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Remote preconditioning improves hepatic oxygenation after ischaemia reperfusion injury

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Summary

Hepatic ischaemia reperfusion injury (IRI) lowers hepatic oxygenation and induces tissue acidosis. Remote ischaemic preconditioning (RIPC) reduces hepatic IRI through increased hepatic blood flow but its effect on hepatic oxygenation and acidosis is not known. This study investigates these effects through near infrared spectroscopy (NIRS). Twenty-four NZ rabbits were grouped into four: sham, RIPC, IRI alone, RIPC + IRI. RIPC was induced through three cycles of 10 min ischaemia and reperfusion to the limb. Total hepatic ischaemia was produced by complete portal inflow occlusion for 25 min. Serum transaminases, bicarbonate and hepatic venous nitrite/nitrate (NO_x) levels were measured 2 h postreperfusion. Hepatic oxygenation was monitored with NIRS. At 2 h post reperfusion, IRI alone resulted in reduced mitochondrial oxygenation (CytOx CuA Redox), serum bicarbonate, hepatic venous NO_x with an increase in serum transaminases and hepatic deoxyhaemoglobin levels. RIPC before IRI caused significant improvement in mitochondrial oxygenation ($P = 0.01$), increased serum bicarbonate ($P = 0.02$), hepatic venous NO_x ($P = 0.025$) with a decrease in serum transaminases ($P = 0.04$) and hepatic deoxyhaemoglobin levels ($P = 0.03$). There was a positive correlation ($P = 0.02$) between hepatic venous NO_x levels and mitochondrial oxygenation. RIPC before IRI improves hepatic mitochondrial oxygenation and reduces acidosis and currently undergoing clinical study.

Introduction

Ischaemia reperfusion injury (IRI) during liver transplantation results in local injury to the liver and systemic organ injury as a result of free radical production, nitric oxide (NO) depletion and release of cytokines and chaemokines. Direct or classical ischaemic preconditioning (IPC) of the liver reduces the adverse effects of IRI [1] and improve hepatic tissue oxygenation [2]. However, direct IPC has been shown to impair liver regeneration [3,4] which may be detrimental in living related liver transplantation. Direct IPC induces a transient hyperaemia which can have an adverse effect on marginal donor livers and this requires further investigation in experimental models of marginal liver transplantation. Novel methods which avoid even minor degrees of direct stress to the organ, such as remote ischaemic preconditioning (RIPC) has been shown to reduce IRI to the [5] myocardium, the liver [6,7] and other organs [8] in experimental studies and recently in patients undergoing cardiac [9] and major vascular surgery [10]. The mechanisms of RIPC are not clear and an understanding of its effects on the hepatic microcirculation and oxygenation is of potential importance in human liver transplantation [11]. This experiment was conducted to study the effect of RIPC on hepatic tissue oxygenation using near infrared spectroscopy (NIRS), a validated noninvasive technique [12] which can be applied clinically.

The severity of IRI following liver transplantation, correlates positively with a low hepatic venous oxygen

saturation [13] and for optimal graft function and survival adequate tissue oxygenation is essential [14]. NIRS monitors hepatic tissue oxygenation [15] and is a reliable measure of microcirculatory impairment and liver dysfunction induced by IRI [16]. These measurements correlate closely with the results of direct hepatic venous O_2 sampling [17] and the severity of liver IRI can be reliably assessed by measuring changes in tissue chromophores; oxyhaemoglobin (O_2Hb), deoxyhaemoglobin (HHb) and the redox state of cytochrome oxidase (CytOx CuA) [18].

The distribution of lactate in body compartments is regulated through complex mechanisms and with the liver producing and utilizing lactate, serum lactate and bicarbonate levels do not reflect hepatic tissue acidosis accurately [19–21]. NIRS derived oxygenation indices of haemoglobin (Hb) can help determine shifts in the O_2Hb dissociation curve and a right shift in the presence of other constant parameters reflects tissue acidosis [21].

The vascular endothelium in the liver plays an important role in maintaining the liver microcirculation through the release of NO which is depleted during liver IRI [22]. In a previous study, we have shown that hind limb RIPC, prior to liver IRI reduces the adverse effects of liver IRI through an increase in NO levels and total hepatic blood flow [6]. However, blood flow measurements do not give information on hepatic tissue oxygenation which is a key determinant of the severity of IRI. The primary hypothesis of this study was to investigate the effects of RIPC on hepatic tissue oxygenation using NIRS, and secondarily on hepatic tissue acidosis.

Materials and methods

Experimental design

The study was conducted under a project licence with ethical approval from the UK home office in accordance with the Animals (Scientific Procedures) Act 1986. Male New Zealand white rabbits (n = 24, 3.2 kg ± 0.3 kg) were allocated to four groups (n = 6/group). In all four groups laparotomy was performed. *Group 1 (Sham operated):* Laparotomy was performed without total portal inflow occlusion or leg ischaemia. *Group 2 (RIPC):* The right hind limb was subjected to three alternate cycles of 10 min ischaemia and 10 min reperfusion, and laparotomy was performed without total portal inflow occlusion. *Group 3 (Total Hepatic IRI):* Total hepatic ischaemia was produced for 25 min followed by 2 h of reperfusion by total portal inflow occlusion which included occluding both the hepatic artery and portal vein [23]. *Group 4 (RIPC + Total Hepatic IRI):* The right hind limb was subjected to three alternate cycles of 10 min ischaemia and reperfusion, followed 5 min later by total hepatic ischaemia for 25 min and 2 h reperfusion. Most animal models for studying hepatic ischaemia involve partial occlusion to blood flow to avoid mesenteric congestion. The present model of total hepatic ischaemia without a porto-systemic shunt closely resembles human operative conditions during liver transplantation where shunts are not used routinely [23]. *Animal preparation:* Animals were maintained in a temperature controlled environment with 12 h light–dark cycle and were fasted (with water allowed) for 24 h before the study. Anaesthesia was induced with an intramuscular injection of 0.3 mg/kg of Hypnorm (0.095 mg/kg fentanyl citrate and 3 mg/kg fluanisone; Janssen Pharmaceutica NV, Beerse, Belgium). A 3 mm endo-tracheal tube was inserted through the trachea and connected to a continuous flow to-and-fro CO_2 absorbing system. Two to 3 L/min of Oxygen (O_2) was delivered to maintain O_2 saturation above 95%. Anaesthesia was maintained with 1–1.5 L/min of Nitrous Oxide and 0.5–1% iso-flurane. Body temperature was maintained at a constant of 37 °C with a heating pad (Harvard Apparatus Ltd., Kent, UK) connected to a rectal temperature probe. A 22 g polyethylene cannula was inserted in the ear artery and connected to a pressure transducer (Type F-LMP1, Datex-Ohmeda, Helsink, Finland) for mean arterial blood pressure monitoring and blood sampling. A 24 g polyethylene cannula was inserted in the marginal vein of the other ear for administration of 0.9% normal saline at a rate of 15 mL/kg/h. *Surgery:* Surgical procedure was the same in all four groups. Following laparotomy the portal vein was mobilized for placement of a 3 mm ultrasonic flow probe (Transonic Systems Inc. New York, USA). An atraumatic vascular loop was then placed around both the hepatic artery and portal vein. Two NIRS probes containing optical fibres were placed on the right lobe of the liver with a 10-mm separation and were held in place using a flexible probe holder for continuous measurement. The abdomen was covered with a plastic wrap to prevent fluid evaporation. A small incision was made over the right calf and a Laser Doppler Flow (LDF) probe (DRT4, Moor Instruments Limited, Axminster, UK) was placed directly on the calf muscle. A pneumatic tourniquet was placed around the right thigh. For inducing RIPC the tourniquet was tightened until LDF was < 5 flux units. The animals were euthanized at the end of the experiment by exsanguination.

Measurements

Blood sampling

Whole blood sampled from the ear artery was collected in precooled tubes at baseline, at the end of liver ischaemia and at the end of 2 h of reperfusion. Serum and plasma were separated and stored at −80 °C. Serum alanine aminotransferase (ALT), lactic dehydrogenase (LDH) and bicarbonate were measured using an automated clinical chemistry analyser (Hitachi 747, Roche Diagnostics Ltd,

Sussex, UK). Hepatic venous blood was taken after 2 h of reperfusion for measuring nitrate and nitrite (NO_x), using a modified Greiss assay. The Griess assay provides an estimate of total NO production by measuring its conversion to the nitrite ion and like all assays for NO has limitations. It is not suitable for *in vivo* detection of NO and sensitivity is limited if plasma is not properly deproteinated. Other assays, such as chemiluminescence is more sensitive, but require purging samples with inert gas, electrochemical methods measure NO only at the tip of the electrode and EPR-based techniques detect only micro molar concentrations of NO. As all techniques for NO measurement have their limitation, a modified Griess technique was chosen for it is validated, allows real time measurement of NO *in vitro* and the sensitivity is increased with proper deproteination of plasma, overcoming some of the drawbacks of the original procedure [24,25]. Plasma was first deproteinated using a 12 kDa centrifugal filter and nitrate reduction conducted by the method of Miranda *et al.* [26]. NO_x levels were measured in a 96 well plate reader (Tecan Sunrise, Maennedorf, Switzerland) and compared against a standard curve.

Measurement of hepatic tissue oxygenation

In all experimental groups changes in chromophore concentration were measured continuously by NIRS instrument (NIRO-500, Hamamatsu Photonics KK, Hamamatsu, Japan) [15] which produces NIR light at four wavelengths [27]. The choice of wavelengths is based on a reference wavelength of 900 nm, the absorption maximum of oxidized Cyt Ox at 845 nm, Hb at 765 nm and a isobestic wavelength of 810 nm, where the extinction coefficients of O_2Hb and HHb are equal and used to calculate Hb concentration independent of O_2 saturation [28].

In the visible region of the spectrum (450–650 nm) light is strongly attenuated, but in the NIR region (700–1000 nm) a significant amount of radiation is transmitted up to a distance of 10 cm [29]. NIRS relies upon the relative transparency of biological tissue to light in this region [28] and the different absorption spectrum of tissue chromophores, such as O_2Hb, HHb and CytOx allow spectroscopic separation of the compounds [15]. Cyt Ox is the terminal enzyme of the mitochondrial respiratory chain and catalyses the reduction of O_2 to H_2O with synthesis of ATP [30]. It contains four redox active groups, two haem iron (haem a and a_3) and two copper (Cu_A and Cu_B) which are optically active with different absorption characteristics, and NIR absorption occurs primarily at the oxidized Cu_A [31]. A change in the redox state of Cu_A reflects changes in mitochondrial oxygenation [32,33] and ATP [34]. Changes in the absorption of NIR light reflect changes in oxygenation of Hb in the microcirculation [35] and changes in HHb represent the state of venous outflow and increases with its impairment [21]. Changes in total Hb (HbT) (Δ [O_2Hb + Hb]) reflect changes in blood volume and increases with impedance to the venous outflow [29]. Changes in (HbD) Oxygenation Index (Δ [O_2Hb-HHb]) reflect net changes in haemoglobin oxygenation independent of blood volume changes [21,29,36].

NIR optical light is transmitted in sequential pulses to the liver through one probe, and emerging photons are collected by the other and detected by a photomultiplier tube. The difference between transmitted and received light intensity is used to determine the optical density changes at each wavelength. These changes are used to compute changes in chromophore concentration by a NIRS algorithm based on diffusion theory [17,18,37]. The absolute concentration of tissue chromophores remains unknown and cannot be calculated, but the calculation of light attenuation and its relationship between optical absorption and chromophore concentration are described by a modified Beer–Lambert's law [15]. This is used to convert the obtained optical densities to concentration changes of HHb, O_2Hb and Δ Cyt Ox in μmole/L per optical path length [28,38]. The sum of the two variables is related to changes in HbT, whereas the difference between the two variables is the oxygenation index (HbD) which correlates highly with venous O_2Hb saturation [21]. The NIRS data were calculated as 1 min averages at baseline, beginning of ischaemia, end of ischaemia and at 2 h reperfusion and measurements expressed as concentration changes (μmole/L) from baseline. These represent volume-averaged values in the segment of tissue under consideration and average Δ [HHb], Δ [O_2Hb], Δ [HbT] and Δ [HbD] are calculated [21].

Data analysis and statistics

Time course data were compared between groups by repeated measures two-way analysis of variance by ranks (ANOVA). Analyses from samples collected at the termination of the experiment were compared using Mann Whitney *U*-test. Bonferroni corrections were applied as appropriate where multiple groups were tested. *P*-values are quoted for each comparison and data expressed as mean ± SD. The relationship between NO_x, hepatic oxygenation and PVF were tested using linear regression and Spearman's rank correlation coefficient analysis. All statistical analyses were conducted using Graphpad Prism V3 (GraphPad Software, San Diego, CA, USA).

Results

Peripheral oxygen saturation (SaO_2%)

In the sham and RIPC only groups SaO_2% remained constant throughout the experiment with no significant

Table 1. Measurement changes in the experimental groups.

	Sham (Gr 1)	RIPC (Gr 2)	IRI (Gr 3)	RIPC + IRI (Gr 4)	RIPC + IRI vs. IRI
Qripheral oxygen saturation (SaO_2%)					
Immediately following occlusion*	99 ± 0.30	99 ± 0.4	95 ± 1.6	96 ± 1.1	$P = 0.001$
of Ischaemia	100 ± 0.32	99 ± 0.3	90 ± 1.9	92 ± 2.2	
A 2 h reperfusion	99 ± 0.37	99 ± 0.8	92 ± 2.1	99 ± 0.7	
Serum ALT (U/L)					
Baseline	22.5 ± 0.9	25.7 ± 5.3	30.8 ± 2.5	30.8 ± 4.3	$P = 0.04$
End of Ischaemia	19.7 ± 1.0	25.0 ± 5.9	35.1 ± 3.6	33.5 ± 3.4	
At 2 h reperfusion	16.8 ± 1.1	16.7 ± 2.7	50.0 ± 9.8	29.4 ± 6.7	
Serum LDH (U/L)					
Baseline	304.2 ± 24.4	321.5 ± 51.5	319.2 ± 34.7	328.2 ± 20.8	$P = 0.01$
End of Ischaemia	324.0 ± 31.6	307.3 ± 19.6	442.5 ± 25.9	415.3 ± 23.6	
2 h reperfusion	322.8 ± 74.7	291.2 ± 23.7	825.4 ± 67.8	484.8 ± 65.0	
Serum bicarbonate (mmol/L)					
Baseline	19.00 ± 1.9	18.17 ± 0.9	18.33 ± 0.5	18.50 ± 0.5	$P = 0.02$
End of Ischaemia	17.83 ± 1.2	17.67 ± 1.3	7.17 ± 0.6	7.67 ± 0.9	
At 2 h reperfusion	16.33 ± 1.4	16.67 ± 1.2	10.00 ± 1.3	14.00 ± 0.5	
Hepatic venous nitrates & nitrites (μmol/L)					
2 h reperfusion	33.4 ± 4.8	35.3 ± 6.5	21.4 ± 2.5	28.7 ± 2.3	$P = 0.025$

*In IRI alone and RIPC + IRI groups portal vein and hepatic artery were occluded.

difference between the groups. In the liver IRI group (Gr 3) SaO_2% reduced below baseline following total portal inflow occlusion, in spite of increasing the FiO_2% and remained lower compared with the other experimental groups at the end of the 2 h reperfusion period. In the RIPC+IRI group (Gr 4) SaO_2% reduced following total portal inflow occlusion, but was significantly better ($P = 0.001$) at the end of the 2 h reperfusion period compared with the IRI only group. Details data has been tabulated in Table 1.

Hepatic tissue oxygenation

Hepatic Tissue oxygenation as indicated by Cyt Ox redox state, HbD, HHb, HbT and HbO_2 levels was well maintained throughout the experiment in the sham and RIPC only groups with no significant differences ($P > 0.05$) between the two groups. With total hepatic IRI (Group 3) and RIPC + IRI (Group 4), the Cyt Ox Redox state fell immediately following total portal inflow occlusion and reduced further by the end of the 25 min ischaemic period (Fig. 1a). At the end of the 2 h reperfusion period Cyt Ox redox state recovered in both groups, but in the RIPC + IRI group recovery was significantly better than in the IRI group. At 2 h post reperfusion, the HbD was significantly lower ($P = 0.001$) in the IRI (Group 3) group compared with the RIPC + IRI group (Group 4) and control groups (Fig. 1b).

In the IRI group (Group 3) HbT was significantly increased ($P = 0.03$) at 2 h postreperfusion compared with the RIPC + IRI group (Group 4) and control groups (Fig. 1c). HHb levels fell initially on total portal inflow occlusion in both the IRI and the RIPC + IRI groups. At the end of the 2 h reperfusion period HHb levels rose ($P = 0.03$) in the IRI group compared with all other groups. In the RIPC+IRI Group (Group 4) HHb levels at 2 h of reperfusion were similar to the control groups (Groups 1 and 2) (Fig. 1d). In both the IRI (Group 3) and RIPC + IRI (Group 4) groups, the HbO_2 levels fell on total portal inflow occlusion and increased to levels similar to Sham and RIPC only groups, post 20 min of reperfusion. Finally, there was no significant difference ($P > 0.05$) between the four groups at the end of the 2 h reperfusion period (Fig. 1e).

Portal vein flow (PVF)

In the Sham and RIPC only groups (Groups 1 and 2) portal venous flow remained constant throughout the experiment with no significant difference ($P > 0.05$) between the two groups. In the RIPC + IRI (Group 4) and IRI (Group 3), PVF fell immediately on total portal inflow occlusion and recovered on release of the inflow occlusion. However, at 2 h postreperfusion PVF was significantly lower ($P = 0.007$) in IRI (Group 3) than the RIPC + IRI (Group 4) and control groups (Fig. 1f).

Serum ALT, LDH and bicarbonate levels

Serum ALT, LDH and Bicarbonate levels were similar in the sham and RIPC only groups throughout the experiment with no significant difference between the groups.

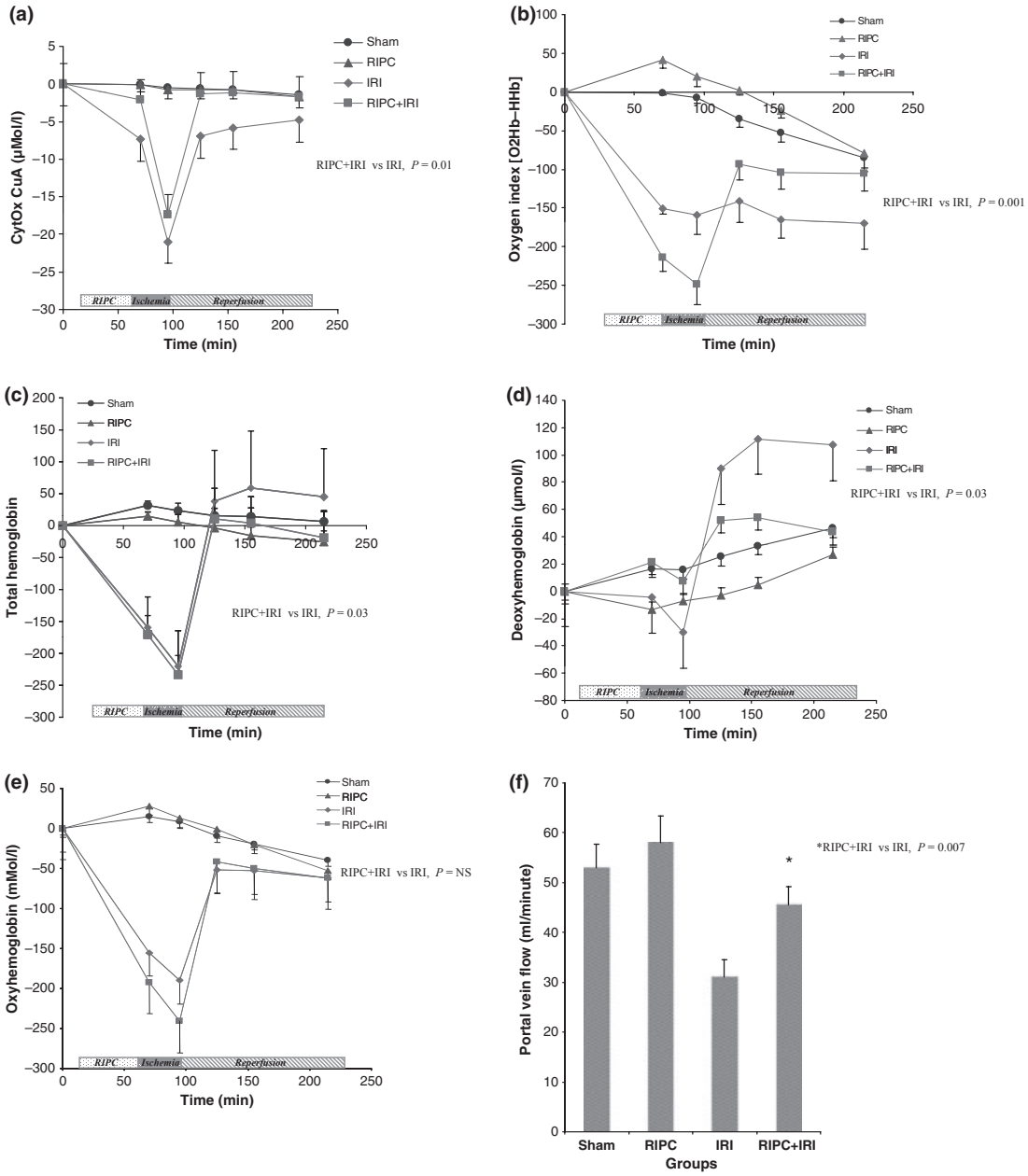

Figure 1 NIRS measurement of hepatic oxygenation and hepatic haemodynamics. (a) Cytochrome oxidase redox state (Cyt Ox Redox), (b) oxygenation index, (c) total haemoglobin (HbT), (d) deoxyhaemoglobin (HHb), (e) oxyhaemoglobin (O2Hb) and (f) portal vein flow (PVF). In the IRI group there is a worsening in mitochondrial oxygenation, an increase in deoxyhaemoglobin levels and a reduction in portal venous flow compared with the other groups at the end of the 2 h reperfusion period (Mann Whitney *U* statistic; $P < 0.05$).

In the IRI group serum ALT ($P = 0.04$) and LDH ($P = 0.01$) levels at 2 h postreperfusion was significantly higher compared with the RIPC + IRI and the control groups (Table 1).

At the end of 25 min of total hepatic ischaemia serum bicarbonate levels were reduced in the IRI and RIPC + IRI groups (Groups 3 and 4). At 2 h postreperfusion the serum bicarbonate level was significantly lower in the IRI group compared with the RIPC + IRI and the control groups ($P = 0.02$) (Table 1).

Hepatic venous nitrate/nitrites (NO_x)

The hepatic vein plasma NO_x levels measured at 2 h post reperfusion was significantly lower ($P = 0.025$) in the IRI group compared with the RIPC+ IRI group and control

groups. There was no significant difference ($P > 0.05$) in hepatic vein plasma NO_x levels between the sham and RIPC only groups (Table 1).

Correlation of NO activity with hepatic oxygenation and Portal vein flow

There was a significant positive correlation between NO_x activity, hepatic mitochondrial oxygenation (Cyt Ox CuA redox state) and Portal vein flow at 2 h postreperfusion in the RIPC+IRI group (Fig. 2a and b).

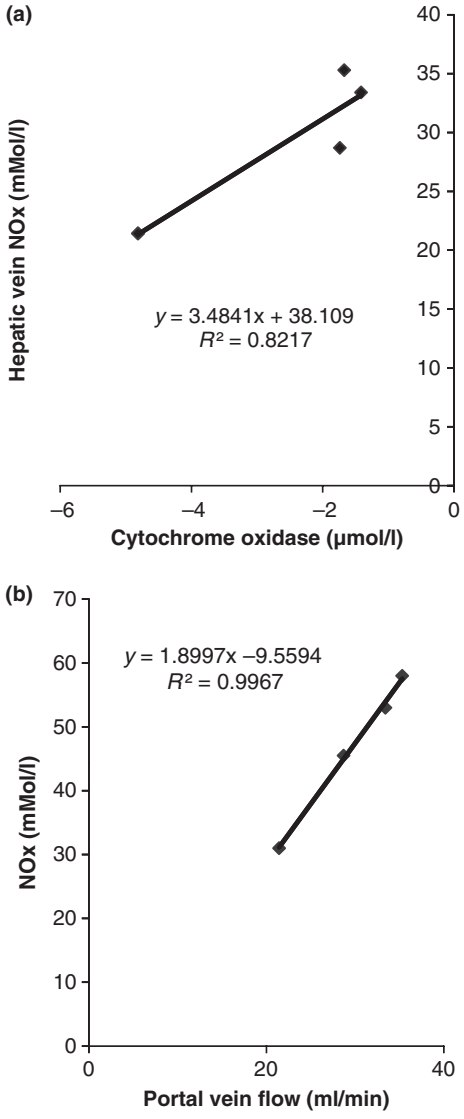

Figure 2 Correlation of NO activity with hepatic oxygenation and Portal vein flow. (a) Hepatic vein NO_x vs Cytochrome Oxidase Redox state (Cyt Ox Redox); (b) Hepatic vein NO_x vs Portal vein flow (PVF). There is a significant positive correlation between NO_x activity, hepatic mitochondrial oxygenation (Cyt Ox CuA redox state) and portal vein flow at 2 h postreperfusion in the RIPC + IRI group.

Discussion

In a previous study, we have demonstrated that hind limb RIPC in rabbits prior to total hepatic ischaemia reduced early liver reperfusion injury (2 h) to the liver compared with animals that underwent IRI without RIPC. In the RIPC+IRI group biochemical and histological markers of liver injury were reduced, mean arterial blood pressure was maintained and total hepatic blood flow was increased as a result of an increase in hepatic venous NO_x levels, suggesting an important role for NO in remote protection [6]. The protocols for RIPC, the cellular mechanisms and the involvement of humoral and neurogenic pathways have been covered in a detailed review by the authors [8]. NO reduces mitochondrial oxidative stress in direct IPC of the liver [39]; however, its role in preservation of hepatic mitochondrial oxygenation in RIPC has not been investigated.

The current study is the first to provide information on the status of hepatic tissue and mitochondrial oxygenation, following RIPC using NIRS. The donation of electrons from Cyt Ox to O_2 accounts for approximately 90% of the O_2 consumed by mitochondrial Cyt Ox in hepatocytes [31]. During ischaemia, electron transfer to O_2 cannot take place and Cyt Ox becomes reduced, and with introduction of O_2 on reperfusion electron transfer results in oxidation of Cyt Ox [31]. At 2 h of reperfusion hepatic IRI alone results in a significant reduction in the Cyt Ox CuA centre, but RIPC before hepatic IRI prevents this marked reduction in the Cyt Ox CuA centre preserving hepatic mitochondrial oxygenation. Oxygenation index (HbD) is another important marker of tissue oxygenation as it is independent of blood volume changes [21] and this is also significantly reduced following IRI, but improved with RIPC prior to IRI.

Near infrared spectroscopy measurement of hepatic oxygenation [40] correlates with liver blood flow [2,17,37] and determines the severity of liver IRI [18]. Hepatic IRI reduces total hepatic blood flow [6] and causes a severe microcirculatory dysfunction predominantly involving post capillary venules which results in venous stagnation, adversely affecting hepatic tissue oxygenation [41]. NIRS may help to differentiate between venous and arterial occlusion with some evidence that venous occlusion alone results in an increase in HbT and arterial occlusion alone results in an increase in HbT [29].

In this study, with both hepatic artery and portal vein occlusion for 25 min in the IRI group, HbT and HHb increased at the end of the reperfusion period, indicating significant impairment to venous outflow and desaturation of Hb at the capillary and venular level [21]. The change in HHb and HbT is more pronounced with both

hepatic artery and portal vein occlusion compared with the change observed following either hepatic artery or portal vein occlusion alone or with partial hepatic lobar ischaemia [2], suggesting a loss in regulatory mechanisms [18]. Remote IPC prior to IRI prevents this increase in HHb and HbT levels confirming that the marked deoxygenation of Hb and venous impairment associated with hepatic IRI is reduced by RIPC.

In the current study hepatic IRI resulted in a significant reduction in systemic serum bicarbonate levels at 2 h of reperfusion. However, systemic acidosis does not reflect acid-base changes within the liver accurately [42]. NIRS by measuring changes in the oxygenation of O_2Hb and HHb gives a relative measure of tissue acidosis through shifts in the O_2Hb dissociation curve [21], and this principle has been applied to study changes in tissue acidosis following hepatic IRI and RIPC for the first time.

The affinity of haemoglobin for O_2 is affected by temperature, hydrogen ion concentration and 2,3 DPG levels, and an increase in these result in a rightward shift of the O_2Hb dissociation curve facilitating O_2 delivery to the tissues without causing a large fall in capillary pO_2 which is the driving force for peripheral O_2 diffusion [43]. Hydrogen ions compete with O_2 for binding to HHb and their accumulation in the hepatic micro-circulation results in an adaptive increase in HHb levels. At 2 h of reperfusion, the HHb levels is significantly higher in the IRI group compared with the RIPC + IRI group, but the changes in O_2Hb levels are similar in both the groups (Fig. 1d and e). This increase in HHb level in the IRI group, in the presence of a constant O_2Hb level and temperature in both groups, indicates a rightward shift of the O_2Hb dissociation curve and significant liver tissue acidosis. This adaptive response to hepatic IRI permits O_2 delivery to acidotic tissues [21]. Direct measurement of pH changes in the liver using pH electrodes would help to further confirm these findings, however, placement of pH electrodes in this experiment could interfere with the absorption spectrum of the NIR instrument and as acidosis was a secondary end point of the study this was not done.

The significant increase in HHb levels with hepatic IRI also suggests the involvement of other adaptive responses to reduce the adverse effect of IRI on the liver. Deoxygenated Hb binds ATP to a lesser extent than O_2Hb and an increase in HHb increases the availability of ATP to ischaemic tissues. Similarly, O_2Hb has a high affinity for NO [44] and an increase in HHb levels reduces the binding of NO to Hb [45–47].

In animals that underwent RIPC prior to total hepatic IRI, there was a significant positive correlation between hepatic venous NO_x levels and hepatic mitochondrial oxygenation and between hepatic venous NO_x levels and PVF which supports our previous finding that NO may be the mediator responsible for the beneficial effects of RIPC [6]. During ischaemia, NO is reduced owing to low levels of NADPH and O_2 and further depleted following reperfusion owing to arginase release [48]. RIPC increases NO levels by unknown pathways [6] and improves hepatic microcirculation through reduced sinusoidal endothelial cell oedema and relaxation of the stellate cells (Ito cells) [49].

In conclusion, this study shows that RIPC has direct benefits in improving hepatic oxygenation and indirectly by reducing hepatic tissue acidosis which can be measured noninvasively in clinical studies on patients. The role of NO in RIPC requires further clarification through pharmacological inhibition of NO, in NOS knockout animal models and through the study of NO synthetic pathways. Clinical application of RIPC during liver transplantation is currently difficult without a clear understanding of the pathway for preconditioning whether humoral or neurogenic, which would determine whether the donor is remotely preconditioned or the recipient [8].

Authorship

SK: performed research and wrote the paper. GG: collected data and analysed. AQ: analysed data. SD: collected data. GF: performed research and wrote paper. BRD: designed research. AMS: designed research and analysed NIRS data.

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