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Ischemic conditioning in organ transplantation

Kristin Veighey^{1,2}

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Abstract: Organ transplantation comes with the inevitability of ischemia reperfusion (IR) injury and the potential for resultant graft dysfunction and acute rejection. Such episodes may limit both graft and patient survival. Ischemic conditioning is a simple, safe and virtually cost-neutral intervention that induces a systemic protective reflex which may protect against IR injury. This review summarizes the current evidence for an effect of ischemic conditioning strategies in the clinical setting of organ transplantation.

Key words: ischemia reperfusion injury, transplantation, ischemic conditioning

Introduction

Loss of perfusion to an organ or tissue leads to cellular injury, which is compounded by an additional insult at reperfusion. This composite injury is termed ischemia-reperfusion (IR) injury, and it limits the amount of tissue which can be salvaged when the blood supply is restored. Organ transplantation presents one of the most complete forms of IR injury, as the organ is completely disrupted from its blood supply in the donor prior to reperfusion in the recipient. Reducing this injury can protect the transplanted organ from damage which may reduce its longevity.

Ischemic conditioning is a phenomenon whereby brief periods of non-lethal ischemia induce an innate systemic protective reflex against later more significant periods of ischemia. The phenomenon was first described in the 1980s when Murry and colleagues demonstrated that brief periods of circumflex artery occlusion in dogs could protect against subsequent more prolonged ischemia (Murry et al., 1986). The magnitude of the protection observed was greater than for any other intervention; moreover, it was reproducible and has since been observed in every animal species in which it has been investigated.

Strategies to minimize IR injury during transplantation have been developed, including cooling by specialized perfusate prior to storage on ice, and the minimization of both cold and warm ischemic times (Guibert et al., 2011). However, these interventions are arguably maximized within the confines of health care delivery systems and the inevitability that some organs will have to travel some distance between donor and recipient because of tissue matching. Therefore, an intervention

that can protect the donor organ from IR injury during transfer from donor to recipient could improve function and prolong transplant life.

Furthermore, there is a mismatch between supply and demand for organs for transplantation, necessitating the consideration of older and more marginal donors (Alexander and Zola, 1996; Foley and Chari, 2007). Minimizing IR injury for these organs, which are at greater risk from its effects, might allow greater numbers of such organs to be transplanted, thus expanding the donor pool.

Organ transplantation is a prime clinical setting to benefit from a conditioning intervention, and as the injury is predictable, an ideal setting for clinical trials. In addition, as the ischemic injury is direct and complete, compared to a partial insult or bystander ischemic insult, it arguably represents a more “pure” IR injury to investigate.

Ischemia reperfusion injury in transplantation

The detailed pathophysiology of IR injury in each organ when transplanted is beyond the scope of this review. It is well documented that organ IR injury during transplantation contributes to inflammation and subsequent allograft fibrosis in all transplanted organ types (Foley and Chari, 2007; Ponticelli, 2014; Zhao et al., 2018). In addition, there is evidence that IR injury stimulates the innate immune system through interaction with toll-like receptors (TLRs), in turn contributing to an increased risk of acute rejection (Zhai et al., 2013; Ponticelli, 2014; Salvadori et al., 2015).

Types of ischemic conditioning

Local ischemic conditioning is the phenomenon whereby

¹Wessex Kidney Centre, Queen Alexandra Hospital, Portsmouth, UK; ²University Hospital Southampton NHS Foundation Trust, Southampton, UK.

Correspondence should be addressed to Dr. Kristin Veighey (kristin.veighey@uhs.nhs.uk).

ischemic conditioning is applied by direct occlusion of the blood supply to an organ, usually by use of arterial cross-clamping. Although arguably this has the potential for maximum conditioning effect, there is concern regarding organ or tissue damage, which is perhaps especially relevant in the field of transplantation.

The protection can also be induced remotely, by occlusion of the blood supply to a separate organ, with the protective effects then disseminated systemically to the area at subsequent risk. The discovery that this *remote ischemic conditioning* could also be effective when applied non-invasively, by use of a limb blood pressure cuff, led to a huge increase in interest in the potential of this intervention (Kharbanda et al., 2002). Thousands of patients have now participated in studies using this method, without any reported serious or prolonged adverse events.

Ischemic conditioning can be performed prior to (pre-conditioning, IPC), during (per-conditioning, IPerC), or after (post-conditioning, IPostC) the IR injury, and can be applied directly or remotely (i.e., RIPC, RIPerC, RIPostC). All of these conditioning strategies have been trialed in various forms in the setting of organ transplantation. While it seems probable that the optimal conditioning strategy will depend not only on the specific organ or tissue at risk, but also on the clinical setting in which the IR injury occurs, there is no current evidence to enable tailoring of the conditioning protocol to these clinical variables.

Mechanism of protection

The mechanism by which the protective effect is conducted systemically from the conditioned organ or tissue to the target organ has still not been fully elucidated. Animal models implicate local factors (Goto et al., 1995; Dana et al., 1998), circulating mediators (Lim et al., 2010; Mastitskaya et al., 2012), and neuronal pathways (Shimizu et al., 2009; Breivik et al., 2011; Mastitskaya et al., 2016), and it is likely that these act collaboratively to induce protection. More recently, the hypoxia-inducible factor pathway and its response to tissue hypoxia has also been implicated; however, the relationship between this and other mediators is again not fully understood (Heyman et al., 2016).

This cascade of mediators activates pro-survival kinase pathways (Hausenloy and Yellon, 2004; Lecour, 2009), which in turn converge upon the mitochondria, acting to stabilize the mitochondrial permeability transition pore (mPTP) in the face of IR injury, thus preventing mitochondrial ion influx and ultimately cell death.

There are two so-called “windows of protection” of ischemic conditioning that have been described in both animal and human models. The first window begins at the time of the conditioning stimulus and lasts for around 4 hours, with a second window of protection returning at around 24 hours and persisting for 48-72 hours (Hausenloy and Yellon, 2010; Yoon et al., 2015).

Types of organ transplantation

There are differing conditions under which organs are harvested for transplantation, and it is useful to first consider these in order to understand potential uses and limitations of ischemic conditioning in this patient group.

When the blood supply to an organ is disrupted during organ harvest, a period of ischemia commences. *Warm ischemia* is defined as the period of time between clamping of an organ until it undergoes cold perfusion prior to being placed into ice, and the period of time from when the organ is removed from ice until it is reperfused in the recipient (Halazun et al., 2007). *Cold ischemia* is defined as the period that the organ spends in ice.

Historically, the latter was thought to be more clinically relevant, contributing to delayed graft function (DGF) and

subsequently acute rejection (ACR) (Stahl et al., 2008; Ponticelli, 2015). However, there is increasing evidence that even small increases in warm ischemic time (of the order of minutes) can contribute to organ dysfunction and poorer short- and longer-term outcomes, perhaps especially in kidney transplantation (Hellegering et al., 2012; Tennankore et al., 2016). Therefore, any benefits of ischemic conditioning should be expected to extend to living donation.

Living donation

Donation of organs from living donors can be performed for a single kidney or single liver lobe.

In kidney transplantation, living donation may be either *directed altruistic*, from a known relative or friend, or *non-directed altruistic*, in which individuals donate their organs to an unknown individual, allocated according to matching criteria. *Paired pooled donations* may also occur, where a relative donates a kidney into a donor pool, and their relative receives a better matched kidney from another patient within the donor pool. *Non-directed altruistic donor chains* may also occur (Human Tissue Authority, 2018).

In liver transplantation, a single lobe can be removed from a donor and transplanted into a recipient. This is more common in the case of pediatric recipients.

Living donation facilitates the shortest possible ischemic time. Where theater capacity allows, organs may only undergo warm rather than cold ischemia, with organs harvested, prepared, and then transplanted into the recipient in an adjacent theater.

Donation after circulatory death (DCD)

This has previously been referred to as donation after cardiac death or non-heart-beating organ donation. There are two situations: *controlled DCD*, where there is a planned withdrawal of life-sustaining treatment in a patient who is deemed to have a poor prognosis; and *uncontrolled DCD*, which follows a spontaneous cardiac arrest from which the patient does not recover or is deemed inappropriate for resuscitation (ODT Clinical - NHS Blood and Transplant, 2018a). The loss of circulating volume that occurs following cardiac arrest, along with a subsequent decreased organ perfusion that results, may contribute to subsequent transplant organ dysfunction. Equally, it could be argued that this period may induce an ischemic conditioning reflex which cannot be quantified or standardized.

Donation after brainstem death (DBD)

This represents patients who are ventilated within the intensive care setting and who have sustained an irreversible neurological injury such that they lack the capacity for consciousness or spontaneous ventilation (ODT Clinical - NHS Blood and Transplant, 2018b). Such patients continue to have functional circulatory systems and perfused organs (albeit some may be supported by inotropes), and therefore organs can be harvested in theater, under conditions of relative stability. Although these organs have arguably sustained less IR injury than DCD organs, the cause of their death may be complex, and their organs may have sustained various insults which may contribute to a conditioning response which again cannot be quantified.

Logistical considerations

In cases of deceased organ donation, organs are retrieved from a very heterogeneous group of donors with differing clinical situations and causes of death. The impact of these clinical precludes to organ harvest may be difficult to quantify, and in a clinical trial setting it is difficult to ensure that the donor population under investigation is truly comparable without the use of a very large (and therefore restrictive) sample size.

This is compounded by the logistical and ethical considerations of having teams in one hospital performing conditioning of donors whose organs might be transplanted

Table 1. Clinical trials of ischemic conditioning in liver transplantation.

Year	n	Authors	Type of IC	Conditioning protocol	Donor/ Recipient	Results
2004	15	Bombuy et al.	IPC	Inflow clamping 5 minutes at start and end of surgery/10 minutes at start and at 1 and 2 hours during resection	Donor	No difference in any outcome.
2005	62	Koneru et al.	IPC	5 minutes of hilar cross-clamping	Donor	No difference in any outcome.
2006	47	Cescon et al.	IPC	10 minutes arterial clamp application followed by 15 minutes reperfusion	Donor	Reduction in ALT and AST but no clinical benefit.
2006	23	Jassem et al.	IPC	10 minutes hepatic pedicle clamping	Donor	Significant reduction in AST in preconditioned group with reduction in length of critical care stay.
2007	60	Amador et al.	IPC	10 minutes hilar cross-clamping	Donor	Reduction in: biochemical markers of liver cell function, apoptosis, AST, need for reoperation, and incidence of primary non-function in IPC group.
2007	101	Koneru et al.	IPC	10 minutes hilar artery occlusion	Donor	Increased aminotransferases in IPC group. No significant differences in clinical endpoints.
2010	44	Andreani et al.	IPC	10 minutes right pedicle clamping	Donor	No differences.
2015	100	Ricca et al.	IPostC	Three x 1-minute arterial occlusions with 1-minute reperfusion in between	Donor	No difference in clinical outcomes; reduced IR on histology.
2014b	78	Kim et al.	RIPostC	4 cycles of upper-limb cuff inflation and deflation (5 minutes each)	Recipient	Significant decrease in AKI, no differences in liver function or other clinical outcomes.
2017	45	Robertson	RIPC	3 x 5-minute cycles of lower-limb cuff inflation and deflation	Recipient	Underpowered to detect a clinical effect but RIPC feasible and safe.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; AKI, acute kidney injury; IPC, ischemic preconditioning; IPerC, remote ischemic preconditioning; (R)IPostC, (remote) ischemic postconditioning; RIPC, remote ischemic preconditioning.

in a different hospital, possibly even in a different country (such as in Europe) where the recipient would also then need to give consent to enter the study. There may be differences in the transplant protocols between these centers, rendering standardization, for example of immunosuppression regimes, difficult.

It is also not yet known whether it is the donor (and therefore the donor organ) that needs to undergo conditioning, or the recipient. It would seem logical that preconditioning the donor organ prior to both ischemia and subsequent reperfusion would have the largest potential for clinical effect. Yet this scenario, outside of living donation, is logistically challenging for the reasons described above.

Ischemic conditioning in the setting of solid organ transplantation

The majority of the literature in this area exists in the fields of liver and kidney transplantation. A summary is presented below. Clinical trials in liver transplantation are listed in Table 1, and those in kidney transplantation in Table 2.

Perhaps what is most evident from these summaries is the heterogeneity of the ischemic conditioning approaches. This leads to difficulty in comparing the studies, and an inability to draw clear conclusions regarding the potential for benefit.

In all areas, there is a wealth of animal research to substantiate the effects of ischemic conditioning in organ or cellular protection. However, in the main this has not been clearly translated into human clinical studies.

Liver transplantation

In 2004, Bombuy and colleagues reported a pilot study of the use of IPC in deceased donor liver transplantation. They recruited 15 patients and randomized them to either control, IPC by inflow clamping for 5 minutes at the beginning and end of the operation, or IPC by inflow clamping for 10 minutes at the start, at one and two hours during resection. They reported no difference in outcomes between the groups (liver biopsy for adenosine levels, hepatic blood flow in the recipient at 1 hour

after reperfusion, liver biopsy at 2 hours post reperfusion, daily liver function or clinical evaluation) (Bombuy et al., 2004).

Following this, in 2005, Koneru and colleagues went on to describe a study in deceased donor liver transplants whereby 62 donors were randomized to receive either 5 minutes of IPC induced by hilar cross-clamping performed shortly following laparotomy, or none. They described no safety issues but equally no effect of IPC (Koneru et al., 2005).

In 2006, Cescon et al. reported a pilot study in whole liver transplantation from deceased donors. Forty-seven patients were randomized to receive either IPC (10 minutes of arterial clamp application followed by 15 minutes of reperfusion) or none. They demonstrated a reduction in median aspartate aminotransferase (AST) levels in the IPC group on postoperative days 1 and 2, and lower alanine aminotransferase (ALT) levels in the IPC group on postoperative days 1, 2, and 3. They concluded that there were “no clinical benefits” in the absence of reduced rates of primary non-function/retransplantation. However, of note, overall 1-year patient and graft survival rates were 100% and 91% in the IPC group, and 92% and 82% in the control group (Cescon et al., 2006).

In the same year, Jassem and colleagues described a small study in deceased donors, in which 23 patients were randomized to IPC by 10 minutes of hepatic pedicle clamping, followed by reperfusion. They observed significantly lower levels of AST (240±98 IU/L vs. 382±163 IU/L; $P>0.016$) and lactate (0.81±0.07 mmol/L vs. 1.58±0.9 mmol/L; $P>0.018$) 24 hr following transplantation in those who had received preconditioning. In addition, the recipients of livers which had been preconditioned spent a significantly shorter time in the intensive care unit following transplantation (1 vs. 2.8±1.6 days; $P=0.0008$). Increases in neutrophil infiltration (43%; $P=0.022$) and CD41 deposition (36%; $P=0.042$) were observed in the control group (Jassem et al., 2006).

The following year, Amador et al. published another study of IPC in deceased donor liver transplantation. In this study, 60 donors were randomized to receive either preconditioning by

Table 2: Clinical trials of ischemic conditioning in kidney transplantation.

Year	n	Authors	Type of IC	Conditioning protocol	Donor/ recipient	Results
2005	20	Loukogeorgakis et al.	RIPC 24 hours pre-transplant	Arm 4 x 5 min	Donor & recipient (same allocation)	Improved MDRD eGFR over 60 months follow up. Reduced urinary biomarkers (RBP) of AKI in RIPC group. Improvement in time for creatinine to fall by half in RIPC group.
2013	60	Chen et al.	RIPC	Leg 3 x 5 min	Donor/recipient/bot h/neither	No difference in urine volumes, urinary biomarkers (MDA, NAG, NGAL, RBP, SOD), or creatinine.
2015	406 pairs	MacAllister et al.	RIPC - Early (immediately pre-transplant)/Late (24 hours pre-transplant)/Dual RIPC	Arm 4 x 5 min	Donor & recipient (same allocation)	No effect on iohexol GFR at 1 year. Significant effect on CKD-EPI eGFR at 3 and 12 months.
2016	225	Krogstrup et al.	RIPC	Leg 4 x 5 min	Recipient	No effect on time for creatinine to fall by ½.
2013	20 (plus 40 historical controls)	Van den Akker et al.	IPostC	2 cycles external iliac artery cross-clamping 1 min, reperfusion 1 min	Recipient	No effect on creatinine or DGF at 3 months.
2015	80	Nicholson et al.	IPerC	Leg cuff 10 min to 200mmHg/SBP plus 25mmHg	Recipient	No effect on MDRD eGFR at 1 or 3 months.
2014a	60	Kim et al.	RIPostC	Arm 3 x 5 min	Recipient	Reduction in time for creatinine to fall by half, no effect on graft function thereafter or complication rates.
2014	48	Wu et al.	RIPC	External iliac artery clamping 3 x 5 minutes	Donor	Improved eGFR and lower NGAL in RIPC group.
2017	321	Bongu et al.	RIPC	Leg 4 x 5 min	Donor	No difference in organs recovered or transplanted. RIPC improved death-censored kidney graft survival.

Abbreviations: AKI, acute kidney injury; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; DCD, donation after cardiac death; DGF, delayed graft function; (e)GFR, (estimated) glomerular filtration rate; IPerC, remote ischemic preconditioning; (R)IPostC, (remote) ischemic postconditioning; MACCE, major adverse cardiac and cerebral event; MDRD, modification of diet in renal disease; MDA, malondialdehyde; NAG, N-acetyl-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; RBP, retinol-binding protein; RIPC, remote ischemic preconditioning.

hilar cross-clamping for 10 minutes immediately prior to organ harvest, or none. They observed that IPC significantly improved biochemical markers of liver cell function (uric acid, hyaluronic acid, and hypoxia-induced factor 1-alpha (HIF-1 α)), and that the degree of apoptosis was significantly lower in the IPC group (assessed via the TUNEL technique and cleavage of caspase-3). IPC significantly improved serum aspartate aminotransferase (AST) levels and reduced the need for reoperation in the postoperative period. Moreover, the incidence of primary non-function (PNF) was lower in the IPC group, although this did not achieve statistical significance (Amador et al., 2007).

A second study by Koneru's group was described in 2007. In this study, 101 deceased donors were randomized to 10 minutes of hepatic hilar artery occlusion early in laparotomy, or none. They concluded that reperfusion injury was increased by IPC, due to the observation that aminotransferases were significantly greater on day 1 and 2 in the IPC group. Other endpoints were similar, although there was a non-significant improvement in patient and graft survival in the IPC group (Koneru et al., 2007).

In terms of living donation, Andreani et al. reported a small study in 2010, where 44 patients were randomized to 10 minutes of right pedicle clamping or none. They observed no difference in terms of length of hospital stay, morbidity/mortality, primary non-function and acute rejection rates, or in post-operative aspartate transaminase or alanine aminotransferase (Andreani et al., 2010).

Studies have also examined the potential effects of IPostC in this setting. In 2015, Ricca and colleagues published the results of a study of 100 patients, in which alternate patients received IPostC, delivered as three 1-minute episodes of arterial occlusion with three 1-minute episodes of reperfusion. Although they did not demonstrate any differences in postoperative liver function or 1-year clinical endpoints (morbidity, mortality and 1-year graft and patient survival), there was a greater tolerance to IR injury on histological examination (Ricca et al., 2015).

In 2014, Kim et al., (2014b) described a study where recipients of living liver transplants were randomized to receive either RIPostC (4 cycles of 5-min upper-arm cuff inflation and deflation, performed immediately after organ reperfusion) or control. They observed a significant decrease ($p=0.006$) in acute kidney injury (AKI) in the postconditioned group. There were no differences in liver graft function or other clinical outcomes (Kim et al., 2014b). IPerC has shown promise in animal models (He et al., 2017), but not as yet been translated into clinical trials in humans.

The Remote Ischemic Preconditioning in Orthotopic Liver Transplantation (RIPCOLT) was a single-center randomized study in which 45 patients were randomized to receive either RIPC, induced by 3 cycles of 5 minutes of lower-limb occlusion using a pneumatic cuff, or a sham procedure (no cuff inflation). This study concluded that RIPC was an acceptable and safe intervention. A slightly higher aspartate transaminase level was reported in those who underwent RIPC, but the study was not powered to look at clinical endpoints (Robertson et al., 2017). A similar study (RIPC-PLDT) is underway in pediatric living-donor transplantation, aiming to recruit 200 patients (ClinicalTrials.gov, 2018).

Kidney transplantation

In 2006, Loukogeorgakis et al. presented a pilot study where a cohort of 20 pediatric living-donor renal transplant recipients and their donors were randomized in a blinded fashion to sham RIPC or RIPC. An upper-limb blood pressure cuff was used to induce 5-minute periods of limb ischemia (3 cycles, applied to both donor and recipient), 24 hours in advance of surgery. RIPC resulted in significant improvement of long-term renal function, in terms of estimated glomerular filtration rate (eGFR). Post-operative excretion of retinol binding protein (RBP) in RIPC patients was significantly reduced compared to controls, and the time for the creatinine to halve was shorter in the RIPC group than in the controls (Loukogeorgakis et al., 2006).

A second randomized controlled study of RIPC in live-donor renal transplantation was subsequently described in a letter to the editor of *Transplantation*. In this study, 60 live-donor kidney transplant recipients and their donors were randomized in pairs to receive either donor RIPC, recipient RIPC or none. The RIPC stimulus was 3 x 5-minute leg cuff inflations to 300mmHg, separated by 5 minutes of reperfusion. In this small study, the authors did not observe any differences in terms of urine volumes, plasma creatinine, AKI biomarkers, length of hospital stay or cost between the three groups. Interestingly, this study is unique in exploring whether it is the donor or recipient (or both) that requires preconditioning (Chen et al., 2013).

The largest study of RIPC in living-donor renal transplantation, Renal Protection Against Ischemia Reperfusion in transplantation (REPAIR), was published in 2015. In this study, 406 donors and their recipients were randomized in pairs in a factorial design to receive either early RIPC (immediately prior to surgery), late RIPC (24 hours prior to surgery), both, or neither (sham procedure). This is the only study to have examined if there is an additive effect of the two windows of preconditioning. RIPC was applied as 4 cycles of upper-arm cuff inflation to systolic blood pressure (SBP) plus 40mmHg for 5 minutes, followed by 5 minutes of cuff deflation. This study demonstrated a trend towards an improvement in formal measured iohexol GFR at 12 months following transplantation in those who received early RIPC; however, this was not significant. Still, there was a significant benefit of early RIPC in improving eGFR at both 3 and 12 months. There was no effect of late RIPC (MacAllister et al., 2015).

In 2014, Wu et al. (2014) reported an effect of RIPC delivered as external iliac artery clamping. This intervention was applied to one kidney of a deceased donor, with the other kidney serving as a control. Each kidney was transplanted into a separate recipient. They observed a higher eGFR and lower neutrophil gelatinase-associated lipocalin (NGAL, a marker of tubular IR injury) in the RIPC group compared to the control group (Wu et al., 2014).

The Remote Ischemic Preconditioning in Neurological Death Organ Donors (RIPNOD) trial recruited 321 neurological death donors to assess the efficacy of four 5-min cycles of RIPC applied immediately following confirmation of brain death and again at harvesting to investigate donor stability, organ quality, organ yield and early post-transplant clinical outcomes. This study did not show an increase in the number of organs which were harvested or transplanted in the preconditioned group. Interestingly, RIPC significantly improved death-censored kidney graft survival (2 years: 96% vs. 88%, $p < 0.01$) and all graft survival ($p = 0.01$). In Cox models, RIPC significantly decreased kidney loss, independent of donor and recipient variables (HR 0.36 [95% CI, 0.16-0.83], $p=0.02$) (Bongu et al., 2017). The associated Remote Ischemic Preconditioning in Abdominal Organ Transplantation (RIPCOT) study has closed to recruitment, and the results are awaited. This study aimed to recruit 580 deceased organ donors and recipients of kidneys, livers and pancreata. Organ donors were randomized to receive either RIPC (leg-cuff inflation on each side for 10 minutes) or no RIPC before organ recovery, performed in the operating room after commencement of surgery. Early postoperative outcomes would then be assessed using markers of organ function and cell injury parameters. Long-term outcomes will be assessed by graft and recipient survival.

Most recently, a study published in late 2017 of 29 renal transplant recipients randomized to receive RIPC by way of 10 minutes of thigh occlusion using a pneumatic tourniquet demonstrated modification of the inflammatory response, but no improvement in short-term kidney function (Zepata-Chavira et al., 2017).

Studies have also investigated the potential benefits of IPerC

and IPostC in kidney transplantation. A randomized controlled trial by Nicholson et al., in which 80 patients undergoing live-donor kidney transplantation were randomized to either RIPerC (4 cycles of leg-cuff inflation to 200mmHg or SBP + 25mmHg for 5 minutes, followed by reperfusion) or a sham procedure (cuff inflation to 25mmHg) performed during ischemia demonstrated no effect of IPerC on kidney function (MDRD eGFR) at 1 or 3 months (Nicholson et al., 2015). A study of RIPerC in deceased donor transplants, CONTEXT, was reported in late 2016 (Krogstrup et al., 2017). This study recruited 225 patients undergoing cadaveric kidney transplantation who were randomized to receive either early RIPC delivered as cycles of 4 x 5-minute leg cuff inflations followed by 5 minutes reperfusion or a sham procedure. The intervention was performed during surgery but prior to reperfusion. The primary endpoint of this study was the time for the baseline creatinine to fall by half following transplantation. An effect on this, or other early outcomes, was not observed in this study. Longer-term outcomes, such as kidney function at 1 year, are awaited (Krogstrup et al., 2017).

A pilot human study in donation after circulatory death (DCD) kidney transplantation recruited recipients to receive IPostC as 3 cycles of clamp release for 1 min followed by re-clamping for 1 min (van der Akker et al., 2014). The control groups consisted of 40 historical controls, and also a paired kidney analysis on the contralateral kidney ($n=11$). The primary outcome was the rate of adverse events, with secondary outcomes of delayed graft function (DGF) and kidney function at 3 months. Of note, donor age and serum creatinine were higher in the IPostC group, and this group experienced more DGF. Also of note, members of the historical control group were younger and had better kidney function than those of the IPostC group. There was no difference in serum creatinine/MDRD eGFR at 3 months between the IPostC and either control group. In addition, one patient had a venous tear which was attributed to the intervention. The authors still concluded that the intervention was safe on the basis that no serious adverse events were observed; however, clearly the incidence of venous damage gives some cause for concern.

Another study randomized 60 recipients of live-donor kidney transplants to receive either RIPostC (3 cycles of upper-limb cuff inflation for 5 minutes, followed by 5 minutes reperfusion) carried out at the time of graft reperfusion, or none. Kidney function (creatinine and eGFR) was assessed 2 hr after surgery, and at 12-hr intervals for 96 hr. Urine output and urine creatinine were assessed until postoperative day 7, and hospital stay and complication rates were compared. The time for the creatinine to reach 50% of its preoperative level was significantly shorter in the preconditioned group [12 (12–24) hr vs. 24 (21–36) hr, $p=0.005$], and the number of patients whose creatinine fell by 50% within 24 hr was also significantly greater in the preconditioned group [$n=26$ (87%) vs. $n=18$ (60%) $p=0.020$]. However, there were no differences in creatinine or eGFR thereafter, incidence of graft dysfunction or complication rates between groups (Kim et al., 2014a).

Cardiac, lung and intestinal transplantation

Animal models have suggested the potential for benefit of preconditioning to improve cardiac function following transplantation (Karck et al., 1996; Konstantinov et al., 2005). However, interestingly, despite the majority of ischemic preconditioning protocols being centered on the prevention of myocardial injury following infarction or surgery, there have been no published clinical studies of ischemic preconditioning prior to cardiac transplantation.

Similarly, in lung transplantation, there is animal literature to support the idea that preconditioning might enhance outcomes (Li et al., 1998), but limited clinical trial evidence in humans. One small pilot single-center study randomized

60 patients to receive either lower-limb RIPC or none. The authors demonstrated no difference in short-term outcomes, but concluded that the intervention was safe and should be further investigated (Lin et al., 2014).

Intestinal transplantation is yet another clinical area with promising outcomes from preconditioning in animal models (Varga et al., 2011), but no human clinical studies as yet.

Non-solid organ transplantation

There has been interest in ischemic or hypoxic preconditioning of stem cells prior to their use, especially in ischemic injuries such as stroke and myocardial infarction. This conditioning strategy can improve the tolerance and regenerative properties of stem cells, and transplantation of such cells suppresses inflammation and immune responses, thus promoting functional recovery (Yu et al., 2013). This is an emerging research area that has shown promise in animal models but that has not yet been tested in human clinical trials.

Barriers to translation

The enduring theme of the above literature is the observation of (often dramatic) protection in animal models, with a lack of translation of this effect into the human clinical setting. There may be several reasons for this. The fact that the mechanism of IPC is still incompletely understood interferes with our ability to plan and conduct scientifically robust clinical studies.

One example of this is the application of RIPC during propofol anesthesia, during which activation of the protective kinase pathways necessary for ischemic conditioning protection does not appear to occur (Kottenberg et al., 2014; Ney et al., 2018; Rossaint, 2018). Of course, the converse is also true, and certain pharmacological agents (including volatile anesthetic agents) are known to mimic ischemic conditioning (Pagel and Crystal, 2018). At present, our incomplete understanding of the conditioning pathways impedes our ability to exploit the potential for pharmacological conditioning, and the known “conditioners” are often of limited utility due to their potential side effects. For example, cyclosporin is known to mimic ischemic conditioning by stabilizing the mitochondrial permeability transition pore (mPTP) (Okorie et al., 2011). However, cyclosporin is an immunosuppressant which has a hemodynamic effect on the kidney microvasculature, increasing risk of acute kidney injury (AKI), especially in clinical conditions where there is a risk of decreased kidney perfusion. Additionally, further elucidation of the conditioning pathway might allow the measurement of a mediator or biomarker that could confirm the effectiveness of the intervention, allowing subsequent modification of the conditioning strategy and its timing to maximize protective effects.

As can be seen above, many studies are underpowered and include heterogeneous groups of patients. There are challenges in recruiting sufficient numbers of comparable patients and a lack of clarity as to what clinical factors must be controlled for. We also do not yet know with certainty if it is the donor that needs to be conditioned to protect the transplant organ from downstream injury, or the recipient, or indeed both.

Many studies deemed to represent a positive effect of IPC are based on a reduction in biomarkers of cell injury, without subsequent clinical correlation. There have also been large negative studies in other clinical areas, and these have fueled the suspicion that there is no benefit of this intervention. For example, the large and well-conducted ERICCA study recruited 1,600 patients undergoing high-risk cardiac surgery and looked at a composite clinical endpoint (MACCE score). They found no benefit of RIPC (Hausenloy et al., 2015a).

The future for IPC in organ transplantation?

Some large studies are in the pipeline and, if positive, they may rekindle interest in this intervention across clinical

disciplines. In particular, the ERIC-PPCI study has recruited 2,800 patients undergoing percutaneous intervention following acute cardiac events and randomized to receive RIPC or none prior to intervention. The associated CONDI2 study is running concurrently in Europe (Hausenloy et al., 2015b).

Conclusions

Organ transplantation is an ideal clinical scenario in which to investigate the effects of an intervention that may protect against IR injury. Ischemic conditioning is a safe and cost-effective intervention which is easy to deliver and has shown promise in animal models. However, the difficulty of translation to the clinical setting has been compounded by the presence of multiple heterogeneous and often underpowered studies, utilizing different conditioning protocols and outcome measures. In addition, as the mechanism is incompletely understood, there remains the risk of trial design being suboptimal in maximizing the potential for an observed clinical benefit. A coordinated multi-center approach to design, funding and recruitment is essential in planning future appropriately sized, robust and definitive studies.

Conflicts of interest statement

The author declares that she has no conflicts of interest.

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