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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

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Remote Ischemic Preconditioning Protects Against Hemodialysis-Induced Cardiac Injury

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The hemodynamic stress induced by hemodialysis (HD) causes recurrent and cumulative ischemic injury to the heart, brain, and other vital organ systems. When the myocardium is subjected to circulatory challenge caused by conventional HD, it results in segmental ischemia,[1] with cumulative changes in ventricular structure and function over time.[1,2]

Murry *et al*. first described the phenomenon of ischemic preconditioning in a canine experimental model: the application of a transient, non-lethal occlusion of the circumflex coronary artery would reduce infarct size after a subsequent, prolonged occlusion of that same vessel.[3] Przyklenk *et al*. described remote ischemic preconditioning (RIPC) in a similar model, where the transient occlusion of the circumflex coronary artery would reduce infarct size after prolonged left anterior descending coronary artery occlusion.[4] A similar effect was subsequently achieved in a swine model by delivering RIPC non-invasively, applying a blood pressure cuff on a peripheral limb to induce brief and transient skeletal muscle ischemia-reperfusion stimuli.[5]

RIPC has recently been the subject of intense human research as a novel cardioprotective strategy. HD represents an attractive model to study RIPC, due to the predictable nature of the hemodynamic stress caused

Table 1. Basic demographics for each group at study start

	Overall cohort (n = 21)	Control (n = 10)	RIPC (n = 11)	*P* value
Age (yr)	58.9 ± 14.6	62.2 ± 14.3	56.2 ± 15	0.37
Gender (m/f)	18/4	8/2	9/2	1.00
Presence of fistula	95	100	91	1.00
HD vintage (mo)	32.0 ± 30.9	27.3 ± 27.7	36.2 ± 34.3	0.36
Diabetes	38	30	45	0.66
CAD	24	40	9	0.15
Kt/V	1.33 ± 0.28	1.29 ± 0.33	1.36 ± 0.23	0.60

CAD, coronary artery disease; HD, hemodialysis; Kt/V, dialyzer clearance of urea × dialysis time/volume of distribution of urea; RIPC, remote ischemic preconditioning.
Values are mean ± SD, or %, unless otherwise indicated.

by it and the importance of preventing HD-induced ischemia-reperfusion injury. We performed a pilot study to investigate the effects of RIPC on HD-induced cardiac injury, to improve conventional HD patients' resilience to ischemia-reperfusion injury and provide cardiac protection.

RESULTS

In total, 21 patients entered the study (Supplementary Figure S1), 10 patients in the control group and 11 patients in the RIPC group. One patient in the RIPC group did not complete the study, owing to transplantation. Table 1 summarizes baseline demographics. No significant differences were observed. The mean HD vintage was slightly higher in the RIPC group (36.2 months ± 34.3 months vs. 27.3 months ± 27.7 months, $P = 0.36$); 4 patients in the control group had a previous diagnosis of coronary artery disease, compared to one in the RIPC group ($P = 0.15$) All patients were adequately dialyzed according to their most recent Kt/V (dialyzer clearance of urea × dialysis time/volume of distribution of urea). The RIPC intervention was well tolerated, and all patients in the RIPC group completed the full 4 ischemia-reperfusion cycles. Three patients in the RIPC group reported paraesthesia and discomfort during initial inflation of the blood pressure cuff.

A comparison of cardiovascular parameters in each group at each visit is shown in Table 2. Both groups showed a moderate reduction in left-ventricular ejection fraction at Screening (40.3 ± 12.1 for control vs. 42.2 ± 10.6 for RIPC, $P = 0.71$). The number of regional wall motion abnormalities (RWMAs) at Screening was comparable between the 2 groups (3.9 ± 1.9 for control vs. 5.4 ± 1.5 for RIPC, $P = 0.1$). Figure 1 summarizes between-group comparisons. A significant reduction in RWMAs was observed in the RIPC group compared with the control group at Intervention (5.2 ± 2.5 for control vs. 2.7 ± 1.8 for RIPC, $P < 0.05$); a non-significant RWMA reduction was observed at 48 hours (4.4 ± 1.7 for control vs. 2.7 ± 2.1 for RIPC, $P = 0.09$) and at 28 days (5.1 ± 1.8 for control vs. 3.7 ± 2.1 for RIPC, $P = 0.13$). Figure 2 summarizes within-group comparisons at each visit. A significant reduction in RWMAs was observed within the RIPC group at intervention (5.4 ± 1.5 for screening vs. 2.7 ± 1.8 for intervention, $P < 0.01$) and at 48 hours (5.4 ± 1.5 for screening vs. 2.7 ± 2.1 for 48 hours, $P < 0.01$) compared with screening. A nonsignificant reduction was observed at 28 days versus screening (5.4 ± 1.5 for screening vs. 3.7 ± 2.1 for 28 days, $P = 0.07$). No significant differences in the number of RWMAs between different visits were observed in the control

Table 2. Cardiovascular and hemodynamic parameters at each study visit

	Control group				RIPC group			
Parameters	Screening	Intervention	48 h	28 d	Screening	Intervention	48 h	28 d
RWMA	3.9 ± 1.9	5.2 ± 2.5	4.4 ± 1.7	5.1 ± 1.8	5.4 ± 1.5	2.7 ± 1.8[a,b]	2.7 ± 2.1[b]	3.7 ± 2.1
Pre-HD GLS (%)	−14.2 ± 2.1	−16.8 ± 4.6	−14.8 ± 5.0	−18.0 ± 5.2	−15.7 ± 3.0	−14.8 ± 3.1	−15.7 ± 4.4	−15.6 ± 4.5
Peak-stress HD GLS (%)	−12.7 ± 5.3	−13.8 ± 4.5[c]	−13.0 ± 4.3	−12.1 ± 3.9[d]	−13.0 ± 3.4[e]	−15.0 ± 4.2	−16.2 ± 4.5	−13.8 ± 3.0
Pre-HD EF (%)	40.3 ± 12.1	41.3 ± 13.8	43.8 ± 14.2	43.2 ± 8.4	42.2 ± 10.6	44.5 ± 9.6	43.9 ± 12.2	42.7 ± 9.6
Pre-HD EDV (ml)	101.4 ± 27.5	124.0 ± 28.0	95.2 ± 9.6	103.6 ± 27.6	111.2 ± 22.1	108.5 ± 20.3	108 ± 15.5	103.6 ± 27.6
Pre-HD ESV (ml)	62.2 ± 23.1	73.3 ± 13.8	53.4 ± 13.1	62.4 ± 33	62.6 ± 21.4	61.3 ± 22.5	61.7 ± 18	60.2 ± 22
UF volume (L)	1.9 ± 0.5	1.3 ± 0.9	1.2 ± 0.8	1.7 ± 0.5	2.2 ± 0.6	2.2 ± 0.7[f]	1.8 ± 0.8	2.0 ± 1.0
UF rate (ml/kg per h)	6.3 ± 2.1	4.0 ± 3.2	4.6 ± 3.1	5.6 ± 1.9	7.8 ± 2.8	7.0 ± 1.9[a]	5.7 ± 2.0	6.1 ± 2.9
Pre-HD SBP (mm Hg)	144.2 ± 22.8	142.1 ± 21.3	152.1 ± 24.9	142.2 ± 27.9	159.4 ± 25.2	146.1 ± 21.1	147.2 ± 17.5	147 ± 27.5
Nadir SBP (mm Hg)	N/A	102.0 ± 12.7	117.6 ± 17.9	109.8 ± 15.8	N/A	124.6 ± 17.1[a]	130.5 ± 14.3	116.3 ± 25.8
IDH (%)	N/A	40	20	40	N/A	36	18	36

EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; GLS, global longitudinal strain; HD, hemodialysis; IDH, intradialytic hypotension; RIPC, remote ischemic preconditioning; RWMA, regional wall motion abnormalities; SBP, systolic blood pressure; UF, ultrafiltration.
[a] $P < 0.05$ vs. control (intervention).
[b] $P < 0.01$ vs. screening (RIPC group).
[c] $P < 0.05$ vs. pre-HD GLS (intervention, control).
[d] $P < 0.05$ vs. pre-HD GLS (28 days, control).
[e] $P < 0.05$ vs. pre-HD GLS (screening, RIPC group).
[f] $P < 0.01$ vs. control (intervention).
Values are mean ± SD, unless otherwise indicated.

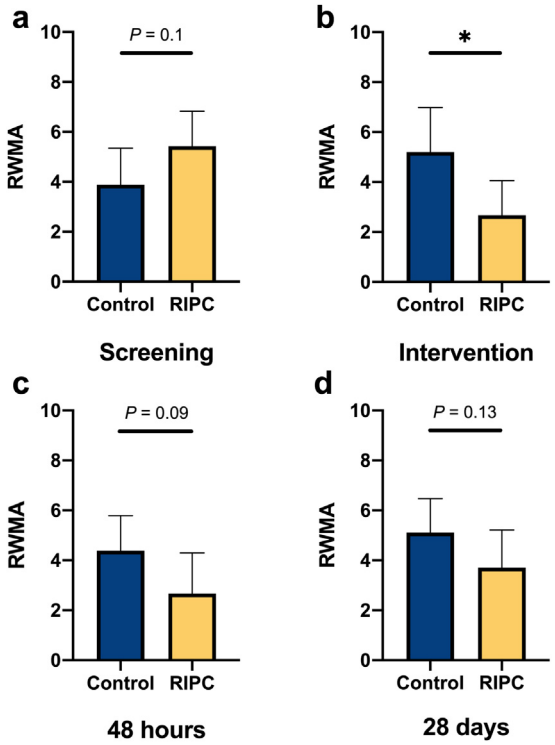

Figure 1. Comparison in regional wall motion abnormalities (RWMA) between groups at each study visit (screening [a]; intervention [b]; 48 hours [c]; 28 days [d]). Columns represent mean RWMA; error bars represent 95% confidence intervals. RIPC, remote ischemic preconditioning. $^{*}P < 0.05$.

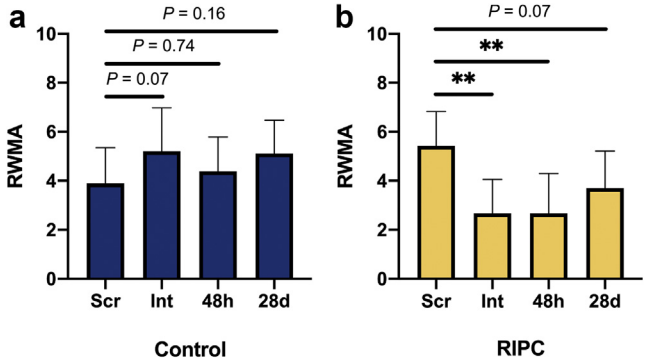

Figure 2. Comparison in regional wall motion abnormalities (RWMA) between study visits in each group (control group [a]; remote ischemic preconditioning [RIPC] group [b]). Columns represent mean RWMA; error bars represent 95% confidence intervals. Int, intervention; Scr, screening. $^{**}P < 0.01$.

group (Figure 2a). Measures of effect size were calculated with Cohen's d for RWMAs between groups: 1.2 for intervention, 0.9 for 48 hours, 0.7 for 28 days.

A reduction in global longitudinal strain at peak-stress HD at screening was observed in both groups, reaching significance in the RIPC group (−15.7 ± 3.0 for pre-HD vs. −13.0 ± 3.4 for peak-stress HD, $P < 0.05$), but not in the control group (−14.2 ± 2.1 for pre-HD vs. −12.7 ± 5.3 for peak stress, $P = 0.77$). No significant differences were observed at the subsequent visits in the RIPC group. A significant difference between pre-HD and peak-stress HD global longitudinal strain was observed in the control group at Intervention (−16.8 ± 4.6 pre-HD vs. −13.8 ± 4.5 peak stress, $P < 0.05$) and at 28 days (−18.0 ± 5.2 pre-HD vs. 12.1 ± 3.9 peak stress, $P < 0.05$).

A greater degree of circulatory stress was observed in the RIPC group compared with the control group at Intervention, as shown by higher ultrafiltration volumes (1.3 ± 0.9 L for control vs. 2.2 ± 0.7 L for RIPC, $P < 0.01$) and ultrafiltration rates (4.0 ± 3.2 ml/kg per h for control vs. 7.0 ± 1.9 ml/kg per h for RIPC, $P < 0.05$); nonetheless, Nadir systolic blood pressure remained significantly higher in the RIPC group (102.0 ± 12.7 mm Hg for control vs. 124.6 ± 17.1 mm Hg for RIPC, $P < 0.05$). No significant differences were observed at subsequent timepoints between groups. Intradialytic hypotension incidence did not differ significantly between groups at any timepoint.

DISCUSSION

This pilot study shows that RIPC has potential to reduce HD-induced cardiac injury and is well tolerated in HD patients. A single 4-cycle RIPC application reduces HD-induced RWMAs for up to 48 hours. As HD-induced RWMAs are associated with higher mortality,[2] RIPC may deserve further research to improve clinical outcomes. Preservation of global longitudinal strain at peak-stress HD provides additional preliminary evidence of cardiac protection provided by RIPC. This finding may also be relevant, as a reduction in absolute global longitudinal strain is associated with worse outcomes in HD patients and is an independent predictor of all-cause mortality.[6]

The significant reduction in RWMA observed at intervention in the RIPC group, associated with higher intradialytic systolic blood pressure despite higher ultrafiltration volume and rates, deserves further attention. RIPC may reduce the negative hemodynamic effects of high ultrafiltration rates by maintaining tissue perfusion.

The efficacy of RIPC in preventing ischemia-reperfusion injury is supported by strong preclinical evidence. Initial results from human trials have been promising in many different clinical settings.[7,S1,S2] However, 2 recent major phase III randomized controlled trials, Effect of Remote Ischemic Preconditioning on Clinical Outcomes in Patients Undergoing Coronary Artery Bypass Graft Surgery (ERICCA)[8] and Remote Ischemic Preconditioning for Heart Surgery (RIPHeart),[9] failed to show any beneficial effect of RIPC in cardiac surgery patients. These trials' results may be explained by the fact that more than 90% of the patients in ERICCA and all patients in RIPHeart

received propofol as part of the anesthesia protocol.[S3] Propofol has been shown to blunt the effects of RIPC in multiple experimental studies, possibly by abolishing its humoral-mediated response.[S4,S5]

In a similar fashion, other drugs, older age, and diabetes have been reported to interfere with the humoral pathways of RIPC: these factors may be relevant in the HD population and need to be taken into consideration when designing new trials.[S6–S8]

The intrinsic nature of HD in delivering a predictable, systemic ischemia-reperfusion insult provides an attractive model to study RIPC, dose–response relationships and its effects on other vascular beds. So far, only 2 small randomized controlled trials have explored the effects of RIPC on cardiac injury biomarkers in conventional HD patients.[S9,S10] Park *et al*. observed a progressive reduction in baseline troponin T levels after 28 days (12 HD sessions with as many RIPC treatments).[S9] Post-HD standard echocardiography was performed at baseline and at 28 days, and no significant differences were observed in left ventricular ejection fraction and mass. Bacci *et al*. failed to demonstrate a reduction in baseline troponin I levels after 3 consecutive HD sessions preceded by RIPC.[S10]

The optimal number of ischemia-reperfusion cycles and frequency of RIPC delivery according to the HD schedule have yet to be identified. At present, there is no clearly defined standard dose, as the number of ischemia-reperfusion cycles used for RIPC differs in the literature, with varying results.[S11,S12] We have chosen four 5-minute ischemia-reperfusion cycles, by analogy with earlier trials suggesting benefits in the setting of cardiac surgery and acute myocardial infarction.[7,S1] We also decided on a higher dose, owing to concerns about potential resistance to RIPC, given the high prevalence of diabetes and older age of the chronic HD population. Time commitment and patient compliance should also be taken into account in the definition of an optimal dosing regimen in chronic HD patients.

In our study, there was evidence of an effect lasting up to 48 hours from a single RIPC application. RIPC results in 2 distinct windows of protection—an immediate one and a later one lasting 48–72 hours.[S13]

Study Limitations

The sample size was small, and the level of HD-induced cardiac injury was unequal at screening. Furthermore, we cannot exclude the possibility that the repeated insults of the HD procedure result in a degree of "chronic" preconditioning, to the benefit of the RIPC group. However, the reduction of RWMA across the entire study period suggests that there is an additive effect from a further dose, even assuming that chronic preconditioning has occurred.

Coronary angiography results were not available in most patients, so the extent of coronary artery disease is unknown. However, the predominant pathophysiological process in HD patients appears to be related to coronary microvascular dysfunction, as HD is capable of generating significant cardiac ischemia in patients with demonstrably normal coronary arteries.[1]

Conclusion

This study suggests that a single application of 4 ischemia-reperfusion-cycle RIPC may reduce HD-induced RWMA for up to 48 hours. Further work is needed to define the optimal RIPC dose and the duration of its effects, guiding the design of suitably robust clinical trials.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

Figure S1. CONSORT chart for trial recruitment.

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Collagen IV Gene Mutations in Adults With Bilateral Renal Cysts and CKD

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Bilateral renal cystic disease presenting as chronic kidney disease (CKD) in adults is most commonly due to autosomal dominant polycystic kidney disease (ADPKD). Genetic investigation of ADPKD cohorts identifies the underlying *PKD1* (77%) or *PKD2* mutation (15%) and up to 8% of cases remain as "no PKD mutation detected" (NMD).[1] Molecular characterization of NMD-ADPKD cases by genome level sequencing such as whole exome sequencing (WES) may identify individuals misclassified as ADPKD based on clinical and imaging criteria. For example, HANAC (*h*ereditary *a*ngiopathy, *n*ephropathy, *a*neurysms, muscle *c*ramps) syndrome caused by heterozygous *COL4A1* mutations can cause bilateral kidney cysts that may phenocopy ADPKD.[2–4]

Thin glomerular basement membrane (TBM) disease usually presents with persistent often familial microscopic hematuria with or without CKD progression. A few reports mention the finding of kidney cysts in patients with TBM disease but this association remains largely underrecognized.[5,6] Because TBM disease is attributed to heterozygous mutations in *COL4A3* and *COL4A4*,[5] we hypothesize that these cysts may be an additional incompletely penetrant consequence of a pathogenic mutation in these type IV collagen proteins. Heterozygous mutations in *COL4A3* or *COL4A4* may thus explain the presence of kidney cysts not due to ADPKD. We report the WES-based genetic investigation identifying type IV collagen mutations in patients with bilateral kidney cysts who were either NMD-ADPKD or were known to carry a diagnosis of TBM disease. A causal effect of these type IV collagen mutations or their modifier role in the progression of a cystic kidney phenotype remains to be established.

RESULTS

We performed WES on 18 patients with findings of multiple bilateral kidney cysts that were either classified as NMD-ADPKD (13 patients) or carried a diagnosis of TBM disease (5 patients). The 13 patients with NMD-ADPKD were part of National Institutes of Health–sponsored longitudinal ADPKD studies and were known to be negative for underlying mutations in *PKD1* or *PKD2* (Supplementary Methods[S1–S17]). Rare variants with an ethnicity-specific population minor allele frequency cutoff of 0.01% for heterozygous variants and 0.1% for recessive variants