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ORIGINAL ARTICLE

Hyperbaric oxygen therapy reduces the severity of ischaemia, preservation and reperfusion injury in a rat model of liver transplantation

Nhut Quang Tran[1], Caterina Malcontenti-Wilson[1], Soukena Hammoud[1], Ian Millar[2], Chris Christophi[1] & Vijayaragavan Muralidharan[1]

[1]Department of Surgery, The University of Melbourne, Austin Health, Heidelberg, Vic, Australia and [2]Hyperbaric Unit, Alfred Hospital, Prahran, Vic, Australia

Abstract

Background: Approaches to increase organ availability for orthotopic liver transplantation (OLT) often result in the procurement of marginal livers that are more susceptible to ischaemia, preservation and reperfusion injury (IPRI).

Methods: The effects of post-OLT hyperbaric oxygen (HBO) therapy on IPRI in a syngeneic rat OLT model were examined at various time-points. The effects of IPRI and HBO on hepatocyte necrosis, apoptosis, proliferation, and sinusoidal morphology and ultrastructure were assessed.

Results: Post-OLT HBO therapy significantly reduced the severity of IPRI; both apoptosis [at 12 h: 6.4 ± 0.4% in controls vs. 1.6 ± 0.7% in the HBO treatment group ($p < 0.001$); at 48 h: 2.4 ± 0.2% in controls vs. 0.4 ± 0.1% in the HBO treatment group ($p < 0.001$)] and necrosis [at 12 h: 18.7 ± 1.8% in controls vs. 2.4 ± 0.4% in the HBO treatment group ($p < 0.001$); at 48 h: 8.5 ± 1.3% in controls vs. 3.4 ± 0.9% in the HBO treatment group ($P = 0.019$)] were decreased. Serum alanine transaminase was reduced [at 12 h: 1068 ± 920 IU/l in controls vs. 370 ± 63 IU/l in the HBO treatment group ($P = 0.030$); at 48 h: 573 ± 261 IU/l in controls vs. 160 ± 10 IU/l in the HBO treatment group ($P = 0.029$)]. Treatment with HBO also promoted liver regeneration [proliferation at 12 h: 4.5 ± 0.1% in controls vs. 1.0 ± 0.3% in the HBO treatment group ($p < 0.001$); at 48 h: 8.6 ± 0.7% in controls vs. 2.9 ± 0.2% in the HBO treatment group ($p < 0.01$)] and improved sinusoidal diameter and microvascular density index.

Conclusions: Hyperbaric oxygen therapy has persistent positive effects post-OLT that may potentially transfer into clinical practice.

Keywords

ischaemia–reperfusion, transplant, resection, liver, transplant outcomes

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Correspondence

Vijayaragavan Muralidharan, Department of Surgery, The University of Melbourne, Austin Health, Level 8, Lance Townsend Building, Studley Road, Heidelberg, Vic 3084, Australia. Tel: + 61 3 9496 3574. Fax: + 61 3 9458 1650. E-mail: muv@unimelb.edu.au

Introduction

Approximately 800 000 people die from end-stage liver disease around the world each year.[1] Orthotopic liver transplantation (OLT) is widely accepted as the definitive treatment in end-stage liver disease, selected liver malignancies and acute liver failure. The major limitation of liver transplantation is the availability of suitable donor organs. Increasing demand and rising mortality in patients awaiting transplantation have led to a number of techniques that increase the availability of donor organs.[2–5] However, these result in the procurement of organs with marginal functional capacity. Marginal donor organs are more susceptible to the effects of ischaemia, preservation and reperfusion injury (IPRI), which leads to an increased incidence of dysfunction and organ loss following transplantation.[6]

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Ischaemia, preservation and reperfusion injury is a complex process characterized by intracellular energy depletion leading to activation of the innate immune system, which ultimately affects parenchymal and non-parenchymal cells of the donor organ.[7] Therapies targeting specific aspects of IPRI have been developed and shown to ameliorate such injury in experimental[8–11] and some clinical[12] settings. Their effects have been demonstrated predominantly when applied prior to the onset of IPRI. Hyperbaric oxygen (HBO) therapy is one such modality that appears to simultaneously impact on multiple aspects of IPRI and to be effective when applied after its onset.[13] The majority of evidence arises from *in vitro* studies and *in vivo* studies of warm ischaemia–reperfusion injury. The effects of HBO in a more realistic model of OLT, particularly in the presence of cold preservation as seen in clinical liver transplantation, remain unknown. This study investigates the impact of HBO therapy in a rat model of OLT, when HBO treatment is delivered after the onset of IPRI.

Materials and methods

In this study, irreversible hepatocyte injury was assessed according to measurements of hepatocyte necrosis and apoptosis. Hepatocyte proliferation was measured as a potential surrogate marker of injury. Liver biochemistry was analysed at the relevant timepoints. Changes in sinusoidal architecture and their relation to hepatocyte injury were assessed with scanning electron microscopy (EM) analysis of microvascular resin casts. Effects on endothelial cell and hepatocyte morphology were determined using transmission EM.

Animals and study design

Male Lewis rats (weighing 250–350 g; Laboratory Animal Services, University of Adelaide, Adelaide, SA, Australia) were housed in a temperature- and humidity-controlled room under a constant 12 : 12 h light : dark cycle. Six animals were randomly assigned to each group for this study. Four animals per group were used for scanning EM analysis. Animals had free access to food and water until surgery. All studies were conducted with the approval of the Austin Animal Ethics Committee and in compliance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

The severity of IPRI (hepatocyte necrosis, apoptosis and proliferation) was assessed in rat treatment and control groups. The effects of HBO therapy were also investigated in normal rat liver. In addition, animals underwent immediate OLT without a preservation period and were assessed at 48 h. Outcomes in this group were compared with those in rats that underwent OLT with 24-h preservation to investigate the effects of prolonged preservation on IPRI. The temporal progression of liver injury severity was investigated in animals receiving livers with a preservation time of 24 h and killed at specific endpoints of 6 h, 12 h, 24 h, 48 h and 7 days after OLT. The effects of IPRI and HBO therapy on liver sinusoidal architecture were assessed by scanning and transmission EM at 48 h after OLT. Treatment with HBO began within 3 h of OLT as this regimen has been shown to significantly reduce IPRI in a rat model.[13] Rats in the HBO treatment groups received two (12 h), three (24 h), five (48 h) or 15 (7 days) treatment sessions.

Donor hepatectomy and storage procedure

An orthotopic syngeneic liver transplant model with a 24-h period of preservation in University of Wisconsin (UW) solution was chosen as the model for IPRI in this study as it is highly reproducible and features the same mechanisms and pathophysiology as human liver transplantation. The donor hepatectomy procedure has been described previously.[14] Briefly, male Lewis rats were anaesthetized with 1.5% isoflurane (David Bull Laboratories Pty Ltd, Mulgrave, Vic, Australia) and continuous anaesthesia was maintained using face masks. Laparotomy was undertaken, the liver mobilized and the vascular system flushed with heparin prior to perfusion with cold UW solution. Flushed livers were placed in 30 ml of UW solution at 4 °C and stored for 24 h at 4 °C prior to transplantation.

Liver transplantation

Recipient rats were anaesthetized and subjected to midline laparotomy. Recipient hepatectomy was performed and recipient vessels prepared for transplantation. Donor livers were removed from the preservation solution and OLT performed using an arterialized technique previously described by Howden *et al.*[14] The anhepatic time during the transplantation procedure was limited to <15 min in order to produce an extremely low mortality rate despite the severity of liver injury.[15]

Administration of HBO therapy

Rats were recovered from anaesthesia and transferred to the HBO chamber within 3 h of liver reperfusion. Treatment with HBO was continued twice daily at 12-h intervals until the selected endpoints. Each treatment session (153 kPa with 100% oxygen) was delivered for 90 min, which is the standard treatment time adopted for rodent experiments by this study group.[16]

Collection of liver samples

The study endpoints were defined as 12 h, 24 h and 48 h after reperfusion. Rats were anaesthetized with an intraperitoneal injection of 400 µl ketamine (100 mg/kg) (Pfizer Australia, West Ryde, NSW, Australia) and xylazine (10 mg/kg) [Troy Laboratories (Australia) Pty Ltd, Glendenning, NSW, Australia]. The liver was removed from the anaesthetized rat, weighed and fixed in 10% buffered formalin (Sigma-Aldrich Corp., St Louis, MO, USA). Because of the hepatic anatomy and the variable sizes of the