Effect of ischaemic preconditioning on hepatic oxygenation, microcirculation and function in a rat model of moderate hepatic steatosis

Rahul S. KOTI∗, Wenxuan YANG∗, Georgios GLANTZOUNIS∗, Alberto QUAGLIA†, Brian R. DAVIDSON∗ and Alexander M. SEIFALIAN∗

∗University Department of Surgery and Liver Transplantation Unit, Royal Free and University College Medical School, University College London and The Royal Free Hospital, London NW3 2QG, U.K., and †Academic Departments of Histopathology, Royal Free Hospital, London NW3 2QG, U.K.

ABSTRACT

IPC (ischaemic preconditioning) may protect the steatotic liver, which is particularly susceptible to I/R (ischaemia/reperfusion) injury. Hepatic steatosis was induced in Sprague–Dawley rats with a high-cholesterol (2 %) diet for 12 weeks after which rats were subjected to I/R (ischaemia/reperfusion; 45 min of lobar ischaemia followed by 2 h of reperfusion). Rats were divided into three study groups (n = 6 each) receiving: (i) sham laparotomy alone, (ii) I/R, and (iii) IPC (5 min of ischaemia, followed by 10 min of reperfusion) before I/R. Hepatic extra- and intra-cellular oxygenation and HM (hepatic microcirculation) were measured with near-infrared spectroscopy and laser Doppler flowmetry respectively. Plasma liver enzymes and hepatic tissue ATP were measured as markers of liver injury. Histology showed moderate-grade steatosis in the livers. At the end of 2 h of reperfusion, I/R significantly decreased extra- and intra-cellular oxygenation concomitant with a failure of recovery of HM (21.1 ± 14.4 % of baseline; P < 0.001 compared with sham animals). IPC increased intracellular oxygenation (redox state of the copper centre of cytochrome oxidase; P < 0.05 compared with rats receiving I/R alone) and flow in HM (70.9 ± 17.1 % of baseline; P < 0.001 compared with rats receiving I/R alone). Hepatocellular injury was significantly reduced with IPC compared with I/R injury alone (alanine aminotransferase, 474.8 ± 122.3 compared with 5436.3 ± 984.7 units/l respectively; P < 0.01; aspartate aminotransferase, 630.8 ± 76.9 compared with 3166.3 ± 379.6 units/l respectively; P < 0.01). In conclusion, IPC has a hepatoprotective effect against I/R injury in livers with moderate steatosis. These data may have important clinical implications in liver surgery and transplantation.

INTRODUCTION

The steatotic liver is particularly susceptible to I/R (ischaemia/reperfusion) injury, resulting in poor outcome following liver surgery [1] and transplantation [2,3]. Impaired microcirculation [4], decreased mitochondrial ATP synthesis [5] and increased neutrophil adhesion [6] are some of the postulated mechanisms of I/R injury in steatotic livers. Furthermore, increased I/R injury in livers of fatty rats was associated with a change from an apoptotic form of cell death to necrosis [7]. Excessive fat accumulation in the liver (steatosis) is a common metabolic disorder seen in humans with an incidence ranging from 6–24 % in autopsy [8,9]. It is

Key words: hepatocellular injury, ischaemia/reperfusion injury, ischaemic preconditioning, liver, steatosis.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CytOx CuA, copper centre of cytochrome oxidase; Hb, deoxyhaemoglobin; HbO2, hepatic oxyhaemoglobin; HbT, total haemoglobin; HM, hepatic microcirculation; IPC, ischaemic preconditioning; I/R, ischaemia/reperfusion; LDF, laser Doppler flowmetry; MABP, mean arterial blood pressure; NIRS, near-infrared spectroscopy; NO, nitric oxide.

Correspondence: Professor Alexander M. Seifalian (email A.Seifalian@rfc.ucl.ac.uk).
caused by a wide variety of conditions and diseases such as alcohol abuse, obesity, malnutrition, hyperalimentation, diabetes, pregnancy and hepatitis, but many are idiopathic [10,11]. There is an increased risk of initial poor function and primary non-function of the graft after transplantation of a fatty liver and the risk increases with the severity of steatosis [12,13]. With the increasing numbers of orthotopic liver transplants and the concomitant lack of suitable donors, many liver transplant programmes increasingly use donor livers of ‘marginal’ quality such as fatty livers [14,15]. The graft as well as patient survival is diminished after use of such organs [16,17]. There is therefore an urgent need for strategies against I/R injury to increase the number of organs available for liver transplantation and, moreover, improve the outcome after transplantation of fatty livers. Serafin et al. [18] have shown that IPC (ischaemic preconditioning) increases the tolerance of fatty livers to I/R injury in rats.

IPC was first described by Murry and co-workers [19] in 1986. It refers to the observation that a brief period of ischaemia improves the tolerance to a subsequent prolonged ischaemia in various organ systems. IPC has been shown to reduce liver injury after warm I/R [20–25] as well as after cold ischaemic storage [26] in normal livers. Clavien and co-workers [27] have demonstrated that IPC significantly decreased plasma transaminase levels in a group of patients with steatotic livers (20–50 % steatosis) undergoing hemicapatectomy under inflow occlusion. Clearly, the effect of IPC on fatty livers may have potential clinical implications for the future.

The aim of the present study was to investigate the effects of IPC on hepatic oxygenation, microcirculation and function in a rat model of moderate hepatic steatosis.

This work was presented as research of distinction at the Digestive Disease Week, held in San Francisco, CA, U.S.A., in May 2002, and subsequently published in abstract form [27a].

**MATERIAL AND METHODS**

**Animal model, anaesthesia and surgical procedure**

The study was conducted under a project license granted by the Home Office in accordance with the Animals (Scientific Procedures) Act 1986. Male Sprague–Dawley rats, weighing 250–300 g, were used in the experiments. All animals were kept in a temperature-controlled environment with a 12 h light/dark cycle and allowed access to tap water. To induce fatty liver, all animals were fed with commercial high-cholesterol (2 %) diet for 12 weeks. The changes in body weight of the animals were monitored weekly. The development of steatosis was examined macroscopically and confirmed by histological examination.

Animals were anaesthetized by inhalation of 4–5 % isoflurane in 50 % oxygen in an induction chamber and maintained with 1–2 % isoflurane in oxygen/nitrous oxide (1:2) via a face mask in a standard anaesthetic circuit. The animals were then prepared for aseptic surgery. Body temperature was maintained at 36–38 °C with a heat pad (Harvard Apparatus Ltd, Edenbridge, Kent, U.K.) and monitored with a rectal temperature probe. SaO₂ (arterial oxygen saturation) and heart rate were monitored continuously with a pulse oximeter (Biox 3740 pulse oximeter; Ohmeda, Louisville, CO, U.S.A.). Polyethylene catheters (PE-50, 0.38 mm inner diameter; Portex, Hythe, Kent, U.K.) were inserted into the right femoral artery and connected to a pressure transducer for monitoring of MABP (mean arterial blood pressure) and for collecting blood samples. Normal saline was administered intraperitoneally to compensate for intraoperative fluid loss.

Laparotomy was carried out through a midline incision. The ligamentous attachments from the liver to the diaphragm were severed and the liver was exposed. Ischaemia of the median and left lateral lobes of the liver was produced by clamping the corresponding vascular pedicle containing the portal vein and hepatic artery branches using an atraumatic microvascular clamp. Other hepatic lobes were not handled during the procedure. This method produces ischaemia to the left and median lobes of the liver (approx. 70 % of the liver) while leaving the blood supply to the right and caudate lobes uninterrupted [28]. At the end of the ischaemia period, the vascular clamp was removed and reperfusion was allowed. Hepatic tissue oxygenation and HM (hepatic microcirculation) were measured continuously over the surface of median and left lateral lobes respectively, throughout the procedure. The abdomen of the animal was covered with a plastic wrap to prevent fluid evaporation. At the end of the experiment, the animals were killed by exsanguination.

**Measurement of hepatic tissue oxygenation**

NIRS (near-infrared spectroscopy; NIRO 500; Hamamatsu Photonics, Hamamatsu, Japan) was used to monitor hepatic tissue oxygenation. An NIRS algorithm specifically developed to measure continuously hepatic HbO₂ (oxyhaemoglobin), Hb (deoxyhaemoglobin), HbT (total haemoglobin; HbO₂ + Hb, which indicates blood volume) and the CytOx Cu A (copper centre of cytochrome oxidase) redox state concentration changes (in µmol/l) was used. NIRS probes were placed with a 10-mm separation on the surface of the median lobe of the liver for continuous measurement of hepatic tissue oxygenation. A flexible probe holder was used to ensure the probes had satisfactory contact with the liver surface and a fixed inter-probe spacing.
NIRS measurements during ischaemia and reperfusion were expressed relative to baseline before vascular occlusion. For comparison between the groups, NIRS measurements at baseline, ischaemia and reperfusion were calculated as the mean of 1-min measurements at the end of each period.

Measurement of blood flow in the HM
HM was measured by surface LDF (laser Doppler flowmetry; DRT4; Moor Instruments, Axminster, Devon, U.K.) in a flux unit. LDF has been shown to be a suitable method for estimation of HM [29]. The LDF probe was placed on a fixed site of the left lateral lobe of the liver and was held in place by a probe holder. LDF data were collected at a sampling rate of 2 Hz. The values at the relevant time points (at baseline, the end of ischaemia and the end of reperfusion) were calculated as the mean of the data after 1 min for each animal, and the reduction in HM after reperfusion was calculated relative to baseline.

Measurement of hepatocellular injury
At the end of the experiment, arterial blood samples were taken for measurement of plasma concentrations of AST (aspartate aminotransferase) and ALT (alanine aminotransferase) using an automated clinical chemistry analyser (Hitachi 747; Roche Diagnostics, Lewes, East Sussex, U.K.).

Measurement of hepatic tissue ATP
At the end of 2 h of reperfusion, liver biopsies of ischaemic lobes were freeze-clamped in liquid nitrogen for ATP determination. ATP levels in ischaemic liver tissue were assayed spectrophotometrically (Unicam UV1 spectrophotometer).

Liver histological examination
Liver biopsies were taken from the ischaemic lobes at the end of the experiment. The tissue sections were fixed in 10 % neutral-buffered formalin, embedded in paraffin and were stained with haematoxylin and eosin. Sections were examined under light microscopy to determine the presence and extent of steatosis, inflammation and necrosis. The grade of steatosis was analysed in a semi-quantitative manner: mild (< 30 %), moderate (30–60 %) and severe (> 60 %), using a clinically applied grading system [3].

Experimental groups and protocol
Three groups of animals (n = 6 each) were used. In the sham laparotomy group (group 1), the liver was exposed for 3 h, but there was no liver ischaemia. In the I/R group (group 2), ischaemia was induced in the median and left lateral lobes of the liver for 45 min, followed by 2 h of reperfusion. In the IPC + I/R group (group 3), the median and left lateral lobes underwent IPC (5 min of ischaemia and 10 min of reperfusion), followed by I/R (as for the I/R group). At termination, blood samples were collected and livers were removed for histological examination. Animals were then killed by exsanguination under anaesthesia.

Data collection and statistical analysis
Data from NIRS, LDF and the pulse oximeter were collected continuously on a laptop computer. The data were calculated as 1-min averages at baseline, at the end of ischaemia and at 30 and 120 min of reperfusion. The values are expressed as means ± S.D. of six animals in each group. The pre-ischaemic baseline was taken as the baseline against which the changes in hepatic oxygenation and HM were compared. Hepatocellular injury was compared relative to values in the sham group. One-way ANOVA and Bonferroni adjustment for multiple comparisons were used, unless otherwise stated, where Student’s t test was used for statistical analysis between the groups. P < 0.05 was considered statistically significant. The relationship between hepatic oxygenation changes, HM and plasma enzyme levels was tested using Spearman’s correlation coefficient.

RESULTS
Induction of fatty liver
All animals tolerated the high-cholesterol diet for 12 weeks with no mortality. The animals maintained a normal body weight during the cholesterol feeding periods, with no significant difference between the groups. The liver weight and the liver weight/body weight ratios were not significantly different between the groups. At laparotomy, the animals showed fat deposition in the skin, liver and spleen. The livers were enlarged, yellowish in colour, with rounded edges and firm consistency. Some animals had minimal ascites, but abdominal varices were not seen. Liver histological examination showed moderate macrovesicular steatosis under light microscopy (Figure 1).

Systemic haemodynamic parameters
The heart rate, MABP, body temperature and oxygen saturation did not change significantly throughout the experiments in any of the animals in the three experimental groups.

Hepatic tissue oxygenation
In the sham laparotomy group (group 1), there were no significant changes in HbO2, Hb and CytOx CuA redox state during the 3 h recording period (all P > 0.05 compared with baseline; Figure 2, and Tables 1 and 2).

In the I/R group (group 2) at the end of 45 min of ischaemia (before unclamping), significant decreases in HbO2, HbT and CytOx CuA redox state were observed (Figure 2 and Table 1) when compared with the respective

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sham group values. There was no significant difference in Hb. Upon reperfusion (after unclamping), HbO₂ decreased further and Hb increased, and there were significant differences in these values when compared with respective sham group values at the end of 2 h of reperfusion (Figure 2 and Table 2). HbT failed to improve and the CytOx Cu₄ redox state remained significantly reduced following reperfusion when compared with sham group value at the end of 2 h of reperfusion.

In the IPC group (group 3) at the end of 45 min of ischaemia (before unclamping), HbO₂ and HbT levels were significantly different from the sham group, but not from the I/R group. Hb was not significantly altered (Figure 2 and Table 1). The CytOx Cu₄ redox state was significantly decreased when compared with the sham group, but not significantly different from the I/R group at the end of 45 min of ischaemia (before unclamping). On reperfusion (after unclamping), HbO₂, Hb and HbT levels improved significantly. HbO₂ and HbT were significantly higher and Hb significantly lower when compared with the I/R group at the end of 2 h of reperfusion (Figure 2 and Table 2). The CytOx Cu₄ redox state showed a downward trend during the initial 30 min of reperfusion, but increased significantly later to above baseline values. At the end of 2 h of reperfusion, the CytOx Cu₄ redox state was significantly higher than the sham and I/R groups (Figure 2 and Table 2).

**HM**

Changes in the absolute values of HM at the end of 45 min of ischaemia and after 2 h of reperfusion in the three groups are shown in Table 2. Figure 3 shows the changes in mean percentage (S.D.) of HM with respect to the pre-ischaemic baseline level. In the sham laparotomy group (group 1), mean HM did not change significantly with respect to baseline throughout the 3 h period of recording. In the I/R group (group 2), at the end of 45 min of ischaemia (before unclamping) mean HM decreased significantly compared with the sham group ($P < 0.001$;
Table 1 Hepatic tissue oxygenation, CytOx CuA redox state and HM at the end of 45 min of warm ischaemia (pre-reperfusion)
Values are means ± S.D. of six animals in each group. *P < 0.05 compared with the sham group; †P < 0.05 compared with the I/R group, as determined using an unpaired Student’s t test.

<table>
<thead>
<tr>
<th></th>
<th>Sham (group 1)</th>
<th>I/R (group 2)</th>
<th>IPC + I/R (group 3)</th>
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<tbody>
<tr>
<td>HbO2 (µmol/l)</td>
<td>11.6 ± 11.5</td>
<td>269.2 ± 46.4*</td>
<td>209.9 ± 57.7*</td>
</tr>
<tr>
<td>Hb (µmol/l)</td>
<td>20.4 ± 7.6</td>
<td>517.0 ± 60</td>
<td>419.0 ± 3.8</td>
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<tr>
<td>HbT (µmol/l)</td>
<td>32.0 ± 16.1</td>
<td>217.4 ± 82.0*</td>
<td>168.5 ± 63.5*</td>
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<tr>
<td>CytOx CuA</td>
<td>-6.8 ± 1.7</td>
<td>-22.2 ± 6.2*</td>
<td>-20.9 ± 4.5*</td>
</tr>
<tr>
<td>HM (flux units)</td>
<td>84.9 ± 5.7</td>
<td>19.9 ± 4.5*</td>
<td>15.5 ± 2.8*</td>
</tr>
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</table>

Table 2 Hepatic tissue oxygenation, CytOx CuA redox state and HM at the end of 2 h of reperfusion
Values are means ± S.D. of six animals in each group. *P < 0.05 compared with the sham group, and †P < 0.05 compared with the I/R group, as determined using Student’s t test.

<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>HbO2 (µmol/l)</td>
<td>10.3 ± 9.8</td>
<td>-855.6 ± 85.3*</td>
<td>264.6 ± 29.7*†</td>
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<tr>
<td>Hb (µmol/l)</td>
<td>14.2 ± 5.7</td>
<td>615.0 ± 57.3*</td>
<td>465.0 ± 47.8*</td>
</tr>
<tr>
<td>HbT (µmol/l)</td>
<td>24.4 ± 13.8</td>
<td>-239.8 ± 59.4*</td>
<td>730.0 ± 30.5*†</td>
</tr>
<tr>
<td>CytOx CuA</td>
<td>-6.9 ± 1.8</td>
<td>-32.2 ± 8.0*</td>
<td>255.0 ± 6.7*†</td>
</tr>
<tr>
<td>HM (flux units)</td>
<td>76.0 ± 4.3</td>
<td>17.4 ± 4.0*</td>
<td>50.5 ± 2.2*†</td>
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Figure 3 HM during 45 min of ischaemia and 2 h of reperfusion as measured by LDF
Values are means ± S.D. of six animals in each group. I, 5 min of ischaemia; R, 10 min of reperfusion.

Figure 3). Upon reperfusion (after unclamping), mean HM failed to recover at the end of 2 h of reperfusion and the response remained significantly different compared with the sham group (P < 0.001; Figure 3). In the IPC group (group 3), mean HM during the preconditioning period of 5 min of ischaemia decreased, but recovered after 10 min of reperfusion (Figure 3). At the end of the subsequent 45 min of ischaemia (before unclamping), mean HM decreased significantly compared with the sham group (P < 0.001; Figure 3). On reperfusion, mean HM recovered progressively to reach a final value of 70.9 % (S.D., 17.1 %) at the end of 2 h of reperfusion, a finding that was significantly different compared with the I/R group (P < 0.001), but not the sham group.

Table 3 Plasma ALT and AST levels and hepatic tissue ATP levels at the end of 2 h of reperfusion
Values are means ± S.D. of six animals in each group. *P < 0.05 compared with the sham group; †P < 0.05 compared with the I/R group, as determined using Student’s t test.

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<th>Sham (group 1)</th>
<th>I/R (group 2)</th>
<th>IPC + I/R (group 3)</th>
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<tr>
<td>ALT (units/l)</td>
<td>46.6 ± 4.8</td>
<td>5435.4 ± 984.7*</td>
<td>474.8 ± 122.3*†</td>
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<tr>
<td>AST (units/l)</td>
<td>59.3 ± 5.1</td>
<td>31663 ± 379.6*</td>
<td>630.8 ± 76.9*†</td>
</tr>
<tr>
<td>ATP (µmol·l⁻¹·g⁻¹ of liver)</td>
<td>16.0 ± 0.5</td>
<td>2.0 ± 0.1*</td>
<td>9.0 ± 0.8*†</td>
</tr>
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Table 4 Correlation between hepatic tissue CytOx CuA redox state and HM (x) and liver enzymes (y) after 2 h of reperfusion following 45 min of ischaemia

<table>
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<th>Regression analysis</th>
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<th>P value</th>
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<tr>
<td>CytOx CuA compared with ALT</td>
<td>y = -67.094x + 1656</td>
<td>0.953</td>
<td>&lt; 0.010</td>
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<tr>
<td>CytOx CuA compared with AST</td>
<td>y = -34.697x + 1115</td>
<td>0.946</td>
<td>&lt; 0.011</td>
</tr>
<tr>
<td>HM compared with ALT</td>
<td>y = -65.762x + 5912</td>
<td>0.972</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HM compared with AST</td>
<td>y = -38.032x + 3556</td>
<td>0.904</td>
<td>&lt; 0.001</td>
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</table>

Hepatocellular injury
I/R significantly increased ALT and AST levels measured at the end of 2 h of reperfusion (both P < 0.05 compared with the sham group), whereas IPC decreased the I/R-induced ALT and AST levels (both P < 0.05 compared with the I/R group; P > 0.05 compared with the sham; Table 3).

Hepatic tissue ATP
I/R significantly decreased ATP levels measured at the end of 2 h of reperfusion (P < 0.05 compared with the sham group), whereas IPC resulted in increased ATP levels in comparison to the I/R group (P < 0.05; Table 3).

Correlation of hepatocellular injury with CytOx CuA redox changes and HM
At the end of 2 h of reperfusion, there was a significant negative correlation between CytOx CuA redox changes and plasma transaminases and between HM and plasma transaminases (Table 4).
DISCUSSION

The present study has investigated the effects of IPC in fatty livers subjected to I/R injury. Rabbits given a high-cholesterol diet, together with diethylstilbestrol, develop a rapid and extensive hepatic fibrosis with fatty infiltration, but this is associated with 27% mortality after 8 weeks of treatment [30,31]. We simplified the method by feeding rats with a cholesterol-rich diet alone for 12 weeks. All animals tolerated high-cholesterol feeding with no mortality. At laparotomy, the livers in the rats were enlarged, yellowish in colour with rounded edges and of firm consistency suggestive of fatty change. High-cholesterol feeding induces the formation of cholesterol fatty liver in which there is accumulation of triacylglycerol (triglyceride) and cholesterol in the liver [30–32]. Morphological changes in cholesterol fatty livers have been investigated in rabbits [32] and, in this species, a high-cholesterol diet for 12 weeks led to macrovesicular fat accumulation with periportal inflammation and necrosis [33]. In the present study model, rats fed with a high-cholesterol diet developed a moderate grade of steatosis with macrovesicular fat accumulation. The patchy distribution of inflammation around centrilobular veins mainly in I/R and IPC groups would be consistent with hepatocellular injury following ischaemia and reperfusion.

The systemic haemodynamic parameters measured in the present study, including MABP, heart rate, body temperature and \( \Delta S_{O_2} \), were not significantly different in the experimental groups, thus excluding any systemic contributions to I/R injury. The model employed was of partial ischaemia and involved clamping the arterial and portal inflow to the median and left lateral lobes and maintaining the blood flow to the caudate and right lateral lobes. This prevents mesenteric ischaemia thus circumventing haemodynamic instability due to portal congestion and subsequent bacteraemia [28].

Both tissue oxygenation and HM were measured in the present study. Maintaining good tissue oxygenation is vital for liver graft function and survival [34]. Liver graft tissue oxygenation correlated with early graft function and survival in both experimental animal models [35] and human liver transplantation [36]. NIRS has been used for monitoring \( HbO_2 \) and \( Hb \) and the CytOx CuA redox state \( m \) vivo [37,38]. It assesses changes in tissue oxygenation at the level of capillaries and intracellular uptake of oxygen [39]. The HM derives its blood supply from both the hepatic artery and portal vein, and determines hepatic tissue oxygenation [40]. The failure of the microcirculation is a major determinant of I/R injury and monitoring HM using LDF to assess the severity of I/R injury has been investigated previously [41]. Furthermore, tissue oxygenation has been shown to correlate significantly with the microcirculatory impairment and liver dysfunction induced by I/R injury [34]. Thus direct measurement of hepatic tissue oxygenation and HM would be good indicators of hepatocyte viability.

The present study showed significant differences in extra- and intra-cellular oxygenation with IPC. It is noteworthy that with IPC the intracellular oxygenation (CytOx CuA redox state) showed a full recovery at the end of reperfusion phase. This suggests an improvement in mitochondrial function or a decreased mitochondrial metabolism and ATP preservation in the preconditioned livers. Further insights may have been obtained by measuring tissue ATP levels at earlier time points throughout the study. In studies on myocardial preconditioning, slowing of metabolism was a feature of the preconditioned myocardium [19]. The results also show an increase in blood volume (HbT) with IPC, which was significantly different from the I/R group. The concomitant failure of improvement in the CytOx CuA redox state and HbT in the I/R group suggests decreased or no flow in the hepatic parenchyma with decreased tissue oxygen availability. This could occur due to tissue oedema, decreased ATP and impaired mitochondrial function or hepatocyte death following I/R, whereas the increased CytOx CuA redox state with IPC indicates viable hepatocytes, suggesting a reduced hepatocellular injury in the preconditioned livers. Viable hepatocytes could be confirmed by methods such as TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling) assay demonstrating decreased apoptosis.

All animals with hepatic steatosis had a reduction in the baseline HM when compared with normal livers (results not shown). The reduction in HM associated with steatosis has been reported in other studies with fatty livers [42,43]. The reduction in HM occurs due to sinusoidal compression and decrease in sinusoidal diameter by the enlarged fat-laden hepatocytes [42]. This alteration in HM by the enlarged hepatocytes is augmented by an increase in fibroblast numbers with the formation of collagen bundles in the perisinusoidal space, which causes further narrowing of the sinusoids [31,32]. Vascular congestion in the narrow and irregular sinusoids and leucocyte adhesion to the sinusoidal walls contribute significantly to the reduction of HM in fatty liver grafts [44]. In the present study, HM changes were measured at different time points. LDF signal was recorded during ischaemia, despite total lobar ischaemia. This has been reported in other studies and can be caused by random motion of residual blood cells, the influence of breathing movements and back flow from hepatic veins. With 45 min of ischaemia, there were no differences between I/R and IPC groups. In the I/R group, flow in the HM during 2 h of reperfusion was not significantly different from ischaemic levels, consistent with failure of the microcirculation. The pathophysiological mechanisms of primary microcirculation failure are no reflow and reflow.
paradox [45,46]. No reflow is a result of postischaemic capillary perfusion failure due to sinusoidal endothelial cell swelling [45]. Recent evidence also supports the contribution of endothelin/NO (nitric oxide) balance in mediating sinusoidal perfusion failure [47]. The reflow paradox is a result of leucocyte adherence and increased macromolecular permeability in postcapillary venules [46]. The fatty liver tolerates ischaemic insult poorly [48], and this evidence is supported by the results of the present study. On the other hand, with IPC the increase in HM was evident within the initial 30 min of reperfusion following which there was linear increase in HM until the end of reperfusion phase. This suggests that IPC has an immediate effect on reperfusion.

The present study has not investigated the mechanism for the preconditioning effect. Since the effect of IPC was evident immediately upon reperfusion, the possible mechanism is likely to be triggered during the preconditioning phase. The preservation of intracellular oxygenation during the subsequent sustained ischaemic phase in the present study would support this hypothesis. Recent studies have indicated NO [49], adenosine [50], HSPs (heat shock proteins) [51] and PKC (protein kinase C) [52] as potential mediators of preconditioning in the liver. Serafin et al. [18] have suggested that NO may mediate preconditioning in fatty livers. The theory of endothelin/NO imbalance in causation or aggravation of I/R injury is gathering favour. Blocking the endothelin-1 receptor in an animal model improves HM, tissue oxygenation, neutrophil infiltration, bile production and survival after I/R [53]. The role of endogenously produced NO in counteracting the action of endothelin has been demonstrated by the fact that blockade of endogenously produced NO during postischaemic reperfusion aggravates microvascular and hepatocyte injury [47,54]. NO is required to maintain perfusion of the HM and we tentatively support that preconditioning might be mediated through NO modulation of microcirculation during I/R injury.

In the present study, plasma liver enzymes measured at the end of reperfusion phase were markers of hepatocellular injury. The enzyme levels correlate with the severity of hepatocyte injury [55], and I/R resulted in a large increase in plasma ALT and AST. Since the fatty liver is particularly susceptible to I/R injury, a severe hepatocyte injury is to be expected. In the present study, the failure of microcirculation and tissue oxygenation with I/R is consistent with the severe hepatocellular injury or hepatocyte death indicated by the significant increases in liver enzyme levels. This is supported further by the fact that there was a significant negative correlation of CytOx Cu A redox changes and HM with plasma transaminases. With IPC, there was a significant decrease in liver enzyme levels. This suggests a reduced hepatocellular injury in the preconditioned livers. This is consistent with the decreased cellular hypoxia secondary to increased flow in HM with IPC and viable hepatocytes indicated by increased intracellular oxygenation in the preconditioned livers.

In conclusion, the present study has shown modulation of severe I/R injury in moderate fatty livers with IPC and provides evidence that IPC has a hepatoprotective mechanism in the ischaemic fatty liver. These data may have important clinical implications in liver surgery and transplantation, as the technique of IPC is easily applicable in clinical situations.

REFERENCES


