BEMS Reports, 2015; 1(2): 41-45
A Multifaceted Peer Reviewed Journal in the field of Pharmacology, Toxicology and Biomedical Reports
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Original Article

Advances in Reducing Liver Allograft Injury

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ABSTRACT

The shortage of organs for liver transplantation has led to the use of more extended criteria donor grafts and grafts recovered from Donation after circulatory death (DCD) donors. The former are more vulnerable to Ischemia-reperfusion injury (IRI) because of pre-existing pathology and the latter experience significant warm ischemia during organ recovery. Reducing the impact of IRI in extended criteria and DCD grafts is key to better outcomes in liver transplantation and could lead to expansion of the donor pool by increasing the utility of suboptimal organs. At each stage of the transplantation procedure there is opportunity to reduce the impact of IRI. This review discusses the underlying pathophysiology of IRI as it occurs in liver transplantation and summarises the advances that have been made in reducing human liver allograft injury through preconditioning, *ex vivo* conditioning and post-conditioning.

Key words: Liver transplantation, Ischemia-reperfusion injury, Organ conditioning.

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DOI: 10.5530/BEMS.1.2.4

INTRODUCTION

Due to the technical process of liver transplantation there is inevitable but varying degrees of ischemia-reperfusion injury (IRI) sustained by the liver graft. Due to a shortage of optimal donor organs, extended criteria liver grafts are increasingly utilised in liver transplantation but these grafts are much more susceptible to IRI due to pre-existing pathology.[1,2] There is no uniform definition of an extended criteria liver graft but risk factors such as an elderly donor, hypernatraemia, hypotension, steatosis and prolonged cold ischemia are accepted as being detrimental to graft function.[3]

Livers from donation after circulatory death (DCD) donors are also increasingly utilised in liver transplantation. In the UK, liver transplantation from DCD donors has increased steadily and during 2010/11 accounted for approximately 20% of liver transplant activity.[4] DCD donors are donors for whom death is declared on the basis of cardiopulmonary criteria rather than cessation of brain function.[5] Compared to procurement of liver grafts from donation after brainstem death (DBD) donors, DCD grafts suffer prolonged warm ischemic times and this contributes to a higher incidence of biliary complications, ischemic cholangiopathy, graft loss and mortality following transplantation.[6]

Reducing the impact of IRI on extended criteria and DCD grafts is therefore key to better outcomes in liver transplantation and could lead to expansion of the donor pool by increasing the utility of suboptimal organs. There are various periods during transplantation when the effect of IRI on the liver graft could be reduced. This includes a) prior to and during organ recovery (preconditioning) b) before and during organ transportation (*ex vivo* conditioning) and c) after implantation (post-conditioning) (Figure 1). This review will briefly discuss the underlying pathophysiology of IRI as it occurs in liver transplantation with particular reference to the DCD setting. Then it will summarise the advances that have been made in reducinghuman liver allograft injury through preconditioning, *ex vivo* conditioning and post-conditioning.

Ischemia-reperfusion injury

IRI is a complex multifactorial event that involves numerous cell populations and a multitude of pathophysiological processes including cell death, microvascular dysfunction, altered transcription and immune activation.[7] During liver transplantation warm ischemia is initiated *in situ* in the donor and this period is prolonged in the DCD setting. Here assent is obtained from the donor's family, then life-sustaining treatments are withdrawnleading to an "agonal" phase wereprogressive hypotension and hypoxia occur until circulatory arrest.[8] Warm ischemia during this period leads to metabolic disturbances including oxygen deprivation, glycogen depletion and adenosine triphosphate breakdown, which resultsin activation of Kupffer cells and initiation of parenchymal cell death.[2,9]

After a no-touch period, a super rapid laparotomy is usually performed and the organs are cooled *in situ* for procurement.[8] A variable period of cold ischemia then follows during *ex vivo* preservation. Hypothermia slows the metabolic activity in the liver by 1.5- to 2-fold for every 10°C drop in temperature and therefore reduces the rate of cell death in the graft.[10] However, it also depletes adenosine triphosphate, damages the actin cytoskeletonand injures the hepatic sinusoidal endothelial cells.[9,11] This disrupts the microcirculation of the graft and upon reperfusion endothelial cell swelling, vasoconstriction, platelet aggregation and leucocyte entrapment lead to impairment of blood flow in the liver sinusoids with subsequent entrapment of active blood components leading to a so called "no re-flow" phenomenon.[1,9]

After organ storage afurther period of warm ischemia occurs during the recipient procedure as vascular anastomoses are performed. Once these are completed and upon reperfusion there rapid cell death resulting in the release of damage associated molecular pattern molecules (DAMPs), activationof the complement system andgeneration of mitochondrial reactive oxygen species.[9] This is the catalyst for an immune response that involves infiltration of Kupffer cells, dendritic cells, T cells, natural killer cells and neutrophils. Pro-inflammatory cascades and

signallingpathways are thenup-regulated leading to cytokine release, expression of adhesion molecules and further reactive oxygen species resulting in additional cell damage and recruitment ofmore peripheral immune cells from the circulation.[1,2,9]

Preconditioning

Preconditioning is the concept of treating an organ in order to protect it, prior to a known impending injury and was first described in the heart by Murry *et al.* in 1986.[12] Since this first description many different preconditioning manoeuvres have been explored in an attempt to reduce the detrimental effects of IRI. In the context of organ transplantation, preconditioning usually refers to a donor treatment or treatment of the organ during procurement. The strategies utilised to date have included ischemic preconditioning, remote ischemic preconditioning, pharmacological preconditioning and normothermic regional perfusion.

Ischemic preconditioning

Ischemic preconditioning is a strategy aimed at reducing IRI in an organ by prior exposure of the organ to a short period of ischemia followed by reperfusion. Typically in the liver, ischemic preconditioning is implemented as one cycle consisting of 10 minutes of clamping of the portal triad, termed Pringle's manoeuvre, followed by 10–15 min of reperfusion (Figure 2). Although the exact protective mechanisms are unknown, ischemic preconditioning is thought to result in nitric oxide and adenosine release, which primes the liver against subsequent more prolonged bouts of ischemia.[13] A meta-analysis performed by Gurusamy *et al.* (2008) of randomised clinical trials (RCTs) comparing ischemic preconditioning versus no ischemic preconditioning during donor liver recoveries identified 3 trials and found no evidence to support the use of ischemic preconditioning.[14] Similarly, a more recent meta-analysis of 11 RCTs of ischemic preconditioning for elective liver resections under clamping also failed to a find a significant benefit of ischemic preconditioning.[15] As a result, attention has turnedto gaining the potential benefits of ischemic preconditioning but using sites remote from organ injury.

Remote ischemic preconditioning

In remote ischemic preconditioning, cellular protection of an organ is attempted by repeated temporary interruption of blood flow to a distant site. This has a benefit over local ischemic preconditioning in that it avoids an additional ischemic injury to the organ. Pilot studies have demonstrated a reduction in acute kidney injury after heart surgery following remote preconditioning of the leg.[16] However, a recent meta-analysis has failed to demonstrate a reduction in acute kidney injury across 10 RCTs.[17] Remote ischemic preconditioning studies in deceased organ donors have not yet been fully reported. Preliminary results of an on-going RCT (NCT01515072) have been presented though. In this trial donors have been randomised to control or remote ischemic preconditioning consisting of 4 cycles of mid-thigh cuff inflation and deflation (5 min/5 min each) after brain death declaration and again at organ recovery. Provisional results from 106 liver transplantations has shown a significant decrease in peak aminotransferases in recipients with cold ischemic times >5 hours.[18]

Pharmacological preconditioning

Pharmacological preconditioning has advantages over a physical preconditioning strategies as an ischemic injury with associated negative consequences is avoided and time-consuming, operator-dependent pre and intra-operative manoeuvres are not required.[19] Despite the development of a multiple of agents protective against liver IRI in experimental models, only few have demonstrated efficacy in human RCTs.[20]

The pharmacological preconditioning strategies used in clinical practice have also varied in terms of the routes used to administer the protective agents. One route of administration of pharmacological preconditioning agents has been via organ flushing. Arora *et al.* (1999) randomly assigned 50 liver grafts to either pre-rinse with standard plasmalyte solution or plasmalyte containing glycine solution prior to reperfusion. A significant reduction in alanine transaminase was found during the first 3 days post liver transplantation in the glycine rinse group.[21]

Alternatively the donor can be systemically pre-treated by intravenous injection. Klein *et al.* (1999) evaluated intravenous donor pre-treatment with the prostaglandin epoprostenol in an RCT of 106 liver donors and observed significantly reduced peak values of transaminases in the recipient after transplantation.[22] Kotsch *et al.* (2008) randomized 100 deceased donors to intravenous methyl prednisolone or control prior to organ donation. Donors treated with methyl prednisolone had a reduction in serum cytokine expression and patients who received grafts from the methyl prednisolone group had significantly reduced levels of aspartate amino-transferase following transplantation.[23]

A combination of organ flushing and systemic treatment of either the donor or recipient has been utilised. Khan *et al.* (2005) administered N-acetyl-cysteine intravenously and via portal flush in a RCT of 18 liver donors but observed no protective effects.[24] Baskin-Bey *et al.* (2007) performed a RCT of 99 liver transplant recipients that assessed the pan-caspase inhibitor IDN-6556 in flush/organ storage solution, intravenously in the recipient and by both routes of administration. There was decreased apoptosis and significantly reduced transaminases only in the study group with IDN-6556 in flush/organ storage solution.[25] Busuttil *et al.* (2011) randomized 47 liver grafts to a pre-implantation flush with the recombinant p-selectin glycoprotein ligand IgG (rPSGL-Ig) or placebo and an intravenous dose of rPSGL-Ig or placebo prior to arterial reperfusion. In recipients with a high donor risk index there was a significant reduction in aspartate aminotransferase after surgery and across the whole treatment group there was an improved biomarker profile (augmented interleukin-10 and reduced C-X-C motif chemokine 10).[26]

Finally, inhalation of anaesthesia is a further method of administering pharmacological preconditioning agents. Minou *et al.* (2012) randomised 60 deceased organ donors to inhaled anaesthesia with the volatile anaestheticse voflurane oranaesthesia without a volatile anaesthetic. Sevoflurane significantly reduced serum transaminases and early allograft dysfunction following liver transplantation. This was the first clinical trial to demonstrate a protective effect of sevoflurane preconditioning during organ procurement on graft function in liver transplantation.[27] However, Beck-Schimmer *et al.* (2008) have previously demonstrated that pharmacological preconditioning with sevoflurane reduces liver IRI following liver resection under continuous inflow occlusion. This was evidenced by a significant reduction in post-operative transaminases and a reduction in post-operative complications.[28]

To date, none of the above pharmacological preconditioning strategies have been widely adopted in clinical practice. Due to the complexity of liver IRI, particularly in the context of transplantation, it may be that pharmacological treatments that are effective in pre-clinical models are challenging translate into human trials. It could also be the case that trials to date have included relatively low numbers of participants and have therefore have been unable to demonstrate the full efficacy of treatments. In numerous preconditioning trials across small numbers of participants, reductions in serum transaminase levels have been seen, but better powered studies are required to demonstrate improvement in clinical endpoints such as early graft function. Better designed multicenter RCTs in transplantation with greater participant numbers could therefore assist with improving the translation of experimental treatments.

Normothermic regional perfusion

In the setting of DCD organ recovery, normothermic regional perfusion is an emerging technique that establishes a regional perfusion circuit including a heat exchanger, oxygenator and pump in the organ donor. Instead of rapidly removing the abdominal organs, normothermic regional perfusion supplies the donor's abdominal organs with oxygenated donor blood, which may enable the organs to better tolerate cold storage prior to transplantation. This technique was established in Barcelona in the setting of uncontrolled DCD donation and now preliminary data from single centre studies suggest that outcomes could also be improved following controlled DCD liver transplantation if normothermic regional perfusionis administered during organ recovery.[29]

Fondevila *et al.* (2007) utilised a normothermic regional perfusion protocol for Maastricht category II uncontrolled DCD donors.[30] The grafts recovered have now been used in 42 liver transplants with a one-year graft survival rate of 73%, one-year recipient survival rate of 81%, biliary complication rate of 17% and a 7% rate of re-transplantation for ischemic cholangiopathy.[29] Jimenez-Galanes *et al.* (2009) also utilised a normothermic regional perfusion protocol for Maastricht category II uncontrolled DCD donors. The grafts recovered were used in 20 liver transplants with a one-year graft survival rate of 80%, one-year recipient survival rate of 86%, re-transplantation rate of 15%, primary non-function rate of 10% and ischemiccholangiopathy rate of 5%.[31]

Rojas-Pena *et al.* (2014) have now utilised normothermic regional perfusion for Maastricht category III controlled DCD donors and performed 13 liver transplants using this technique observing a one-year graft survival rate of 86%, a two-year graft-survival rate of 71% and a 14% primary non-function and biliary stricture rate.[32] Concomitant dual temperature organ recovery is novel approach that could further expand the use of normothermic regional perfusion in the controlled DCD setting. This technique has been applied and enabled prolonged periods of normothermic regional perfusion but with rapid lung procurement thus avoiding topical cooling of the liver by non-ventilated lungs.[33]

Ex vivo conditioning

Currently static cold storage is the standard method of preserving liver grafts for transplantation. Organs are flushed and cooled with preservation solutions at 4°C to reduce metabolic activity and prevent cellular swelling.[10] Hypothermic machine perfusion has now surfaced as an alternative preservation technique. During preservation, hypothermic machine perfusion provides a continuous circulation of filtered preservation solution and metabolic substrates, anti-oxidants and colloids to the vasculature of the liver graft. This stabilises the microvascular tone and improves adenosine triphosphate availability on reperfusion. A 'washout effect' removes and dilutes waste products preventing direct endothelial and parenchymal cell contact with toxic substrates.[11,34] In kidney transplantation hypothermic machine perfusion is more established than in liver transplantation and appears to reduce delayed graft function compared with static cold storage.[35]

Guarrera *et al.* (2010) performed the first prospective hypothermic machine perfusion study in liver grafts. In total they included 20 adults who had received hypothermic machine perfusion-preserved livers from DBD donors. There were no cases of primary non-function, early allograft dysfunction rates were 5% and biliary complications occurred in 10%. Compared to a matched group of patients, transplanted with cold storage livers serum injury markers were significantly lower and so was mean hospital stay in the hypothermic machine perfusion group.[34] Dutkowski *et al.* (2014) have since went on publish the results of eight liver transplants using Maastricht category III controlled DCD liver grafts that received hypothermic oxygenated perfusion for 1–2 h prior to implantation through the portal vein. They observed outstanding early graft function after transplantation with the release of liver enzymes, kidney function, intensive care unit and hospital stay comparable or better than in matched DBD liver grafts. Furthermore, no evidence of intrahepatic biliary complications occurred during a median follow up of 8.5 months.[36] Sub-normothermic and normothermic *ex-vivo* perfusions are newer machine perfusion options that respectively offer partial and full metabolic supportto the liver graft with the possibility to assess graft viability before transplantation. However, to date these techniques have only been used experimentally on discarded human livers unfit for transplantation.[37,38]

Treating transplant donors raises ethical questions, and a detrimental impact on other organs by systemic treatments must be excluded in the context of multi-organ donation.[1] However, with the emergence of *ex vivo* conditioning there is now the potential for liver grafts to be optimized during organ storage.[38] Pharmacological intervention without potential negative systemic effects could be possible during *ex vivo* conditioning. If normothermic *ex-vivo* perfusion is developed further this could be particularly promising area of drug development since test compounds are more likely to be fully activeat normal temperatures.[1] Indeed, with the advent of normothermic oxygenated perfusion systems such as Organox (Organ Ox Ltd, Oxford, UK) and Organ Assist's (Groningen, Netherlands) Extra Corporal Organ Procurement System significant opportunities for *ex vivo* pharmacological conditioning may develop in the near future.

Pharmacological post-conditioning

A number of studies have assessed treatments administered to the recipient of the liver graft either by pre-rinse of the organ prior to implantation or systemically by intravenous injection. St Peter *et al.* (2003) randomly assigned 20 human liver grafts to either pre-rinse with standard plasmalyte solution or plasmalyte containing tacrolimusprior to reperfusion. They showed that after liver transplantation peak changes from baseline aspartate aminotransferase and activated partial thromboplastin time values were significantly improved by tacrolimus.[39] Kristo *et al.* (2011) performed a RCT of 26 liver transplant recipients comparing an intraportal perfusion of tacrolimus or placebo during liver transplantation. Tacrolimus treatment suppressed inflammation and immune response in the transplanted liver on a genome-wide basis but did not result in a reduction in serum transaminases.[40]

In a RCT of 22 cadaveric liver transplant recipients, Bogetti *et al.* (2005) assessed the ability of thymoglobulin to protect against IRI when administered intravenously during the anhepatic phase of transplantation and post-operatively. Significantly decreased levels of alanine aminotransferase were noted on day 1 after surgery.[41] Lang *et al.* (2007) performed a RCT of 20 patients comparing inhaled NO and placebo during liver transplantation and found significantly decreased hospital length of stay, serum transaminases and coagulation times in the NO group.[42] Finally, a meta-analysis performed by Cavalcanti *et al.* (2011) assessed 10 RCTs comparing perioperative prostaglandin E1 or E2 versus placebo or standard treatment for adult patients undergoing liver transplantation but failed to demonstrate any reduction in mortality, primary non-function or re-transplantation.[43]

In common with pharmacological preconditioning, none of the above pharmacological post-conditioning strategies described have been implemented routinely in clinical practice.

CONCLUSION

The shortage of organs for liver transplantation has led to the use of more extended criteria donor grafts or grafts recovered from DCD donors. The former are more vulnerable to IRI because of pre-existing pathology and the latter experience significant warm ischemia during organ recovery.

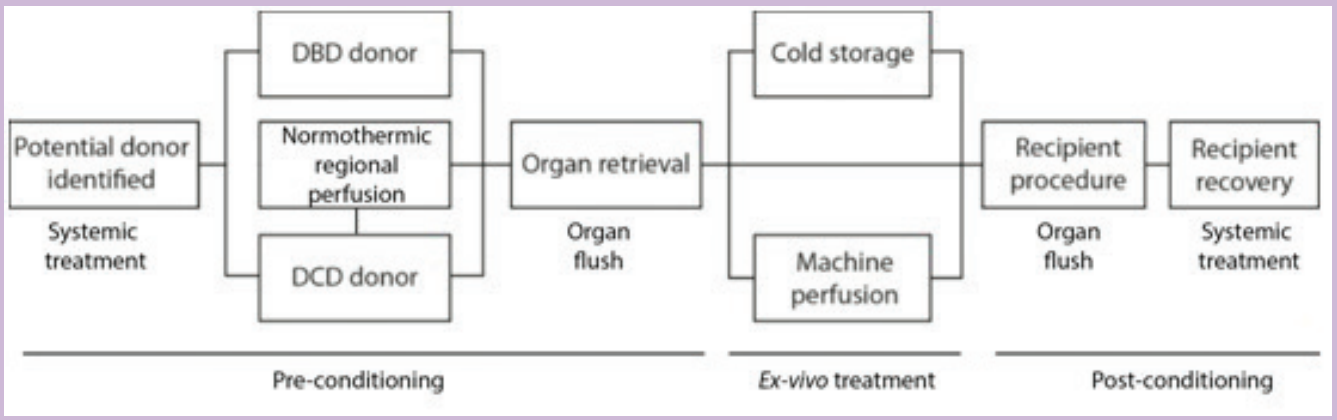

Figure 1: Periods during transplantation when the impact of IRI on the liver graft could be reduced.

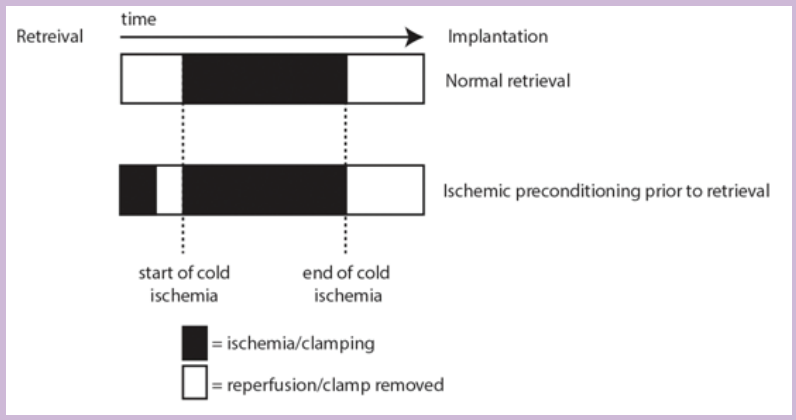

Figure 2: Ischemic preconditioning used prior to organ retrieval.

It is imperative liver allograft injury is reduced and at each stage of the transplantation procedure there is opportunity to reduce the impact of IRI. In future, a combination of preconditioning, *ex vivo* conditioning and post-conditioning strategies could be employed to improve liver graft function. This may involve multiple modalities and potentially overlapping such as pharmacological therapies, normothermic region perfusion systems and *ex-vivo* machine perfusion.

ABBREVIATIONS USED

IRI: Ischemia-reperfusion injury, **DCD:** Donation after circulatory death, **DBD:** Donation after brainstem death, **RCTs:** Randomised clinical trials.

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SUMMARY

- Reducing the impact of IRI in extended criteria and DCD grafts is key to better outcomes in liver transplantation.
- At each stage of the transplantation procedure there is opportunity to reduce the impact of IRI through preconditioning, *ex vivo* conditioning and post-conditioning.

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