http://dx.doi.org/10.1016/j.hpb.2017.05.005 HPB

ORIGINAL ARTICLE

Remote ischaemic preconditioning in orthotopic liver transplantation (RIPCOLT trial): a pilot randomized controlled feasibility study

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Abstract

Background: Ischaemia Reperfusion (IR) injury is a major cause of morbidity, mortality and graft loss following Orthotopic Liver Transplantation (OLT). Utilising marginal grafts, which are more susceptible to IR injury, makes this a key research goal. Remote Ischaemic Preconditioning (RIPC) has been shown to ameliorate hepatic IR injury in experimental models. Whether RIPC can reduce IR injury in human liver transplant recipients is unknown.

Methods: Forty patients undergoing liver transplantation were randomized to RIPC or a sham. RIPC was induced through three 5 min cycles of alternate ischaemia and reperfusion of the left leg prior to surgery. Data on clinical outcomes was collected prospectively. Per-operative cytokine levels were measured.

Results: Fourty five of 51 patients approached (88%) were willing to enroll in the study. Five patients were excluded and 40 randomized, of which 20 underwent RIPC which was successfully completed in all patients. There were no complications following RIPC. Median day 3 AST levels were slightly higher in the RIPC group (221 IU vs 149 IU, p = 1.00).

Conclusions: RIPC is acceptable and safe in liver transplant recipients. This study has not demonstrated evidence of a reduction in short-term measures of IR injury. Longer follow up will be required and consideration of an altered protocol.

Received 12 March 2017; accepted 8 May 2017

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Introduction

Liver transplantation is the treatment of choice for both acute and chronic end stage liver disease. As outcomes following transplantation have improved, the indications for liver transplantation have been widened and a shortage of suitable organ donors has developed. This has resulted in the increased use of grafts from marginal donors such as the elderly, those with fatty liver disease and those from donors following cardiac death (DCD). The use of liver DCD grafts in the UK has increased from 6.9% in 2005[1] to 19.1% of grafts implanted in 2013.[2]

Ischaemia Reperfusion (IR) injury is the damage that happens to an organ when its blood supply is interrupted and reconstituted. It is a major cause of morbidity and mortality following liver transplantation and is believed to account for up to 10% of early graft loss.[3]

Grafts from "extended criteria" or "marginal" donors are particularly prone to IR injury and in UK centres, the implantation of DCD grafts is associated with a 2 fold increase in risk of graft loss and recipient mortality which is maintained for 3 years post transplantation.[1] Due to these poor clinical outcomes, grafts from

extended criteria donors are often discarded. The ability to ameliorate IR injury would improve outcomes following liver transplantation, reduce early graft loss and the need for re-transplantation and allow the safe implantation of more marginal grafts increasing the potential donor organ pool. There is no current accepted treatment for IR injury and therefore the development of strategies to treat IR injury remains a key clinical concern.

Ischaemic Preconditioning (IPC)[4] is the process by which short periods of ischaemia to the target organ protect that organ during further more substantial ischaemic periods. Despite robust evidence of the protective benefit of direct IPC in small animal models,[5,6] IPC has been shown in small animal models to impair liver regeneration.[7,8] Although not shown on donor grafts in human liver transplantation, a multivariate analysis found that direct IPC is an independent predictor for post-operative morbidity[9] following hepatic resection surgery in humans.

Ten small studies have investigated the effect of direct IPC of donor livers deceased donor liver transplantation and a recent meta-analysis found that donor IPC results in a large reduction in recipient mortality and incidence of primary graft non-function although this difference was not statistically significant.[10]

Remote Ischaemic Preconditioning (RIPC)[11] is the process by which preconditioning of one organ or vascular bed provides protection to distant organs or vascular beds during a sustained period of ischaemia. RIPC has been shown by our own group and others to ameliorate hepatic IR injury in small animal models.[12–14] It has been translated into clinical benefit in patients undergoing both cardiac surgery[15] and major vascular surgery.[16] In major liver surgery, RIPC was shown in a pilot RCT to reduce liver IR injury as indicated by a reduction in post operative transaminases and increased ICG clearance.[17] Successful use of RIPC in liver transplant recipients would avoid the complexity of preconditioning donors at multiple hospitals and the potential risk of impairing graft regeneration following implantation.

Although the mechanism by which RIPC and IPC protect organs from IR injury remains unknown, the process of performing RIPC in the recipient more closely resembles successful animal models in which preconditioning is performed in the individual in which the reperfusion injury occurs and therefore may be more efficacious than IPC of donor livers.[18] RIPC of renal transplant recipients has been investigated in 2 recent trials including both living[19] and deceased[20] donors with 1 trial demonstrating an improvement in early graft function.[20]

There are no previous human clinical trials of liver transplant recipient RIPC and there are fundamental issues to be addressed. These include the willingness of patients, their clinicians and the transplant anaethetists to support such a trial. Patients undergoing liver transplantation mainly have end stage cirrhosis and the risk of limb conditioning in patients with jaundice and a coagulopathy are unknown. Finally the conditioning protocol which has been used in other clinical applications may not be optimal with the altered metabolism and haemodynamics of end stage cirrhosis.

The aim of this study was to perform a prospective randomized controlled feasibility study to address these issues and to obtain preliminary data on which to design a further prospective trial to determine efficacy and cost effectiveness.

Methods

A single centre double blinded open prospective randomized sham controlled trial was performed at the Royal Free Hospital following approval by the NHS National Research Ethics Service (11/H0720/4) and the Royal Free Hospital/University College London medical school ethical committee (8191). The trial involved randomization of adult recipients undergoing deceased donor liver transplantation and was registered with ClinicalTrials.gov: Number NCT00796588. The protocol has been previously published.[21]

Patients above the age of 18 undergoing first elective deceased donor liver transplantation were enrolled with informed consent in the study for randomization. All graft types were included. Trial exclusion criteria are contained in Table 1.

Fifty-one patients undergoing assessment for liver transplantation were approached for recruitment to the study of which 6 patients were unwilling to recruit to the trial and 5 patients were excluded. Forty patients were randomized to a sham control group or a RIPC group. Randomisation was performed, following induction of anaesthesia, using a sealed envelope method by the study fellow who was not involved with the transplant surgery or post operative care (CONSORT flowchart, Fig. 1).

Both patients and the clinical teams, including the transplant surgeon and anaesthetists, were blinded as to which group the patient was randomized to.

Endpoints

The primary endpoints were:

1: Feasibility of recruiting patients with end stage cirrhosis to undergo limb ischaemic preconditioning immediately prior to commencement of liver transplantation.

Table 1 Exclusion criteria

Exclusion criteria:	Re-transplantation
	Patients under 16 years of age
	Super-urgent transplantation
	Lack of informed consent
	Combined liver and kidney transplantation
	Peripheral vascular disease
	Varicose veins
	Localized limb infection
	Prior history of thrombo-embolic disease
	Inclusion in another interventional trial

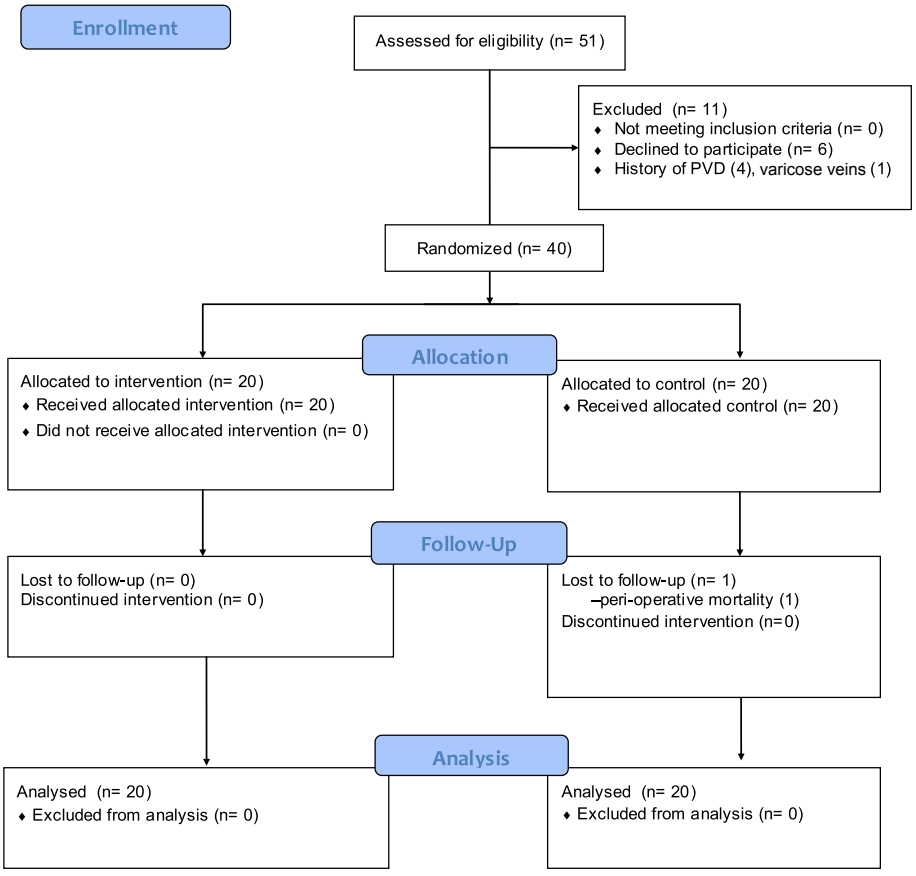

Figure 1 CONSORT flow diagram

2: Safety of limb pre-conditioning in patients with end stage cirrhosis.

This included the possible development of pain, neuropraxia or deep vein thrombosis due to the pneumatic tourniquet or any harmful systemic effects of the remote preconditioning stimulus. Secondary endpoints are listed in Table 2 and include clinical outcomes of patient and graft survival, complications as measured using Clavien Dindo classification, graft function including day 3 aspartate transferase (AST) levels[22] and early allograft dysfunction (EAD) as defined by Olfthoff *et al.*[23]

The stimulus

Following induction of anaesthesia but before skin incision, the left lower limb was covered with 2 layers of stockinette and a wide pneumatic tourniquet was applied to the left middle thigh in accordance with safe and recommended practices by the Association of Peri-operative Registered Nurses (AORN).[24] To induce RIPC through transient ischaemia, the tourniquet was inflated to 200 mmHg for 5 min and then deflated for 5 min to reperfuse the leg. This was repeated 2 more times and completed prior to the abdominal incision for the transplant

Table 2 Primary and secondary trial endpoints

Primary endpoints:	Ability to recruit patients to the trial
	Feasibility of performing RIPC in liver transplant recipients
	Safety of RIPC in liver transplant recipients
Secondary endpoints:	90 day recipient mortality
	90 day graft loss
	AST levels on the third post-operative day[22]
	Incidence of Acute Kidney injury and need for Renal Replacement therapy
	Length of stay in Intensive Care and total hospital stay
	Incidence of vascular thrombotic events
	Incidence of biliary complications
	Incidence of post-operative infections
	Incidence of acute rejection in the first months post transplantation
	Circulating cytokine levels 2 h post reperfusion of the liver graft

procedure. A sham consisted of placing the tourniquet as above but not inflating it.

Liver transplantation

Grafts were identified and retrieved through the dedicated UK National Organ Retrieval Service (NORS) according to national standards of organ retrieval from deceased donors[25] (NHSBT). Following aortic cannulation all grafts were perfused in situ with cold University of Wisconsin (UW) solution (Bridge to Life, Chicago) at a maximum pressure of 200 mmHg. On removal the grafts were further flushed with ice cold UW solution on the back bench via the hepatic artery, portal vein and the bile duct. The grafts were then sterile packaged in cold UW solution and transported to the recipient hospital on ice. Grafts which were stored and transported using normothermic perfusion using the OrganOx system were excluded.

The recipients were monitored intra-operatively via arterial and central venous catheters with availability of trans-oesophageal echo as required. Implantation of the liver graft was performed by standard piggy-back and caval replacement techniques. Veno-venous bypass was not employed in any patient randomized in this trial. Grafts were flushed with 500–1000 mls warm 4.5% human albumin solution (Bio Products Laboratory) via the portal vein immediately prior to blood re-perfusion to remove residual UW solution and waste material accumulated during cold ischaemia. One gram of methylprednisolone (Pharmacia) was given intravenously during the anhepatic phase as part of standard anaesthetic protocol.

Post operative management

Post-operatively all patients were managed in the intensive care unit.

Haemoglobin levels were maintained below 10 g/L. Platelets and fresh frozen plasma were administered if there was a coagulopathy associated with active blood loss. Patients were routinely started on subcutaneous thromboprophylaxis on the first post operative day. All patients underwent a Doppler ultrasound scan of the liver vessels on the first, third and fifth post-operative day. Daily blood tests included clotting profiles, renal function, bilirubin and serum liver enzymes.

Patients were extubated on the first post-operative day unless there was a clinical need for ongoing respiratory support and triple therapy immunosuppression was commenced on day 1 post-operatively. If there was evidence of early renal impairment, monoclonal antibody therapy was given in place of triple therapy immunosuppression.

Blood oxygenation levels during preconditioning

Two 2 mls bloods samples were collected simultaneously in 21 patients (10 RIPC, 11 Control). A venous blood sample was collected from the recipients left foot in lithium heparin gas syringes (BD Preset, UK), after 4.5 min of lower limb vascular occlusion while the tourniquet was still inflated or at the same time point in the sham group. A simultaneous peripheral arterial blood sample was collected from the arterial line and identically managed. Oxygen levels, lactate levels and acid base status were measured instantly from both samples on a RAPIDPoint 500 blood gas analyser (Siemens, Surrey, UK).

Measurement of cytokines

In both groups, 10 mls of peripheral arterial blood was collected at the following time intervals:

1 baseline (following induction of anaesthesia but before abdominal incision),
2 immediately before the recipient's portal vein and vena cava were cross clamped,
3 2 h post reperfusion of the portal vein,
4 24 h post-operatively.

Blood was collected in BD vacutainer plasma tubes (BD, UK). Samples were immediately centrifuged at 1000 g for 10 min and the plasma was stored at −80 °C until analysis. IL2, IL6, IL10, TNFα and IFNγ were measured by legendPLEX (Biolegend, UK)- Human Th Cytokine Mix and Match Subpanel. IL8 (Biolegend, UK) and IL17A (Biolegend, UK) were measured by ELISA via commercial kits.

Statistical analysis and power calculation

A power calculation is not required for a pilot feasibility study.[26] Statistical guidance would suggest that 40 patients are a suitable sample size.[27]

Continuous variables were expressed as median (+interquartile range) or mean (±standard deviation) as appropriate and comparisons between the groups were analysed by Mann Whitney-U test or Students' T-Test. Binary outcomes were expressed as frequency counts and percentages and comparisons between the groups were analysed by Chi-squared tests on Statistical Package for Social Sciences (SPSS) (IBM, Chicago, IL, USA) and Prism 5 (Graphpad, USA).

Results

Feasibility and recruitment

Fifty-one patients were approached of which 45 (88%) wished to be involved in the trial. Five patients were subsequently excluded

due to risk factors for complications of the limb conditioning. Four of the five patients had a prior history of thromboembolic disease and 1 patient had varicose veins of the left lower limb. The remaining 40 patients were randomized with 20 allocated to RIPC and 20 to a sham control. The patient groups were well matched at baseline. The characteristics of the recipients and donors are shown in Tables 3 and 4.

All patients randomized to undergo preconditioning successfully completed the preconditioning protocol prior to the abdominal incision. There was no evidence of systemic haemodynamic instability or vagal response during the cuff inflation or after reperfusion of the limb. Visual inspection of the limb following preconditioning showed no evidence of bruising/haematoma formation.

No patient complained of pain, parasthesia or limb weakness post-operatively. There was no clinical evidence of DVT or PE formation in any patient.

Secondary end-points

1: 90 day graft and patient survival

One patient in the control group died peri-operatively as a result of significant intra-operative haemorrhage and primary graft non-function (PGNF). There was no 90 day mortality in the RIPC group. One patient in the control group required retransplantation on the 4th post-operative day following the discovery of an incidental adeno-carcinoma in the donor's gallbladder.

2: Complications:

There was no significant difference in incidence of post-operative complications between the groups. 15 of 20 RIPC patients vs 14 of 19 sham control patients developed a post operative complication (Table 5).

3: Graft function:

Aspartate transferase (AST) levels on the 3rd post operative day were trending to be higher in the preconditioning group but this did not reach significance (221 IU (82–434) vs 149 IU (103–370), p = 1.00). There was also a trend to higher incidence of EAD in the preconditioned group but this was not significant (10 vs 7, p = 0.523). Although median transaminase levels, in the

Table 3 Base-line recipient characteristics (mean ± SD)

Recipient characteristics	RIPC	Control	P value
Gender (M:F)	18:2	16:4	0.661
Age	55 (±10)	54 (±9)	0.758
MELD	15 (±5)	13 (±5)	0.190
UKELD	55 (±5)	52 (±5)	0.085

Table 4 Baseline donor and Transplant characteristics (mean ± SD)

Donor and transplant characteristics	RIPC	Control	P value
Gender (M:F)	16:4	13:7	0.731
Age	43 (±14)	47 (±16)	0.376
Type of graft			0.723
DBD	15	17	
DCD	3	2	
Domino	1	0	
Split	1	1	
Cold ischaemic time (mins)	470 (±140)	455 (±157)	0.750
Warm ischaemic time (mins)	44 (±14)	42 (±11)	0.546

Table 5 Clinical outcomes in RIPC and control groups

	RIPC	Control	P value
3 month mortality	0	1	1.00
3 month graft loss	0	2	0.487
Day 3 AST	221 (82–434)	149 (103–370)	1.00
EAD	10	7	0.523
Mean days ventilated	2 (±1)	2 (±1)	0.758
Mean ITU stay (days)	4 (±2)	3 (±3)	0.372
Mean hospital stay (days)	31 (±46)	21 (±14)	0.409
Complications			
Grade I			
Wound infection	4	7	0.303
Urine infection	2	3	0.517
Grade II			
Chest infection	0	1	0.349
Bacteraemia	3	2	0.549
Abdominal infection	3	2	0.549
Fungal infection	1	0	0.368
Portal vein thrombosis	0	0	1.00
Hepatic artery thrombosis	0	0	1.00
Grade IIIa			
Bile leak	1	1	1.00
Biliary stenosis	3	1	0.37
Grade IIIb			
Bile leak	1	1	1.00
Biliary stenosis	0	0	1.00
Grade IV			
Acute kidney injury	10	7	0.523
Need for haemofiltration	5	3	0.695
Retransplanted	0	1	1.00
Grade V			
Death	0	1	1.00

first week post transplantation, were higher in recipients that underwent RIPC, median bilirubin (27 μmol/L (19–37) vs 41 μmol/L (23–74), p = 0.087) and alkaline phosphatase levels (215 IU/L (168–293) vs 275 IU/L (218–351), p = 0.126), at day 7 post transplantation, were trending to be lower in recipients who were preconditioned although this was not statistically significant. By 3 months post transplant both groups were similar in all measurements (Fig. 2).

4: Acute cellular rejection:

Incidence of acute cellular rejection was low with only one episode proven by biopsy in the control group and no episodes in the preconditioning group.

5: ITU and total hospital stay:

Patients in the preconditioning group spent longer in ITU post operatively (4 days vs 3 days, p = 0.372) and in hospital (20 days vs 16 days, p = 0.409), although this was not statistically significant.

Limb oxygenation during RIPC

Arterial oxygen levels measured from the radial artery during the preconditioning stimulus were similar between the preconditioned and control groups (28.87 (±9.73) kPa vs 30.43 (±12.63) kPa, p = 0.757). Venous oxygen levels measured at the same time in the lower limb during the preconditioning stimulus were significantly lower in the preconditioning group than the control group (7.53 (4.94–9.28) kPa vs 15.06 (8.67–19.00) kPa, p = 0.004) (Fig. 3).

Plasma cytokine levels

Plasma levels of IL6, IL8, IL10 and IL17a were significantly raised from baseline at 2 h post reperfusion and had returned to near baseline levels within 24 h (Fig. 4). Plasma levels of IL2, IFN-γ and TNF-α did not change during the peri-transplant period (Table 6). The median IL-6 levels in the preconditioned group were significantly lower than in the control group (487.99 (221.65–1232.37) pg/ml vs 1062.3 (221.5–25903.85) pg/ml, p = 0.013) (Fig. 5). Median levels of all other cytokines measured were similar between both groups and are shown in Fig. 4.

Discussion

This is the first trial to investigate the feasibility of RIPC in liver transplant recipients. It has demonstrated that RIPC is feasible, acceptable to patients and safe in this group of patients.

Recruitment to the trial was satisfactory with a consent rate of 88% and a post randomization drop out of 0%. The RIPC protocol was successfully completed in all patients. No patient suffered a complication secondary to RIPC and in no patient was surgery delayed as result of undergoing RIPC. This study has satisfied the primary objectives of the feasibility study.[21]

Ninety day patient survival was 100% in the RIPC group and 95% in the control group and ninety day graft survival was 100%

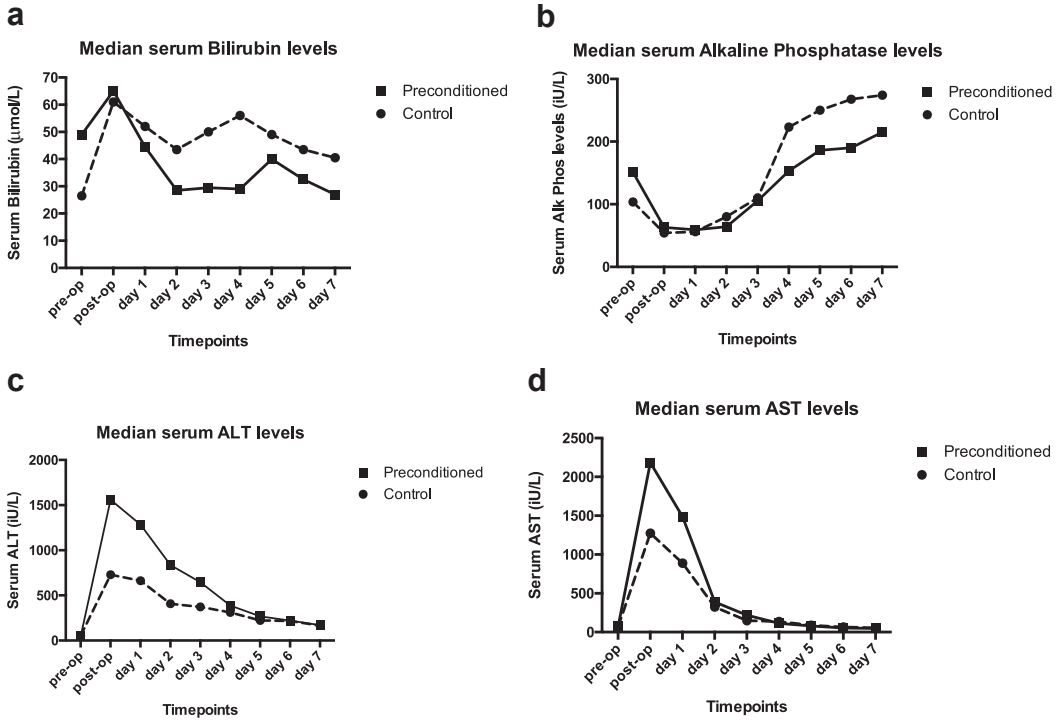

Figure 2 Serum transaminase, bilirubin and alkaline phosphatase levels in the first week post liver transplantation

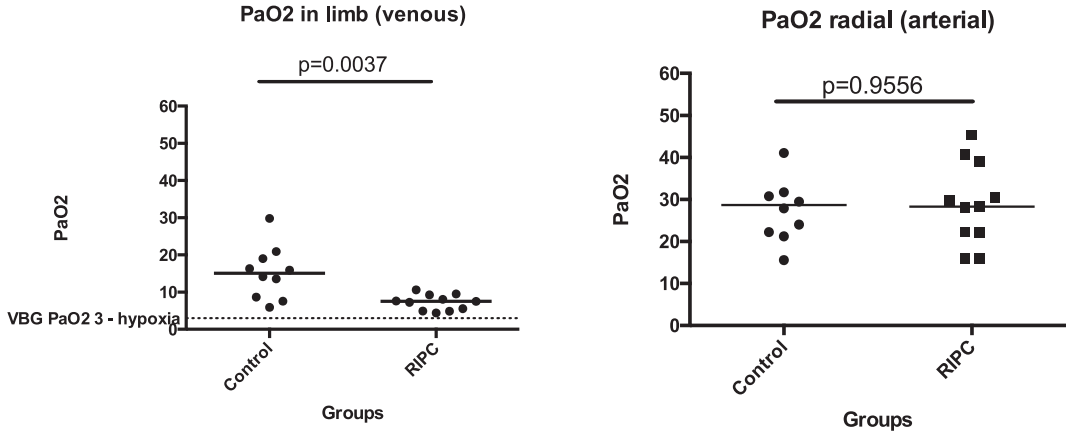

Figure 3 Venous oxygen levels in the limb during the preconditioning cycle

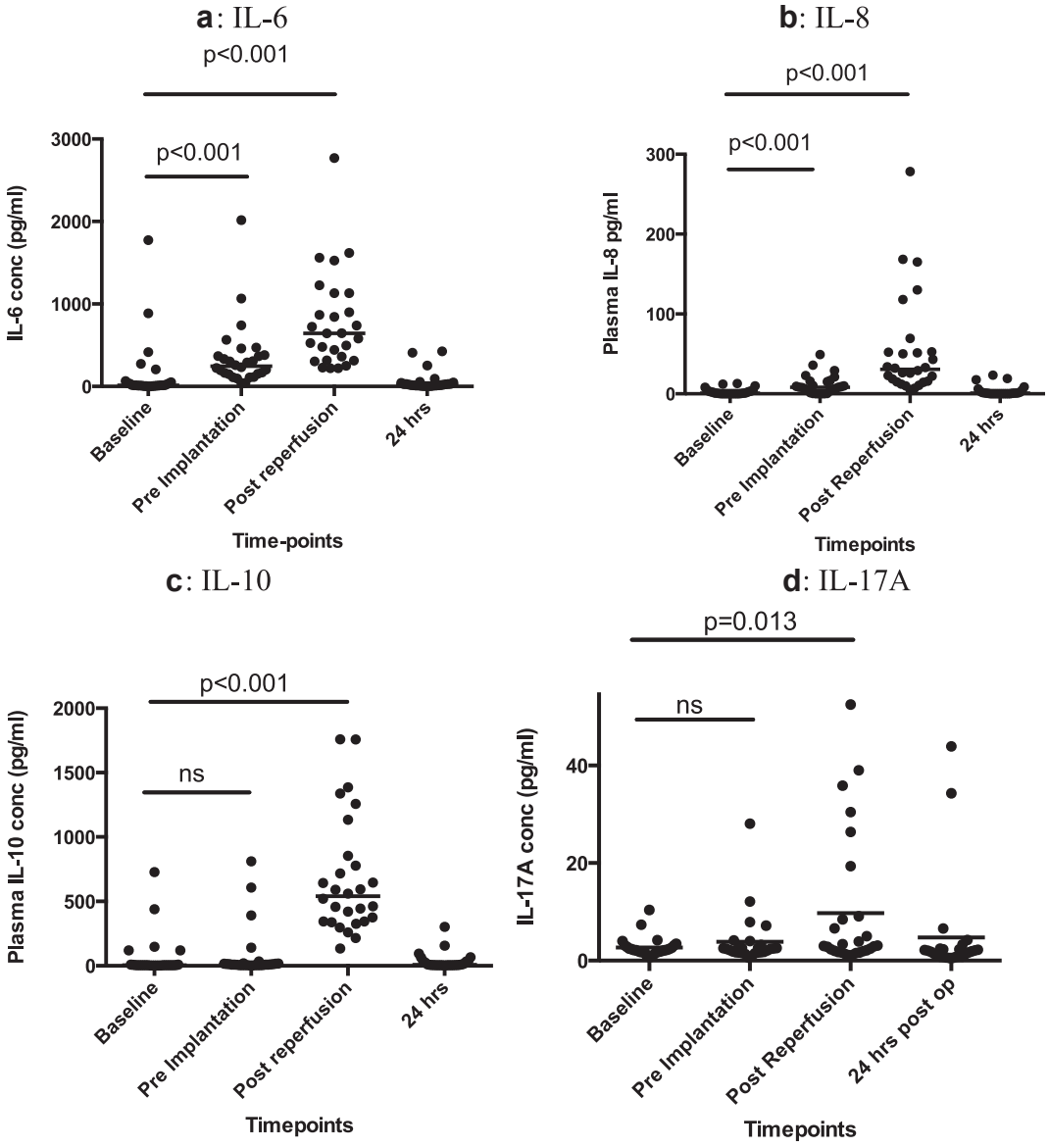

Figure 4 Circulating cytokine levels that showed a significant increase during liver transplant and at 24 h post op

in the RIPC group and 90% in the control group. Neither of these were significantly different as would be anticipated with a small pilot study. Secondary end points were chosen that may allow differences between the RIPC and control group within a pilot study. These are mainly end points which are influenced by graft IR injury.

The overall complication rate was not significantly different between the groups with 15 of 20 patients undergoing RIPC developing a complication and 14 of 19 patients undergoing a sham developing a complication.

Patients who underwent preconditioning had a higher incidence of post operative acute kidney injury (10 vs 7) and need for renal replacement therapy (5 vs 3) neither of which were significant. The mean days ventilated post operatively was similar between the 2 groups (2 vs 2). Severe IR injury results in a systemic inflammatory response and end organ damage. AKI is a particular problem post OLT and documented rates in the literature range from 14% to 94%.[28–33] A recent audit of incidence of AKI at the Royal Free Hospital found that AKI occurs in around 50% of patients undergoing liver transplantation. Seventeen patients (43%) of patients developed an AKI so this is representative of our patients. This data would suggest that RIPC did not reduce the incidence of end organ dysfunction following IR injury.

Another secondary end point used in this study was the AST levels on the 3rd post-operative day which have been shown to correlate strongly with both graft and recipient survival following liver transplant.[22] Median AST levels on day 3 were non-significantly higher than in the control group again suggesting that in its current form RIPC has not reduced IR injury in the liver graft and is not associated with an improvement in graft or recipient outcome.

EAD which has also been shown to be associated with an increased risk of graft loss and recipient mortality[23] was measured. There was a non-statistically significant higher incidence of EAD in the RIPC group (50% vs 37%, p = 0.523), again suggesting that RIPC did not improve early graft function following OLT.

Taken together this data shows that although RIPC is safe and feasible to be performed in patients undergoing liver transplantation, in its current form it does not provide evidence of clinical benefit to liver transplant recipients in this small patient group.

In this study we investigated the preconditioning stimulus which is likely to be mediated by limb hypoxia, ischaemia and acidosis. Although the venous PO_2 in the limb was significantly lower in patients undergoing RIPC compared with controls, true hypoxia (PO_2<3 kPa) was not achieved in the limb during the preconditioning stimulus. There could be several reasons for this including errors in cuff inflation. However the standard deviation of the venous PO_2 levels is small (6.19 kPa) suggesting that this is not the case. It may be that the high FiO_2 delivered to the patients during the transplant procedure including during the period of the preconditioning stimulus prevented significant tissue hypoxia in the conditioned limb. This would suggest that 5 min of tourniquet inflation was insufficient to create localized ischaemia in the limb in these patients. This mirrors the results from direct preconditioning of liver grafts in donors prior to organ retrieval in which 5 min of ischaemia was found not to be of any benefit[34] but 10 min of ischaemia was shown to provide a degree of protection demonstrated by a reduction in markers of liver injury.[35] The optimal protocol for RIPC in humans remains to be established. A pilot study of RIPC in patients undergoing liver resection using 10 min cycles to perform the preconditioning stimulus showed evidence of a reduction in liver injury as demonstrated by a reduction in post operative transaminases and improve ICG clearance and as such a further trial with 10 min cycles is warranted.[17]

IR injury results in systemic cytokine release resulting in end-organ damage.[36,37] TNFα, is a key cytokine shown to be upregulated early following IR injury and to promote recruitment of lymphocytes to the ischaemic injury.[38] Other cytokines that have been implicated in IR injury in small animal models include IL-2,[39] IL-6,[40] IL8,[41] IL-17[42] and IFNγ[40] and these were measured in this study. Our results show that circulating levels of IL-6, IL-8, IL-10 and IL-17A were significantly raised at 2 h post reperfusion but had returned to undetectable levels within 24 h post operatively. In contrast circulating levels of IL-2, TNFα and IFNγ were not detectable in our patients at 2 h post reperfusion or at 24 h post operatively. A previous observational study in humans undergoing liver transplantation[43] measured circulating serum

Table 6 Plasma cytokine levels during the transplant period (Median + IQR) *denotes significance identified with Kruskal–Wallis

Cytokine	Baseline (pg/ml)	Pre implantation (pg/ml)	Post reperfusion (pg/ml)	24 h post-op (pg/ml)
IL-2	9.34 (4.08–35.11)	7.50 (4.08–40.40)	6.19 (4.08–17.13)	12.16 (4.08–42.29)
IL-6*	13.98 (8.27–44.80)	245 (150.97–375.12)	644.98 (338.31–1132.01)	21.58 (10.11–43.29)
IL-8*	0.88 (0–3.26)	8.23 (1.28–15.84)	30.59 (15.37–52.42)	0.88 (0–3.11)
IL-10*	4.22 (3.72–7.69)	9.83 (4.38–16.96)	540.74 (344.21–815.48)	7.37 94.56–35.26)
IL-17A*	2.14 (1.74–2.96)	2.40 (1.68–3.2)	2.94 (1.85–8.78)	1.86 (0.81–2.33)
IFNγ	57.13 (18.05–176.09)	32.66 (17.15–73.29)	31.41 (10.92–107.48)	17.35 (6.50–44.20)
TNFα	7.97 (3.5–53.16)	7.17 (3.5–33.79)	7.15 (5.46–8.58)	6.46 (3.5–8.58)

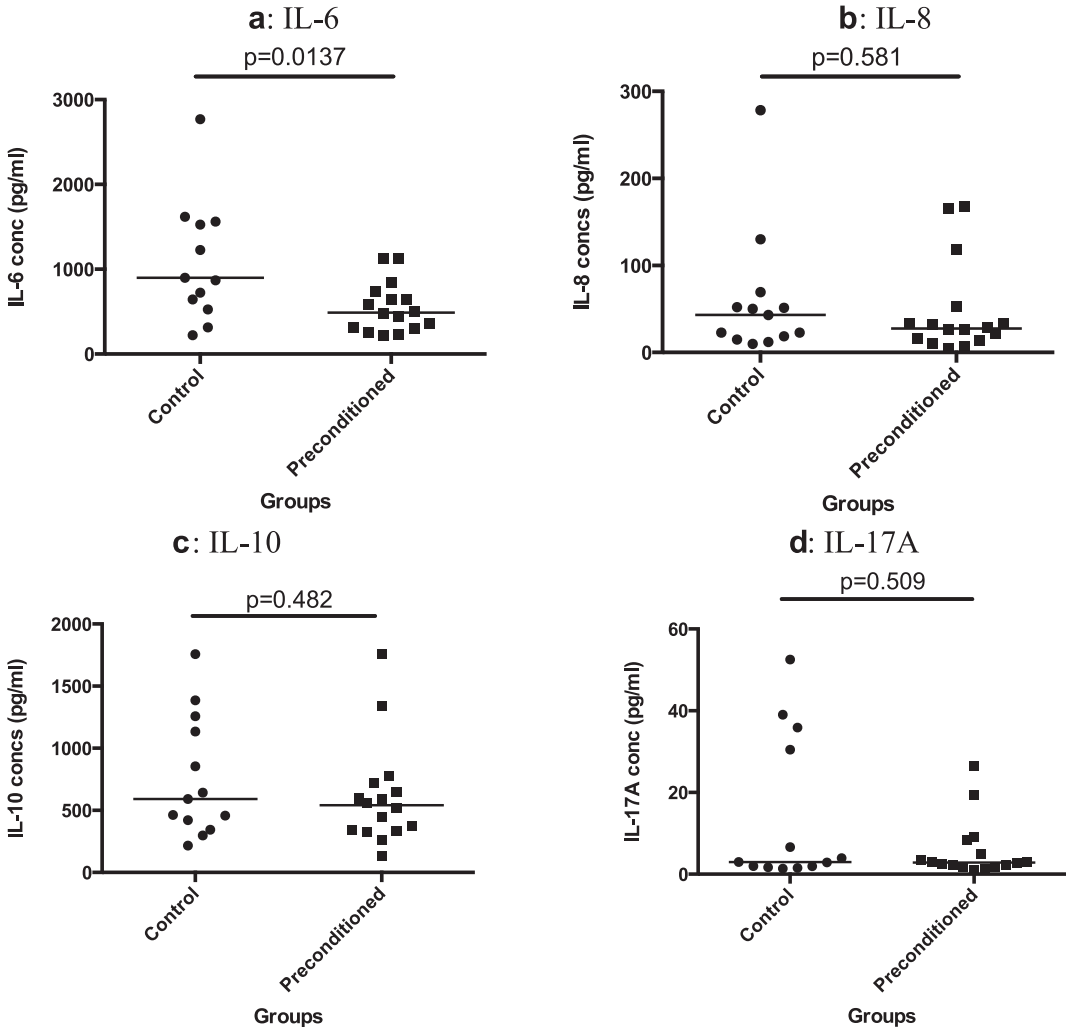

Figure 5 Circulating cytokine levels between the groups

cytokine levels at 24 h post transplantation and found that in 99%of patients, IFNγ was not detected and in 77% of patients, TNFα was not detected. Although 17 patients developed an AKI as measured by the AKIN criteria – suggesting a systemic inflammatory response and end organ damage, plasma levels of IL2, IFNγ and TNFα were not significantly raised from base line in our patients even 2 h post reperfusion suggesting that these cytokines may not be involved in the systemic inflammatory response post transplant. This is in keeping with results from canine lung IR injury which showed no elevation in serum IL-2, IFNγ and TNFα although they were significantly elevated in bronchial alveolar lavage samples[44] showing they may be involved in the local inflammatory response.

In the current study, circulating levels of IL-6, IL-8, IL-17A and IL-10 were elevated at 2 h post reperfusion and were similar to baseline levels by 24 h. Similar peaks of circulating levels of IL-6 and Il-8 were seen in patients at 2 h post liver resection[45] however circulating levels remained elevated at 24 h post operatively in comparison to this study when levels returned to near baseline. This may reflect the fact that post liver transplant patients are immunosuppressed whilst they are not following liver resection surgery. Similarly the peak of plasma Il-10 levels at 2 h in our patients likely represents the anti inflammatory effect of the intravenous dose of methylprednisolone given during the anhepatic phase of the transplant. In this study there were significantly lower levels of circulating IL-6 in patients who underwent RIPC. However the significance of this result is unclear. Plasma IL-6 levels post reperfusion showed a positive correlation with the calculated donor risk index[46] suggesting that IL-6 levels vary with the quality of donor organ. Higher IL-6 levels post liver resection have been shown to be associated with increased risk of post-operative complications and bile leaks.[45] However plasma IL-6 levels were not higher in patients who developed either EAD or AKI which are associated with poor quality grafts. Furthermore although patients undergoing RIPC had lower levels of IL-6 post reperfusion, there was no

evidence that this was associated with a reduction in graft injury or an improvement in clinical outcomes.

In conclusion, to our knowledge, this is the first trial to investigate RIPC of liver transplant recipients and has shown that RIPC is feasible and safe in liver transplant recipients. In its current form it does not appear to provide any clinical benefit detectable within the first 3 months post transplantation and longer term follow up will be required. Venous blood gas measurements taking from the limb during the preconditioning period suggest that 5 min cycles are insufficient to create localized ischaemia in the limb.

An altered preconditioning protocol for example of three 10 min cycles requires to be evaluated before considering a larger trial to measure efficacy.

Acknowledgements

Mr Francis Robertson is the recipient of a Wellington/HCA research fellowship. We gratefully acknowledge the support of the transplant coordinators, hepatologists and the transplant anaethetists who helped perform this study.

Funding sources

No funding.

Authorship

FR designed the study, collected and analysed the samples and data and wrote the final manuscript. RG helped design the study, collect data and reviewed the final manuscript. GW helped design the study, analyse the data and reviewed the final manuscript. CI, DS and MM helped collect samples and data and reviewed the final manuscript. BF and BD designed the study, reviewed the data prior to manuscript preparation and reviewed the manuscript.

Conflict of interest

None declared.

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