Effect of Remote Ischemic preConditioning on liver injury in patients undergoing LIVER resection: The ERIC-LIVER trial

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ABSTRACT

Objective

Acute ischemia/reperfusion injury during liver surgery leads to poor outcomes. Novel hepatoprotective strategies are needed to improve clinical outcomes. There is mixed data on the clinical role of remote ischemic preconditioning (RIPC) in liver surgery. We investigated RIPC in patients undergoing partial hepatectomy for primary hepatocellular carcinoma (HCC). The primary hypothesis was that RIPC would reduce acute liver injury following surgery as indicated by serum alanine transferase (ALT) levels 24 hours following partial hepatectomy in patients with primary HCC, compared to sham control.

Methods

This was a Phase II, proof-of-concept, single-center, sham-controlled, randomized controlled trial (RCT). Patients were randomized to receive either four cycles of 5minute/5minute arm cuff inflation/deflation following anaesthesia and immediately prior to commencing surgery, or control. The primary endpoint was ALT at 24 hours. Secondary endpoints included clinical, biochemical and pathological outcomes. Liver function measured by Indocyanine Green pulse densitometry was performed in a subset of patients.

Results

24 and 26 patients were randomized to RIPC and control groups respectively. The two groups were well balanced for baseline characteristics, except the duration of operation was longer in the RIPC group. Median ALT at 24 hours was similar between RIPC and control groups (196 IU/L IQR 113.5-419.5 versus 172.5 IU/L IQR 115-298 respectively, p=0.61). RIPC and control groups were similar in all secondary endpoints analyzed.

Conclusion

This small RCT of HCC patients did not demonstrate any beneficial effects with RIPC on either serum ALT levels 24 hours or any of the secondary endpoint after partial hepatectomy.

INTRODUCTION

Liver cancer is the sixth most common cancer worldwide and the third leading cause of cancer death.[1] There is great geographic variation in the distribution of liver cancer with some 80% of cases found in the Asia-Pacific region, and this is closely linked to the increased prevalence of chronic hepatitis B and C in this part of the world.[2,3] Liver resection (partial hepatectomy) is the treatment of choice for primary hepatocellular carcinoma (HCC).[4] Surgical resection is advisable if the tumor is localised, and can be removed completely, leaving an adequate liver remnant. Liver resection is cost effective and has improved outcomes in patients with primary HCC worldwide.[4]

However, during liver surgery, a significant amount of acute ischemia/reperfusion injury (IRI) occurs to the liver. Warm ischemia can arise from vascular clamping of the hepatic territory and subsequent unclamping[5–7] (for example, Pringle maneuver to reduce operative blood loss during parenchyma transection), hemorrhage during surgery and subsequent blood transfusion[7], or simply mobilization or retraction of the liver.[8] This results in acute liver injury evidenced by an increase in liver enzymes, impaired post-operative liver function, and increased risk of liver failure, resulting in significant morbidity and mortality in patients undergoing partial hepatectomy.[9,10] The reported incidence of liver failure after partial hepatectomy varies widely from 0.7% to 33.8% mainly related to patient selection, inadequate residual liver tissue, and functional capacity.[9] This is especially pertinent in HCC patients, who are especially prone to acute IRI owing to high prevalence of chronic liver disease and cirrhosis from chronic hepatitis B or C infection, and pre-existing impaired hepatic functional reserve.[4]

Novel therapies are therefore urgently required to protect the liver against the detrimental effects of acute IRI during partial hepatectomy and to improve surgical outcomes in HCC patients.[11] Remote ischemic preconditioning (RIPC) describes the phenomenon in which the application of brief episodes of non-lethal ischemia and reperfusion to an organ (such as the kidney, liver or small intestine) or tissue (such as the skeletal muscle) is protective against acute

lethal IRI.[12,13] RIPC is non-invasive, low-cost, and has been shown to reduce organ injury in acute ischemic conditions[14,15] including myocardial injury from coronary bypass graft surgery[16,17], and has the potential to reduce liver injury after liver surgery.

While promising experimental data supports the protective effect of RIPC on liver IRI, its clinical role is unclear. Lai et al performed the first experimental study to demonstrate a beneficial effect on the liver with RIC, and found that four 10-minute cycles of limb ischemia/reperfusion reduced the serum liver enzyme alanine aminotransferase (ALT) in a rat model of partial hepatic ischemia.[18] Since then, the protective effects of limb RIC against acute liver IRI were subsequently also demonstrated in rat, rabbit and mouse models.[19–21] More recently, RIC was shown to promote liver regeneration in small liver grafts in a rat model of liver transplantation.[19] However, clinical trials in this area are fairly recent and there has been mixed clinical data from a small number of trials guiding the use of RIPC in liver surgery.[22–24]

In this randomized controlled trial, we aimed to evaluate whether remote ischemic preconditioning (RIPC), could reduce acute liver injury following partial hepatectomy.

METHODS

Trial design

This was a Phase II, proof-of-concept, single-center, single-blinded, sham-controlled, randomized controlled trial (RCT) to investigate whether RIPC can reduce liver injury and preserve liver function in patients undergoing partial hepatectomy for primary HCC. The primary hypothesis was that RIPC would reduce elevations in serum ALT levels 24 hours following partial hepatectomy in patients with primary HCC, compared to sham RIPC.

The protocol was registered prospectively on https://clinicaltrials.gov (NCT03594929). No major changes to design were implemented and there was no deviation to the original statistical plan. The trial was conducted in accordance with the Declaration of Helsinki 1964 as revised in 2013, the International Conference of Harmonization Guidelines for Good Clinical Practice and the Singapore Good Clinical Practice Guideline. Ethics approval was granted by the SingHealth Centralized Institutional Review Board (Reference 2015/3167).

Eligible subjects were patients aged 21 years and above, and scheduled for partial hepatectomy for primary HCC in the Singapore General Hospital (SGH). Exclusion criteria were: significant pulmonary disease (Forced Expiratory Volume in one second <40% predicted), known severe renal failure with a Glomerular Filtration Rate<30 mL/min/1.73 m^2, on sulphonylurea or nicorandil (as these medications may interfere with the protective effect of RIPC), recruited into another study which may impact on this study, significant peripheral arterial disease affecting the upper limbs, and repeat liver resection surgery.

Intervention

Patients were screened by clinical research coordinators at the surgical pre-admission clinic where informed consent was obtained. Consented patients were randomized 1:1 to RIPC or control. Randomization was carried out on the day of surgery, using a computer-generated list of randomised numbers, and allocation concealment achieved using sequentially-numbered opaque sealed envelopes allocated by the unblinded research staff. The treatment allocation was concealed from the subject, the anaesthesiologist, the liver surgeon(s), pathologist, ITU and ward staff, and the research staff collecting the data and clinical endpoints.

Both groups had a manual pneumatic blood pressure cuff applied to the upper arm. Following induction of general anesthesia, the RIPC group received four-5 min cycles of alternating cuff inflation to 200mmHg and deflation. For patients with a systolic blood pressure (SBP)≥175mmHg, the cuff was inflated to 25mmHg above SBP. This RIPC protocol was modified from protocols used in previous studies.[24] RIPC was performed by unblinded study coordinators, who were otherwise not involved in patient recruitment, data collection or analysis. In the sham control group the cuff was applied but not inflated for the same total duration. These procedures were commenced before surgical incision, and did not interfere with the commencement of surgery.

All patients underwent partial hepatectomy, either via laparotomy or laparoscopy. Open liver resection was performed through a right upper abdominal incision. Inflow vessels on the side of the resection were divided extra-parenchymally. Portal vascular inflow occlusion (Pringle maneuver) was performed selectively as clinically indicated. Liver transection was performed selectively as clinically indicated using an ultrasonic dissector in all cases. All patients received antibiotic prophylaxis pre- and post-operatively. Laparoscopic liver resection was performed in a similar manner, with the exception of access trocars being placed as per the surgeon's

preoperative planning, and parenchymal transection being performed with an additional energy device.

10 ml of whole blood in pre-cooled tubes were collected from the arterial line from each subject at each of three time-points: baseline (just after anaesthesia), six hours following liver resection, at 24 hours and 48 hours later. Measurements were performed in the hospital's Chemical Pathology laboratory.

Endpoints

The primary endpoint was acute liver injury as measured by serum ALT at 24 hours post-randomisation.

The secondary endpoints were: ALT at end of resection, Aspartate Aminotransferase (AST) at end of resection and at 24 hours, acute liver function assessed by Indocyanine Green (ICG) pulse densitometry at baseline in pre-admission clinic and at 24 hours, incidence and grade of liver failure assessed at day five, episodes of culture-confirmed sepsis, acute kidney injury, hospital length of stay, quality of life at baseline and at three weeks measured by Short Form (SF)-30 health survey questionnaire with the mental and physical component scales (SF-36 MCS and PCS), and 30-day mortality.

Acute kidney injury was graded as follows: Grade 1 was rise of serum creatinine by 150-200% or urine output <0.5ml/kg/hour for >6 hours; Grade 2 was rise of creatinine 200-300% or urine output <0.5ml/kg/hour for >12 hours; and Grade 3 was rise of creatinine >300% or urine output <0.3ml/kg/hour for >24 hours or anuria for 12 hours.

Postoperative liver failure was defined as present when prothrombin time (PT) was <50% normal and serum bilirubin was >50μmol/L at postoperative day 5. Its grading was defined as: Grade A requires no change in patient management; Grade B was requires deviation from usual management but does not require invasive therapy; Grade C requires invasive treatment.

Other postoperative complications were graded according to the commonly-used Clavien-Dindo classification.

Indocyanine Green pulse densitometry

ICG is a fluorescent dye eliminated exclusively by the liver and its elimination rate is used to evaluate global liver function. A slow intravenous bolus of 5mg/ml ICG (Limon, Pulsion, Munich, Germany) was given intravenously at a dose of 0.5 mg/kg. The blood concentration of ICG was measured via peripheral blood sampling 15 minutes after ICG bolus was administered. . Measurements were made at two time-points: baseline during pre-operative workup for surgery, and 24 hours following completion of liver resection. The results were recorded as plasma disappearance rate of ICG [ICG-PDR (%/min)] and ICG retention rate after 15 min [R15 %]. This was measured in a small subset of patients (n=17, selected based on the clinical discretion of the primary surgeon, who determined the need for ICG measurement for pre-operative determination of liver function based on clinical practice).

Sample size

Sample size calculation was based on the results from a previous trial of RIPC in major hepatectomy in patients with colorectal liver metastasis in which RIPC reduced the serum level of ALT by 41% when compared to control (412 ± 144 IU/L vs 698 ± 137 IU/L; P=0.026).[22] Although there was a reduction of 41% in that study we had powered for a more conservative

effect size of 20%. In order to reduce the 24-hour serum ALT level from 698 IU/L to 558 IU/L (SD 137 IU/L) with 90% power and a significance level of 0.05 (two-tailed test), we needed 21 subjects in each group or 42 subjects in total. We targeted to recruit 50 subjects to allow for 19% loss to follow-up.

Statistical analysis

Statistical analysis was carried out by an independent researcher blinded to the specific groups under evaluation. STATA version 15 (College Station, Texas, USA) was used for all analyses. The primary analyses were intention-to-treat. Clinical characteristics were presented as mean ± standard deviation (SD) for continuous data and counts and percentage for categorical data. Continuous outcome variables were expressed as median (interquartile range, IQR). Differences between RIPC and sham were tested using the Mann-Whitney U test for continuous data and Fisher's exact test for categorical data. The 48 hours area under the curve (AUC) was calculated for the liver enzymes.

Pre-specified subgroup analyses were performed based on age, diabetes comorbidity, presence of cirrhosis, performance of Pringle maneuver, major liver resection (three segments or more).

RESULTS

Trial profile and baseline characteristics

Figure 1 shows the CONSORT flow diagram. 247 patients were assessed for eligibility, of which 50 subjects were recruited. 156 patients were excluded for the following reasons: Resection for indications other than HCC (n=99), repeat resection (n=25), on metformin (n=21), impaired creatinine clearance (n=6) and no resection performed (n=5).

24 were randomized to the RIPC group and 26 to the control group. There was no cross-over or drop-out post-randomization. 17 patients had baseline ICG levels measurements, of whom 15 patients had a post-operative day 1 ICG levels measured.

The two groups were well-balanced for baseline characteristics (Table 1), except the duration of operation was incidentally found to be longer in the RIPC group (233±121.2 versus 178±82.1 minutes, p=0.046).

Primary and secondary endpoints

Table 2 shows the comparison of primary and secondary endpoints. Median ALT at 24 hours was not significantly different between RIPC and control groups (196 IU/L IQR 113.5-419.5 versus 172.5 IU/L IQR 115-298 respectively, p=0.61).

Figure 2 shows the absolute ALT concentrations over the 48 hours post partial hepatectomy in the RIPC and control groups. There was no significant difference in the areas under the curves for RIPC and control groups (p=0.38)

Table 3 shows the comparison of the primary endpoint in preplanned subgroups. There was no significant difference in median ALT or AST at 24 hours between RIPC and control groups in subgroups of age, presence of diabetes mellitus, presence of cirrhosis, and use of Pringles maneuver.

There were no significant differences between RIPC and control groups in any of the secondary endpoints analyzed. There were no adverse events arising from RIPC.

DISCUSSION

This sham-controlled randomized controlled trial of 50 HCC patients did not demonstrate a reduction of serum ALT levels 24 hours after partial hepatectomy with the use of RIPC.

These findings concur with the RIPCOLT trial[24], which randomized 40 liver transplant patients to either three 5-minute cycles of RIPC on the leg or sham RIPC, and found no significant difference in median AST at 72 hours. Our study, along with the RIPCOLT trial, was unable to reproduce the hepatoprotective effects found by two previous trials. Kanoria et al randomized 16 patients to RIPC (three 10-minute cycles on the leg) or sham and demonstrated significant reduction in ALT and AST immediately post-operatively and at 24 hours after resection of liver colorectal metastasis.[22] Similarly, Rakić et al found reduction in transaminases and bilirubin in both RIPC (three 5-minute cycles on the arm) and IPC (15-minute of portal triad clamping then 10-minute reperfusion) in resection of liver colorectal metastasis.[23]

However, this trial cannot conclude the absence of clinical hepatoprotective effect from RIPC, as there may be alternative reasons to explain the neutral results. First, a key consideration is the ubiquitous preference for propofol induction and inhalational maintenance in liver surgery among the anesthesiologists in our center. Unfortunately, these factors, especially propofol, have been strongly implicated with attenuation of the organ protective effect of RIPC.[12,13,17,25] In the same vein, co-morbidities (such as diabetes and neuropathy) and co-medications (such as beta-blockers) have too been suggested to abrogate the effect of RIPC.[12,13,25–27] The high proportion of diabetes mellitus in our sample population is especially problematic in this regard as diabetes has been shown to blunt the effect of RIPC in animal studies.[27] Even though evidence of this might have been apparent in the subgroup analyses, the sample size in the subgroups was small, increasing the risk of false negative findings. Finally, there was a finding that operative time was longer in the RIPC group, which may have affected the hepatoprotective

effect of RIPC. This could possibly be related to the higher proportion of laparoscopy versus open surgery in the RIPC group.

Second, the RIPC protocol used may be suboptimal. If RIPC were considered as one would a drug, its dosing, pharmacokinetics, and pharmacodynamics are poorly characterized.[15] In explaining the neutral results from their randomized trial of RIPC (three 5-minute cycles on leg) on liver transplantation, Robertson et al observed that hypoxia (venous $P0_2$<3kPa) was not achieved in the conditioned limb during the preconditioning stimulus. While the use of 5-minute RIPC cycles were successfully used in clinical cardioprotection trials, its extrapolation to surgical RIPC trials may be inadequate owing to high-flow oxygen used during induction and maintenance of anesthesia. In support of this view is that Kanoria et al's positive trial in resection of hepatic colorectal metastasis had employed a different dosage of three 10-minute cycles.[22] However, this explanation is contradicted by the Rakić trial which demonstrated benefit with only three 5-minute cycles.[23]

Third, the subjects enrolled in this trial were not high risk for severe IR injury. Two thirds of our subjects only received minor resections (defined as less than three segments of liver resected) while prior positive trials have demonstrated benefit in major resections.[22] In Rakić et al's positive trial, all patients received Pringle's maneuver, which in itself induces IR injury. This view is supported by the finding of low rates of complications including PLF in our subjects, and comparatively much lower levels of baseline and subsequent transaminitis compared to the positive trials. In particular, our study was powered to detect a reduction the 24-hour serum ALT level from 698 IU/L to 558 IU/L. The significantly lower baseline ALT levels in our patient population indicate that the possibility of the study being underpowered.However, as noted in the results, there was no significant difference in median ALT or AST at 24 hours between RIPC

and control groups in the pre-specified subgroups, including the performance of Pringle's maneuver.

There are several limitations to our study. First is the absence of standardization of anesthetic strategy in terms of anesthetic drugs, as well as triggers and patterns of blood product transfusion, as a difference in these factors between treatment groups, if present, would affect the endpoints measured. Secondly, as the optimal RIC protocol is yet to be characterized, it is difficult to conclude whether the neutral results are due to a suboptimal protocol. A strength of this study is that it is the first clinical trial investigating RIPC in HCC resection in which the majority have a background of chronic hepatitis. This trial also adds to a very small pool of trials which have investigated RIPC in liver surgery, and is, to our knowledge, the largest recruitment to date.

The presence of a hepatoprotective effect from RIPC has been postulated based on the demonstrated protection from IRI in many other organs, ranging from the heart, kidneys, brain, soft tissue grafts and lungs in a variety of experimental and clinical settings.[14,25] At the same time, local (non-remote) IPC (such as from portal triad clamping) have demonstrated benefits in liver surgery, with a 2016 Bayesian network meta-analysis suggesting reduced blood transfusion requirement and lesser operative time, but too few trials and patients to make conclusions on patient-oriented outcomes such as mortality, hospital stay or Intensive Therapy Unit (ITU) stay.[11] The mechanism of hepatoprotection by RIPC is not fully understood. From rodent models, it has been postulated that RIPC suppresses cytokine release, enhances production of hepatoprotective adenosine, and increases adenosine triphosphate (ATP) availability by slowing the rate of ATP depletion, thus leading to up regulation of the process of cellular ATP production and liver regeneration, and also reduce the liver apoptotic process.[28,29]

It should be pointed out that RIPC has an excellent safety profile, with large studies finding a small proportion of patients not being able to tolerate the resulting tourniquet pain, but no other adverse events reported.

CONCLUSION

This sham-controlled, single-blind randomized controlled trial of 50 HCC patients did not demonstrate a reduction of serum ALT levels 24 hours after partial hepatectomy with the use of RIPC. However, a patient population was heterogenous, and sample size may have been too small to show a statistically significant difference. Larger studies, with a well-defined patient population, focusing on clinically-relevant outcomes are warranted, although these are likely to require an exponentially larger sample size.

CONFLICT OF INTEREST

The authors declare that there are no potential conflicts of interests in relation to this study.

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