need to establish the clinical benefits of RIPC as well as 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 individual to individual (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 set up, it was shown that plasma from remotely preconditioned animals is cardioprotective when perfused into an isolated naïve 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 release of damage-associated molecular patterns (DAMPs) that interact with pattern recognition receptors on renal tubular epithelial cells (Figure 1). However, a number of other mechanisms have been proposed and it is possible that different organs are effected 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 involvement of neuronal pathways. Reducing the protective effect of RIPC with spinal cord transection at T₇-T₁₀ (54, 55) or intrathecal spinal opioid receptor blockade with naloxone (56) and infarct size decrease by spinal cord stimulation by C₈-T₂ (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. Protein kinase 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 (PI3K), Akt, endothelial nitric oxide synthase (NOS), cyclic guanosine monophosphate, and PKG. These signaling pathways may open the 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 non-selective NOS inhibitor abolishes the protective effects of ischemic preconditioning. This evidence suggests that nitric oxide (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 (L-NAME), 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, 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 protein kinase (MAPK)-activated protein kinase 2, MAPK kinase kinase 2, and MAPK kinase kinase 8, were decreased. Ischemic

preconditioning activated TNF receptor 1 (TNFR1), which induces manganese SOD production, a strong protector against reactive oxygen species and antioxidant (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 renal replacement therapy, and length of intensive care 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] 0.5 (ng/ml)²/1000 before surgery had a significantly reduced rate of AKI compared to patients with lower urinary [TIMP-2]•[IGFBP7] concentration (RR, 67%; 95% CI, 53% to 83%, 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 G₀. 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 G₁ or G₂, it favors a hypertrophic and fibrotic phenotype (88, 89). Conversely, exit from cell cycle in late G₁ 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 both inducers of G₁ 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 G₁ cell cycle arrest phase following stress due to a variety of insults (95). Induction of cell-cycle arrest is not only associated with increased risk for AKI but may also serve 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 and colleagues have demonstrated that TIMP-3 protects the cells from damage, whereas TIMP-2 appears to promote injury through matrix metalloproteinase activation (96).

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). Pre-emptively 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 ≥ 0.5 , and only in this group was the intervention effective (25). Conversely, 24% of patients exhibited a urine [TIMP-2]•[IGFBP7] to ≥ 0.5 before surgery and these patients appeared not to benefit from RIPC (Figure 2).

As discussed above, cell cycle arrest is a protective mechanism (98, 99), suggesting that temporary G₁ 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 damage associated molecular patterns (DAMPs) from the remote tissue (Figure 1). These DAMPs are subsequently filtered by the kidney and signal through pattern-recognition receptors (PRR) 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 for AKI while post-RIPC HMGB-1 (OR, 0.75; 95% CI, 0.61 to 0.91; P=0.005) and [TIMP-2]•[IGFBP7] (OR, 0.57; 95% CI, 0.35 to 0.94; P=0.03) were associated with lower risk for AKI. Furthermore, both HMGB-1 and RIPC were significant predictors of post-RIPC [TIMP-2]•[IGFBP7] ≥ 0.5 (ng/ml)²/1000 (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 HMGB1 is the only or most important DAMP released after RIPC and to what extent released HMGB1 is filtered at the glomerulus. Since many of the affected patients likely have a reduced glomerular filtration rate, 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 needs to be investigated in considerably greater detail.

Conclusion

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 appear to benefit most from this intervention and indeed the renoprotective effects may be limited 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 “theragnostics” is certainly intriguing.

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Figure 1.

Remote ischemic preconditioning (RIPC) reduces the occurrence of AKI by inducing release of damage-associated molecular patterns (DAMPs) which bind to pattern recognition receptors (PRRs) on surface of renal epithelial cells. Next, alarm markers, tissue inhibitor of metalloproteinases-2 (TIMP-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. While subsequent surgical stress results in injury to renal epithelium with release of both alarm biomarkers as well as damage markers, this injury is attenuated in the RIPC condition compared to control.

Figure 2.

Mean (SEM) urinary [TIMP-2]•[IGFBP7] concentrations before and after RIPC or control and start of cardiopulmonary bypass (CPB) stratified by development of AKI within 72hrs. Note that patients receiving RIPC but still developing AKI (Triangles/solid line 45 pts (38%) had higher baseline biomarker concentrations and responded less to RIPC. CPB, cardiopulmonary bypass. Circles with solid line = sham with AKI; triangles with solid line = RIPIC with AKI; diamonds with long dashed line = sham no AKI; squares with dotted line: RIPIC no AKI.

Table 1

Clinical trials on the effect of RIPC on AKI

Reference	Clinical setting	Outcome parameters
Surgery patients		
Ali <i>et al.</i> (48)	Abdominal aortic aneurysm repair (N=82)	RIPC reduced the incidence of AKI (30% versus 7%; P=0.01)
Walsh <i>et al.</i> (49)	Abdominal aortic aneurysm repair (N=51)	No differences in renal outcome
Walsh <i>et al.</i> (50)	Endovascular aneurysm repair (N=40)	RIPC did not reduce the occurrence of AKI, but the release of biomarkers
Rahman <i>et al.</i> (51)	Cardiac surgery (CABG) (N=162)	No differences in renal outcome
Venugopal <i>et al.</i> (52)	Cardiac surgery (CABG) (N=78)	RIPC decreased the incidence of AKI
Huang <i>et al.</i> (53)	Laparoscopic partial nephrectomy (N=82)	RIPC reduced the rate of GFR reduction at 1 month
Zimmerman <i>et al.</i> (55)	Cardiac surgery (N=120)	RIPC reduced the occurrence of AKI compared to control (20% versus 47%; P=0.004)
Thielmann <i>et al.</i> (56)	Cardiac surgery (N=120))	RIPC reduced peak creatinine serum concentration
Gallagher <i>et al.</i> (57)	Cardiac surgery in patients with CKD (N=86)	RIPC did not reduce the rate of AKI
Zarbock <i>et al.</i> (21)	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
Pedersen <i>et al.</i> (58)	Congenital heart surgery (N=113)	No differences in AKI incidence
Choi <i>et al.</i> (59)	Cardiac surgery (N=76)	No differences in AKI incidence
Candilio <i>et al.</i> (60)	Cardiac surgery (N=180)	Secondary analysis: No differences in renal outcome
Young <i>et al.</i> (61)	Cardiac surgery (N=96)	Secondary analysis: No differences in renal outcome
Chen <i>et al.</i> (63)	Renal transplantation (N=60)	RIPC did not improve early renal Function
Wu <i>et al.</i> (64)	Renal transplantation (N=48)	RIPC enhances early recovery of renal Function
Non-surgery patients		
Er <i>et al.</i> (20)	Coronary angiography (N=200)	RIPC decreased CI-AKI (40% in the control group versus 12% in the RIPC group)
Deftereos <i>et al.</i> (54)	Patients with myocardial infarction undergoing PCI (N=225)	RIPC significantly reduced the rate of AKI (12.4% versus 29.5%; P=0.02)

Table 2

Clinical trials on the effect of RIPC on AKI: patient selection, nature of maneuver, and definition of AKI

Reference	Nature of the maneuver	Definition of AKI	Patient selection
Surgery patients			
Ali <i>et al.</i> (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 $\mu\text{mol/L}$ (2.0 mg/dL)	Low risk patients and procedures
Walsh <i>et al.</i> (42)	Location: lower limb, number of cycles: 2, duration of cycle: 10 min.	Serum creatinine Concentrations	Low risk patients and procedures Low risk patients and procedures
Walsh <i>et al.</i> (43)	Location: iliac artery, number of cycles: 2, duration of cycle: 10 min.	Serum creatinine concentrations and biomarkers	Low risk patients and procedures
Rahman <i>et al.</i> (40)	Location: upper limb, number of cycles: 3, duration of cycle: 5 min.	Creatinine levels and creatinine between day 0 to 4	Low risk patients and procedures
Venugopal <i>et al.</i> (34)	Location: upper-limb, number of cycles: 3, duration of cycle: 5 min.	AKIN within the first 72 hours after cardiac surgery	Low risk patients and procedures Low risk patients and procedures
Huang <i>et al.</i> (44)	Location: lower limb, number of cycles: 3, duration of cycle: 5 min.	Serum creatinine concentrations and biomarkers	Low risk patients and procedures
Zimmerman <i>et al.</i> (32)	Location: lower limb, number of cycles: 3, duration of cycle: 5 min.	AKIN	High risk patients: CKD (<60 ml/min per 1.73 m ²)
Thielmann <i>et al.</i> (33)	Location: upper limb, number of cycles: 3, duration of cycle: 5 min.	Serum creatinine concentration over 72 h after surgery	High risk patients: several co-morbidities
Gallagher <i>et al.</i> (38)	Location: upper-limb, number of cycles: 3, duration of cycle: 5 min.	AKIN within 48 hours after cardiac surgery	and/or complex surgical procedure
Zarbock <i>et al.</i> (25)	Location: upper limb, number of cycles: 3, duration of cycle: 5 min.	KDIGO criteria within 72 hours after cardiac surgery	Low risk patients and procedures Low risk patients and procedures
Choi <i>et al.</i> (36)	Location: lower limb, number of cycles: 3, duration of cycle: 10 min.	AKIN within 48 hours after cardiac surgery	High risk patients/ procedures: Ejection fraction below 50%, re-
Candilio <i>et al.</i> (39)	Location: upper and lower limb, number of cycles: 2, duration of cycle: 5 min.	RIFLE	operation, complex cardiac surgery
Young <i>et al.</i> (37)	Location: upper-limb, number of cycles: 3, duration of cycle: 5 min.	RIFLE	Low risk patients and procedures Low risk procedures
Chen <i>et al.</i> (46)	Location: lower limb, number of	Serum creatinine concentrations	Low risk patients

Reference	Nature of the maneuver	Definition of AKI	Patient selection
Wu <i>et al.</i> (47)	cycles: 3, duration of cycle: 5 min. Location: iliac artery, number of cycles: 3, duration of cycle: 5 min.	Serum creatinine levels and biomarkers	Low risk patients and procedures
Non-surgery patients Er <i>et al.</i> (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 hours after contrast medium administration AKI: defined as an	
Deftereos <i>et al.</i> (35)	Location: coronary artery, number of cycles: 4, duration of cycle: 30 sec.	absolute increase in serum creatinine of 0.5 mg/dl or a relative increase of 25% compared with baseline within 96 h after PCI	