Remote Ischemic Preconditioning Living Donor Renal Transplant

Study Protocol & Statistical Analysis Plan

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Remote Ischemic Preconditioning Study / Renal Transplant

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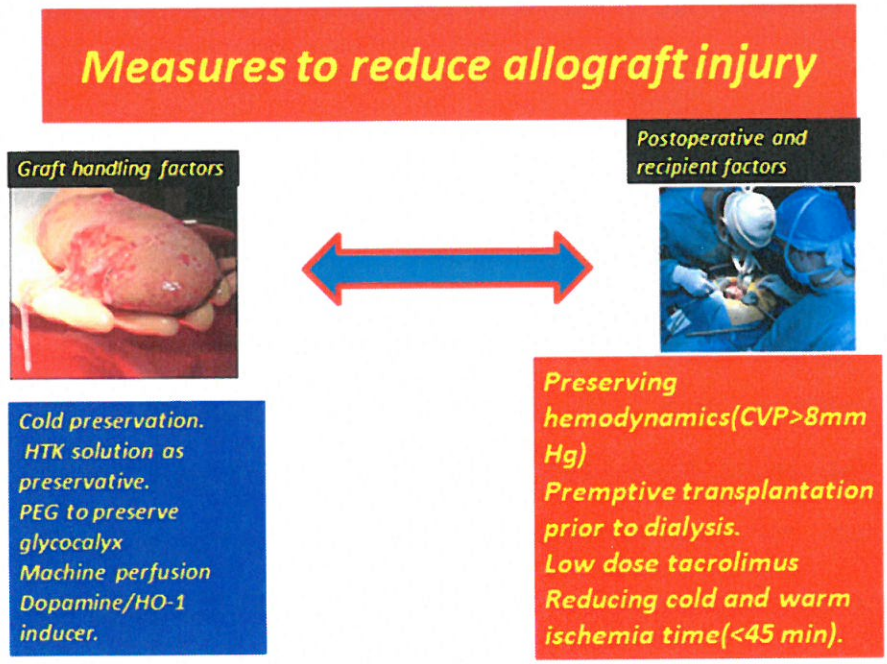
Specific aim:

Remote ischemic preconditioning (RIPC), a phenotype of ischemic preconditioning, has been found to provide protection from I/R in humans. Several proof of concept clinical trials have shown that transient ischemia reperfusion of lower extremities confers protection to kidneys in patients undergoing endovascular or open surgical repair of an abdominal aortic aneurysm and in patients undergoing cardiac surgery. Furthermore, a randomized pilot clinical trial demonstrated that application of RIPC in high risk patients with kidney dysfunction undergoing coronary angioplasty reduced the incidence of procedure related contrast media induced AKI. To date the efficacy of RIPC in protecting renal allografts from I/R injury during renal transplantation has not been systematically investigated. **In this current proof of concept proposal we will test the central hypothesis that application of RIPC in donors and recipients during live donor kidney transplantation will reduce the severity of graft injury and improve outcome. We propose the following specific aim: To examine the effect of RIPC on clinical and biochemical markers of graft function in patients undergoing live donor kidney transplantation.**

In this study, patients undergoing live donor kidney transplantation will be allocated to the control group or RIPC group. Before allograft implantation, RIPC will be accomplished both in the donor and the recipient by inducing intermittent extremity ischemia through four five cycles of oscillometric blood pressure cuff inflation. The monitored clinical end points will include total urine output following kidney reperfusion over three days, plasma creatinine declination over five days, initiation of dialysis, and development of graft injury. Magnitude of graft injury will be measured using biochemical markers, such as, plasma and urinary concentration of neutrophil gelatinase associated lipocalin (NGAL), IL-18, and KIM-1. By rejecting our null hypothesis, RIPC may serve as a safe, cost-effective protective strategy to prevent allograft injury in the clinical setting of live donor kidney transplantation.

2.1 Background

Kidney transplant is the ultimate treatment for end stage renal disease. It improves the quality of life and survival of the patient. Acute kidney injury (AKI) caused by ischemia reperfusion occurs to some extent in cadaveric renal allografts , invariably in the non-beating heart setting and in some live donor transplants leading to varying degrees of delayed graft function (DGF). DGF due to AKI is defined as the need for dialysis in the first week after transplantation and complicates 4-10% of live donor and 5-50% of cadaveric kidney transplants. [i] Several lines of experimental evidence suggest that ischemia reperfusion injury (I/R) plays a critical role in the pathogenesis of DGF. The kidney with its high adenosine triphosphate (ATP) consumption and complex vascular architecture is particularly vulnerable to I/R injury. A variety of therapeutic measures have been implemented to decrease the incidence of DGF. [ii] These measures include maintenance of normovolemia, avoidance of arterial hypotension, and avoidance of nephrotoxic drugs in the recipient. Minimizing cold and warm ischemia time have also been advocated to minimize graft injury; however, these measures have not significantly decreased the rate of occurrence of DGF after kidney transplantation. [iii],[iv] Given the increasing demand for kidney allografts, new diagnostic and therapeutic measures are needed to reduce AKI and DGF following kidney transplant and to improve outcome. [v],[vi],



(Fig#1) STRATEGIES TO MINIMIZE ALLOGRAFT INJURY DURING KIDNEY TRANSPLANTATION

2.2.1 Remote Ischemic Preconditioning (RIPC):

Remote ischemic preconditioning (RIPC) represents a novel therapeutic strategy for limiting I/R injury by applying non-lethal episodes of ischemia and reperfusion to an organ or tissue distant from the target organ. [vii,viii] Przyklenk et al demonstrated brief ischemic episodes of the left circumflex coronary artery significantly reduced myocardial infarct size following sustained occlusion of the left anterior descending coronary artery. [ix] In subsequent experimental studies, short periods of bowel or renal ischemia conferred protection to the myocardium known as remote inter-organ conditioning. [x,xi,xii] The discovery that transient ischemia and reperfusion of extremities could also elicit remote ischemic conditioning facilitated translation of this endogenous cardio-protective phenomenon into the clinical setting. Indeed, there is now clinical evidence suggesting this novel form of protection can be achieved by simple inflation and deflation of a blood pressure cuff around the arm. [xiii,xiv] Several clinical trials examining RIPC in the

setting of percutaneous coronary intervention and coronary artery bypass grafting have shown reduction in myocardial injury. [xv ,xvi, xvii]

2.2.2 Ischemic preconditioning: Underlying mechanisms

The precise mechanism by which brief periods of ischemia reperfusion of remote site confers protection to distant organs is not evident at this time. According to Hausenloy and Lim etal, three main mechanisms –humoral, neural, and systemic –hypothetically interact with each other to confer target organ protection from a remote site. [xviii,xix] It has been postulated that remote preconditioning result in activation of pro-survival kinase elements of the reperfusion injury salvage kinase (RISK) cascade. The RISK pathway seems important in mediating the protective effects of different pre- conditioning procedures. Activation of some RISK elements in local ischemic preconditioning -P13K, Akt, e-NOS, c GMP, PKG pathways- result in opening of mitochondrial potassium sensitive ATP channels. This in turn leads to reactive oxygen species (ROS) production and potentiation of survival kinase activation. [xx]

2.2.3 Ischemic preconditioning in the animal kidney model

In a recent meta-analysis, Wever et al examined the efficacy of RIPC in renal ischemia reperfusion injury. [xxi] Three clinical outcome measures were assessed: serum creatinine, serum BUN, and histologic renal damage. All three outcome measures improved by the application of ischemic preconditioning; however, IPC was more effective when applied twenty four hours before the index ischemia, especially in regards to serum BUN and renal histology. Moreover, IPC was more protective in males than in females.[xxii] Some inter-species difference was also observed with greater IPC efficacy in mice models as opposed to rat models.[xxiii] The translation of animal data to the clinical arena is limited because of heterogeneous experimental design and the use of multiple animal species. Serum creatinine was used in multiple studies as a measure of renal function; however, rodent serum creatinine as a measure of renal function is significantly limited by increased tubular creatinine excretion on creatinine in mice. [xxiv]

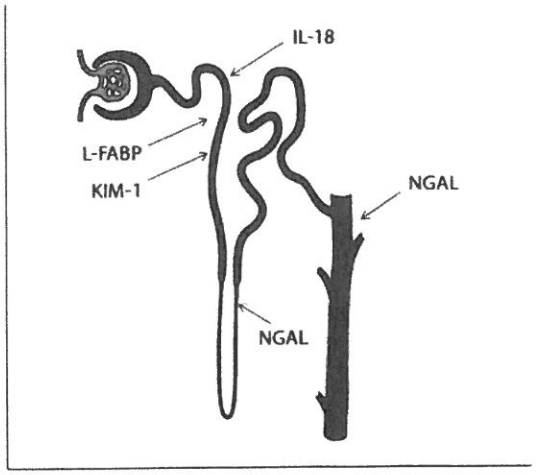
2.2.4 Clinical trials demonstrating protective effect of RIPC on the kidney

Several proof of concept studies have examined protective effects of RIPC in patients undergoing vascular surgery, cardiac surgery, and percutaneous coronary angiography receiving contrast media.[xxv,xxvi,xxvii ,xxviii, xxix, xxx] Two experimental studies showed patients undergoing elective abdominal aortic aneurysm surgery with intermittent cross clamping of the common iliac artery for ten minutes resulted in less postoperative myocardial injury, myocardial infarction, and renal impairment. [xxxi] Venugopal etal in a retrospective secondary analysis of two RIPC randomized placebo controlled trials found a statistically significant difference in AKI NETWORK staging in patients undergoing coronary artery bypass graft surgery. [xxxii] Incidences of AKI stages 1, 2, 3 were 25%, 0%, and 0% in the control group and 3%, 8% and 0% in the RIPC group, respectively. This study had several limitations including a highly selective sample population. Patients with renal disease were excluded from original studies, and diabetic patients were excluded from the analysis. Patients at higher risk of AKI, who could potentially benefit from RIPC, were not represented in this study. The study also had limitations inherent to any retrospective analysis. Finally, AKI incidence was not assessed by biomarkers but rather relied on creatinine measurements that can potentially be influenced by extracellular fluid volume distribution and perfusion pressure changes associated with cardiopulmonary bypass (CPB). In a recent double-blinded study, Er et al examined the potential benefit of RIPC on AKI development in patients with impaired renal function receiving contrast medium for elective coronary angiography.[xxxiii] Half of the patients received transient non-lethal repetitive upper extremity ischemia. The primary endpoint was development of contrast-induced nephropathy (CIN) defined as an increase in serum creatinine >25% or >0.5/D L above baseline at forty-eight hours after contrast-medium exposure. RIPC resulted in protection against AKI with CIN occurring in 40% of the control group and 12% of the RIPC group, respectively. Levels of urine NGAL at twenty-four and forty-eight hours in both groups with greater increases measured in the control group. There was also a greater increase in serum cystatin–c in the control group at twenty-four and forty-eight hours after contrast administration. Lower levels of serum cystatin-c and urine NGAL were consistent with preserved glomerular filtration rate (GFR) and attenuated kidney injury.

2.3 The role of biomarkers in assessment of Graft Injury/Acute Kidney Injury

Reliance on creatinine for the assessment of graft injury is inappropriate because creatinine is a measure of filtration function rather than true injury. Furthermore, serum creatinine is influenced by non-renal factors such as body weight, muscle mass, protein intake, and extracellular fluid volume. Secretion accounts for 10-40% of creatinine clearance, which could mask decreases in creatinine clearance. Most importantly, elevation in serum creatinine shows significant lag time after kidney injury.[xxxiv]

Multiple novel biomarkers are now available with potential to accurately diagnose graft injury in a timely fashion. There is evidence to suggest that biomarker expression precedes the clinical syndrome of renal failure. Therefore, by measuring kidney specific biomarkers we may identify graft injury before DGF develops. [xxxv,xxxvi ,xxxvii] This may provide a unique opportunity for early intervention including aggressive resuscitation of patients with reversible renal hypoperfusion and early initiation of dialysis in patients with severe ATN based upon biomarker evidence. Indeed, the time delay between graft injury and loss of graft function resulting in serum creatinine elevation may explain the high morbidity and mortality associated with allograft failure. NGAL, IL-18, and KIM-1 have been measured in urine for early diagnosis of graft injury and as markers of graft recovery and function. [xxxviii,xxxix, xl, xli, xlii]



2.3.1 Neutrophil gelatinase associated lipocalin (NGAL)

Neutrophil gelatinase associated lipocalin (NGAL) is normally secreted by renal tubular cells, lymphocytes, and cardiomyocytes in response to cytosolic or pericellular catalytic iron liberated during ischemic or toxic injury. NGAL released in blood is freely filtered by the glomerulus; however, most NGAL is reabsorbed in the proximal tubule by megalin-dependent endocytosis. This is supported by experimental evidence that systemic injection of labeled NGAL becomes enriched in the proximal tubule but does not appear in the urine of animals. [xliii] The presence of urine NGAL is a reflection of proximal tubular injury that precludes NGAL reabsorption and/or increased *denovo* NGAL synthesis. Gene expression studies in AKI have demonstrated massive up-regulation of NGAL mRNA in the distal nephron segments specifically in the thick ascending limb loop of Henle and collecting ducts. This increased synthesis of NGAL in the distal nephron segments accounts for most of the NGAL observed in the urine. [xliv,xlv] Mishara etal examined protocol biopsy specimens in thirteen cadaveric and twelve living- related allografts within one hour of reperfusion for NGAL expression by immunochemistry. Staining intensity for NGAL correlated well with cold ischemia time, peak post-transplant serum creatinine, and requirement of dialysis. [xlvi] In another study of 33 cases, urine NGAL on the day of transplant surgery was a predictor of DGF with an AUC of 0.9 (Needs reference). Importantly, serum creatinine level peaked after two to four days. Kusaka etal found urine NGAL as a good predictor of recovery from DGF (Needs Reference). In addition, clinical studies suggest that measurement of urine NGAL has several advantages: urine NGAL permits prediction of DGF with adequate urine output and decreasing creatinine, low urine NGAL levels in oliguric patients after kidney transplantation suggests reversible hypovolemia and hypoperfusion, urine NGAL predicts prolonged DGF, and urine NGAL provides a simple non-invasive test to quantify recovery from kidney injury. If urine NGAL reaches a normal value, subsequent rises suggest new kidney injury. [xlvii] Indeed, emerging evidence suggests urine NGAL is also a marker of chronic kidney disease severity. Urine NGAL levels are elevated in CKD and significantly correlate with serum creatinine, GFR and proteinuria. Levels of urine NGAL in these situations are significantly blunted compared with values measured in AKI. [xlviii, xlix, l]

NGAL limitations include increased sensitivity and specificity in homogeneous patient populations with temporally predictable forms of AKI.

2.3.2 Interleukin-18 (IL-18)

Interleukin-18 (IL-18), a proinflammatory cytokine originating from proximal tubular epithelial cells, is one injury mediator involved in ATN experimental models. Early expression of IL-18 in ischemic, septic and other clinical settings allows for early AKI detection. [li, lii] Parikh et al reported IL-18 elevations in AKI patients but not in chronic kidney disease, urinary tract infection, nephritic syndrome, or pre-renal azotemia. [liii] In critical ARDS patients, urine IL-18 was found to be significantly elevated at twenty-four and forty-eight hours. These values correlated well with patient mortality in ARDS populations; however, IL-18 has inconsistently predicted AKI in post-CPB surgery patients. [liv],[lv] A recent study by Hall etal demonstrates that urine NGAL and IL-18 are accurate predictors of need for dialysis within the first week of kidney transplantation and 3-month recovery of graft function. (needs reference)

2.3.3 Kidney Injury Molecule-1 (KIM-1)

KIM-1 is a trans membrane protein expressed in the proximal convoluted tubule upregulated by toxemic and ischemic insults. [lvi] A cross sectional study by Han et al demonstrated KIM-1 elevations in AKI patients. [lvii] A comparative analysis of biomarkers in post-CPB patients at two hours found KIM-1 to be a better predictor of AKI as compared to NGAL,IL-18 and cystatin C ,but it loses this differentiation at twenty-four and forty-eight hours. [lviii, lix] Coca etal concluded that KIM-1 cannot predict AKI but is useful in diagnosing established AKI. The combination of KIM-1, NGAL and IL-18 has been found to be a better predictor of AKI. [lx] A recent study by Malyszko etal showed a strong correlation between KIM-1, serum creatinine, and estimated GFR in renal allograft recipients. (needs reference)

3. Significance

Ischemia –reperfusion (I/R) is inevitable when the kidney allograft is connected with the recipient vascular system initiating a cascade of cellular and molecular events. High energy consumption and a complex vascular pattern make the kidney particularly vulnerable to I/R injury. [lxi, lxii] Main consequences of renal I/R injury are allograft injury resulting in primary non-function (PNF) and delayed graft function (DGF). Currently there are a number of strategies pre and post transplantation to attenuate or minimize I/R injury and to improve outcome (See figure# 1). These measures have had minimal impact on the rate of occurrence of DGF. [lxiii] New approaches are needed to improve recovery and preserve kidney graft function. [lxiv, lxv]

4. Experimental Design

This will be a randomized controlled trial to evaluate the influence of RIPC administered intraoperatively on the magnitude of allograft injury in patients undergoing live donor kidney transplantation. The study will be conducted after the institutional board approval and informed consent will be obtained from each patient (both the donor and the recipient). Patients undergoing live-donor kidney transplants >19 years of age will be enrolled in this study. Perioperative immunosuppressive agents will be administered according to the institution protocol, and all patients will undergo general endotracheal anesthesia.

4.1 Randomization and intervention

Patients undergoing live-donor renal transplant will be randomized to the RIPC or the control group in a 1:1 ratio using manually shuffled blocks of ten generated by the study coordinator. The allocation sequence will be concealed until the study group is assigned. The study coordinator will open the opaque sealed envelope containing the patients' group before the patient arrives at the operating room. For patients randomized to RIPC, a tourniquet (Stryker, Kalamazoo MI) will be placed on the lower or upper extremity avoiding the intraoperative blood pressure cuff and any functioning dialysis-access fistula. The

tourniquet will be inflated 3 times to a pressure of 200mmHg for five minutes with five minutes of reperfusion between inflations. The RIPC regimen will be administered while patient monitors, intravascular catheters and indwelling bladder catheters are being placed in order not to affect the time from induction of anesthesia to initial skin incision compared with the control group. Tourniquet cuff pressures of 20 mmHg applied to control group patients will provide some level of compression without ischemia. All patients will receive standard perioperative and postoperative care as directed by the anesthesiology and surgical staff with all receiving isoflurane as part of the anesthetic technique.

4.2 Measurements

For each patient, approximately ten milliliters of urine will be collected at zero, six, and twelve hours after transplantation, as well as, postoperative day one and two. Time zero will be defined as the time surgery is completed per documentation in the anesthesia flow sheet. Each sample will be placed in a centrifuge at 3300 RPM for three minutes. The urinary supernatant will be frozen at -80 Celsius and assayed in batches for NGAL and IL-18. Both urine NGAL and IL-18 will be quantified using ELISA testing. Serum and urine creatinine concentrations will be measured by the Jaffe assay using the P-module of a Cobas analyzer (Roche diagnostics, Indianapolis, IN) and the Beckman creatinine analyzer system (Beckman Instruments Inc., Fullerton CA).

4.3 Outcomes

The degree of allograft injury will be assessed by the levels of urinary biomarkers NGAL, IL-18, and KIM-1. The primary endpoints will be the level of graft function in the early post transplantation period categorized as follows: delayed graft function (DGF) (defined as the need for dialysis within the first week of transplantation), slow graft function (SGF) (defined as <40% decrease in serum creatinine by POD 3), and immediate graft function (IGF) (defined as >40% reduction in serum creatinine by POD3). Incidence of allograft primary malfunction will be based on urine output during the first three days after

transplantation, the one month serum creatinine concentration, one month estimated GFR calculated according to diet modification. Additional secondary endpoints include 90 day mortality, length of ICU stay, and length of hospital stay.

4.4 Statistical Analysis

The sample size calculation is based on a projected difference of NGAL levels between the two study arms. Hall et al reported a mean NGAL level of 49 mg/mL (SD = 37 mg/mL) for a group of patients that had immediate graph function and a mean NGAL level of 248 mg/mL in a group of patients with slow graft function. (which Hall refernce is this) Based on these data we consider a conservative estimate of a mean difference between study groups to be 35 mg/mL NGAL. Using these assumptions, an alpha level of 0.05 and 80% power, we calculate a sample size of n= 19 per study group. Variables such as biomarkers, blood pressure, creatinine, and urine output that are expressed at multiple time points will be compared between the groups using repeated measures of ANOVA. Categorical variables will be expressed as percentages and will be compared using chi-squared analysis. A p value <0.05 will be considered to be a statistically significant difference.

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Ref #2 (old ref #11)

Ref#3Rabb,H(2012) The promise of immune cell therapy for acute kidney injury.journal of clinical investigation122(11):3852-54(old ref #38)

Ref#4 Perico P (2004) Delayed graft function in kidney transplantation Lancet 364(9447):1814-1827

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Ref#6 (OLD REF#6)

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