N-Acetylcysteine ameliorates the late phase of liver ischaemia/reperfusion injury in the rabbit with hepatic steatosis


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ABSTRACT

Steatotic livers are highly susceptible to I/R (ischaemia/reperfusion) injury and, therefore, the aim of the present study was to evaluate the in vivo effect of NAC (N-acetylcysteine) on hepatic function in the early and initial late phase of warm liver I/R injury in steatotic rabbits. Twelve New Zealand White rabbits were fed a high-cholesterol (2%) diet. The control group (n = 6) underwent lobar liver ischaemia for 1 h, followed by 6 h of reperfusion. In the treated group receiving NAC (n = 6), an intravenous infusion of NAC was administered prior to and during the 6 h reperfusion period. Systemic and hepatic haemodynamics were monitored continuously. ALT (alanine aminotransferase) activity and bile production were measured. NMR spectroscopy was used to analyse bile composition. Oxidation of DHR (dihydrorhodamine) to RH (rhodamine) was used as a marker of production of reactive oxygen and nitrogen species. Moderate centrilobular hepatic steatosis was demonstrated by histology. The results showed that NAC administration significantly improved portal flow, hepatic microcirculation, bile composition and bile flow after 5 h of reperfusion. NAC administration was also associated with less hepatocellular injury, as indicated by ALT serum activity, and decreased the oxidation of DHR to RH. In conclusion, NAC administration decreased the extent of I/R injury in the steatotic liver, particularly during the late phase of reperfusion.

INTRODUCTION

Hepatic I/R (ischaemia/reperfusion) injury always occurs following liver resection, trauma and transplantation, due to temporary complete or partial cessation of hepatic blood flow [1]. The pathophysiology of I/R injury is complex with the activation of many different metabolic pathways. Several mechanisms have been suggested to explain the I/R phenomenon, including endothelin/nitric oxide imbalance [2], humoral induction of inflammatory mediators, such as cytokines and eicosanoids, and up-regulation of adhesion molecule expression [3] with increased leukocyte–endothelial cell interaction [4]. Although discordance on the I/R pathophysiology exists, it is widely accepted that the key feature of I/R injury is the formation of ROS (reactive oxygen species) [5].

There are two distinct phases of liver reperfusion injury: the early phase (up to 2 h post-reperfusion), and the...
late phase (4–24 h post-reperfusion) [6,7]. The early phase is characterized mainly by activation of complement and Kupffer cells, and the production of free radicals. The main event during the late phase is the accumulation of activated neutrophils and the production of free radicals and proteases. Most liver injury occurs during the late phase [4] and can lead to increased morbidity and mortality and remote organ injury, leading to multi-organ failure [8].

Hepatic steatosis occurs with obesity, alcohol abuse and metabolic disorders [9], and an incidence of up to 11 % has been reported in autopsy studies on accidental deaths [10]. It is now well recognized that steatotic livers are more susceptible to I/R injury than normal livers [11]. The mechanisms involved are still poorly understood but, as fat accumulates within the hepatocytes, cell volume increases resulting in a decreased sinusoidal space [12] and impaired microcirculatory blood flow [11]. Sinusoidal blood flow can be reduced by 50 % in fatty livers [13,14]. Steatosis represents a risk factor in liver surgery, with a mortality rate of up to 14 % in patients undergoing major hepatic resections [15]. Similarly, the use of steatotic grafts for transplantation is associated with a much higher risk of primary graft non-function or dysfunction [16].

As a period of ischaemia is often necessary in liver surgery, and is inevitable in organ retrieval and transplantation, pharmacological modulation could potentially protect steatotic livers, minimizing the detrimental effects of I/R injury. NAC (N-acetylcysteine) is a thiol-containing compound that interacts and detoxifies free radicals by non-enzymatic reactions, and is deacetylated to form cysteine, which supports biosynthesis of glutathione, one of the most important components of the intracellular antioxidant system [17]. Nakano et al. [18] demonstrated that perfusion with NAC prior to organ retrieval reduced the extent of I/R injury after 24 h of cold storage in an isolated perfused rat steatotic liver. Bucillamine, another thiol antioxidant, has more recently been shown to prevent reperfusion injury in rat models of liver transplantation with both normal and fatty livers [19].

Recent in vivo experimental work from our group in rabbits with normal liver undergoing 1 h of lobar ischaemia [20] has shown that continuous infusion of NAC during reperfusion significantly reduces liver injury. The aim of the present study was to determine whether administration of NAC to rabbits with fatty livers reduced liver I/R injury.

MATERIALS AND METHODS

Animal model

The study was conducted under a licence granted by the Home Office in accordance with the Animals (Scientific Procedures) Act (1986). A rabbit liver lobar I/R model was used where both the early and late phases of reperfusion injury could be studied. Twelve New Zealand White rabbits with a mean body weight of 3.8 ± 0.5 kg were used. Steatosis was induced by feeding the animals with a high-cholesterol (2 %) diet for 8 weeks. Anaesthesia was induced by intramuscular injection of 0.5 ml/kg of body weight fentanyl-fluanisone (Janssen Animal Health).

Tracheostomy was performed, and anaesthesia was maintained with isoflurane (0.5–2 %) via an anaesthetic circuit. Temperature was measured by a rectal thermometer and maintained at 37–38.5 °C with a warming blanket (homeothermic blanket control unit; Harvard Apparatus). Arterial oxygen saturation and heart rate were recorded continuously by a pulse oximeter (Biox 3740 pulse oximeter; Ohmeda) applied to the tail. A venous cannula (20 guage) was inserted into the ear artery to collect blood samples and connected to a pressure transducer to monitor MABP (mean arterial blood pressure) and heart rate. Ear marginal veins were cannulated in both ears with radiopaque catheters (22 guage) for the administration of fluids and drugs. Normal saline was infused at a rate of 15 ml·kg⁻¹·h⁻¹ to replace the intra-operative fluid losses.

Laparotomy was performed through a bilateral subcostal (roof-top) incision. The ligaments from the diaphragm to the liver were divided and the liver was fully exposed. The bile duct was cannulated with a polyethylene catheter (PE-50, 0.58 mm inner diameter; Portex). Bile flow was measured and calculated as µl·min⁻¹·100 g⁻¹ of liver wet weight. Following dissection of the portal vein, a perivascular Doppler probe (HT207; Transonic Medical System) was positioned around it to monitor the portal blood flow. Lobar ischaemia was induced by clamping the vascular pedicles of the median and left lobes of the liver by using an atraumatic microvascular clip. The microvascular clip was removed after 60 min of ischaemia, and reperfusion was allowed for 6 h. At the end of this period, the animals were killed by exsanguination.

Experimental groups and protocol

Two animal groups (n = 6 each) were used. In the treated group, NAC (150 mg/kg of body weight; Parvolex; Medeva Pharma) in 20 ml of 5 % dextrose was infused intravenously through the ear vein over the 15 min immediately before reperfusion and maintained at 10 ml·kg⁻¹·h⁻¹ of body weight·h⁻¹ in 5 % dextrose during the 6 h reperfusion period.

In the control group, 20 ml of 5 % dextrose was infused intravenously 15 min before reperfusion and continued at a rate of 10 ml·kg⁻¹·h⁻¹ of body weight·h⁻¹ during the reperfusion period.

In both groups, after laparotomy and a stabilization period of 10 min, systemic and hepatic haemodynamics, oxygen saturation, body temperature and bile flow were recorded continuously. Arterial blood samples were
taken before the induction of liver ischaemia (baseline) and at 2, 5 and 6 h after reperfusion for measurement of ALT (alanine aminotransferase) activity. An equal volume of normal saline was used to replace the volume of blood taken. Serum was separated from the samples and stored at –20 °C until assayed. The measurements were done using an automated clinical chemistry analyser (Hitachi 747; Roche Diagnostics). A biopsy was taken from the left liver lobe at baseline and 6 h after reperfusion. Formalin-fixed liver tissue samples were embedded in paraffin and stained with haematoxylin and eosin for subsequent microscopy (digital light microscope CLF60 optical system; Nikon). Formalin-fixed, but not paraffin-embedded, tissue was stained for fat using the Swank and Davenport modification of the Marchi method [21].

Measurement of blood flow in the hepatic microcirculation

To determine the effect of NAC in the hepatic microcirculation, a LDF (laser Doppler flowmeter; DRT4; Moor Instruments) was used. The LDF probe was placed on a fixed site on the surface of the left hepatic lobe and held in place by a probe holder. Doppler signal varies linearly with the product of the total number of moving red blood cells in the measured volume of a few cubic millimetres by their mean velocity. Flow in the hepatic microcirculation was expressed in units of flux and averaged over a period of 2 min.

Bile flow and composition

To determine the effect of NAC therapy on bile production and excretion, bile flow was measured and bile composition was analysed by 1H-NMR. Bile samples were taken at baseline and each hour thereafter and stored at –80 °C. Bile volume was expressed as μl·min⁻¹·100 g⁻¹ of liver weight.

1H-NMR analysis was performed on an 11.7 Tesla (500 MHz for protons) spectrometer (Varian Unity+; Varian) at 25 °C. Bile was thawed at room temperature and placed in a 5 mm NMR tube. For a field/frequency lock, a co-axial capillary insert was used (Wilmad). This capillary insert was filled with a deuterium oxide solution of TSP (sodium 3-((trimethylsilyl)−[2,2,3,3-2H4])-1-propionate) that acted both as a chemical shift reference and quantification standard. This capillary was calibrated by using a series of known concentrations of deoxycholate and a calibration curve was obtained. This was then used for quantification of bile components [lactate, acetate, pyruvate and PC (phosphatidylcholine)], which enabled an accurate comparison of bile levels between the groups. One-dimensional NMR spectra were obtained at 500 MHz with a sweep width of 6 kHz. Presaturation of bile was carried out to attenuate the intensity of water signal. The spectra were analysed using software from MestRe-C version 3.1.1 (Universidad Santiago de Compostela, Spain). All spectra were integrated using a fixed range for each peak and the published peak assignments [22] shown in Table 1.

Table 1 1H-NMR peak assignments

<table>
<thead>
<tr>
<th>Peak assignments</th>
<th>p.p.m.</th>
<th>Range of integration</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSP</td>
<td>0.0</td>
<td>−0.02 to +0.02</td>
</tr>
<tr>
<td>C18 bile acids proton peak</td>
<td>0.7</td>
<td>0.61−0.73</td>
</tr>
<tr>
<td>Lactate methyl group (CH3)</td>
<td>1.3</td>
<td>1.25−1.38</td>
</tr>
<tr>
<td>Acetate methyl group (CH3)</td>
<td>1.9</td>
<td>1.89−1.95</td>
</tr>
<tr>
<td>Pyruvate methyl group (CH3)</td>
<td>2.3</td>
<td>2.34−2.38</td>
</tr>
<tr>
<td>PC head group -N+(CH3)3</td>
<td>3.2</td>
<td>3.20−3.27</td>
</tr>
<tr>
<td>Anemeric glucose proton peak</td>
<td>5.2</td>
<td>5.20−5.25</td>
</tr>
</tbody>
</table>

DHR (dihydrorhodamine) 123 oxidation

To determine the effect of NAC on the production of ROS and RNS (reactive nitrogen species), such as peroxynitrite, the in vivo oxidation of DHR 123 to RH (rhodamine) was studied. DHR 123 is a chemical compound and its oxidation to RH is partially peroxynitrite dependent [23]. RH is a fluorescent substance that gives a characteristic colour to plasma, easily detected by fluorescence. At 6 h after reperfusion, DHR 123 (Sigma) was administered intravenously (2 μmol/kg of body weight in 0.8 ml of 0.9 % saline) to the animals. After 20 min, plasma samples were collected for evaluation of RH fluorescence. For fluorescence measurements, a fluorimeter (Thermo Electron) was used at an excitation wavelength of 485 nm and emission wavelength of 538 nm. The concentration of RH formation was calculated using a standard curve obtained with authentic RH (1−40 nmol/l) prepared in plasma obtained from untreated rabbits.

Data collection and statistical analysis

Data from the pulse oximeter, blood pressure monitor, transonic flowmeter and LDF were recorded continuously on a laptop computer. The data were averaged for 2 min before the induction of ischaemia (baseline), at the end of ischaemia and hourly during a reperfusion period of 6 h.

Results are expressed as medians (range). Medians were compared using the Wilcoxon rank-sum test for differences in values within the same group. The differences in values between the two groups were calculated using the Mann–Whitney U test. P < 0.05 was considered statistically significant. All statistical analysis was done using SPSS for Windows version 11.0.

RESULTS

All animals fed with a high-cholesterol (2 %) diet for 8 weeks developed moderate hepatic steatosis, and histological examination revealed centrilobular steatosis.
Figure 1 A section of liver stained with the Marchi method showing a moderate amount of centrilobular fat (stained black). The arrows indicate two portal tracts. Magnification, ×100.

Figure 2 Portal flow in the NAC and control groups. Values are medians (range). 0 h, baseline; 1 h, ischaemia; 2–7 h, reperfusion for 1–6 h. *P < 0.05 compared with control group at 6 h post-reperfusion.

Systemic haemodynamics
In both groups, MABP and heart rate fell during the reperfusion period compared with baseline. There was no significant difference in MABP, heart rate and oxygen saturation values between the two groups throughout the experimental period.

Hepatic haemodynamics
Portal flow values are shown in Figure 2. There was no significant difference in baseline values between the two groups. Portal flow was higher in the NAC group after reperfusion for 3 h and this difference reached significance at 6 h (P = 0.03).

Hepatic microcirculation
During ischaemia, LDF values were significantly decreased in both groups and were almost reduced to zero (Figure 3). In the control group, flow in the hepatic microcirculation was significantly reduced from baseline at 5 and 6 h of reperfusion (P = 0.03 and P = 0.04 respectively; Figure 3). In the NAC group, no significant difference was recorded in LDF between baseline and reperfusion (Figure 3). Flow in hepatic microcirculation was better in the NAC group compared with control group after 3 h of reperfusion, and this difference was significant at 5 and 6 h of reperfusion (P = 0.05 and P = 0.03 respectively; Figure 3).

Liver function tests
No significant difference between baseline ALT values in the two groups was recorded (Figure 4). ALT values were significantly greater in the control group at 5 and 6 h of reperfusion (P = 0.019; Figure 4).
Ischaemia/reperfusion injury in the steatotic liver

Figure 5  Bile flow in the NAC and control groups
Values are medians (range). 0 h, baseline; 1 h, ischaemia; 2–7 h, reperfusion for 1–6 h. *P < 0.05 compared with control group at 5 and 6 h post-reperfusion.

Bile flow and 1H-NMR spectroscopy
Baseline bile flow was similar in the two groups (Figure 5). Bile flow was significantly greater in the NAC-treated animals at 5 and 6 h of reperfusion (P = 0.016 and P = 0.008 respectively; Figure 5).

Baseline control bile spectra are shown in Figure 6. The results of the integration of the area under curve for the assigned peaks are shown in Table 2. Bile lactate levels were lower, across all time points, in the control group compared with the NAC group (Table 2). Bile acetate levels rose in the NAC group to approx. twice the baseline level at 6 h post-reperfusion, whereas, in the control group, the levels fell to almost half of their baseline levels. Acetate levels were significantly higher (P = 0.021) in the NAC group compared with the control group at 5 and 6 h post-reperfusion, as shown in Table 2. Pyruvate levels were significantly higher in the NAC group compared with controls at 2 and 5 h of reperfusion (P = 0.009 and P = 0.021 respectively; Figure 7 and Table 2).

During ischaemia there was a 2-fold rise in PC levels in the control group compared with only a slight elevation in the NAC group (P = 0.047). On reperfusion, PC levels fell gradually in controls, whereas, in the NAC group, PC levels rose to peak after 2 h reperfusion then fell to close to baseline values at 6 h reperfusion.

DHR oxidation
RH values were significantly lower (P = 0.008) at 6 h post-reperfusion in the NAC group [1.2 (0.63–1.44) nmol/l] than in controls [2.92 (1.37–4.07) nmol/l], indicative of less production of RNS and ROS in the NAC group at this time point.

DISCUSSION
In the present study, we have shown that NAC administration reduces I/R injury in steatotic liver following a 1 h period of partial inflow occlusion, and the benefit is apparent mainly in the late phase of I/R injury. Although there have been previous in vitro studies on the effect of NAC on hepatic I/R injury [18], this is the first in vivo study to investigate the effect of NAC in warm liver I/R injury in steatotic livers.

Rabbits were fed a high-cholesterol (2%) diet for 8 consecutive weeks in order to induce moderate steatosis [24]. Unlike the commonly used low-choline/methionine diet, which produces perportal fatty infiltration [25,26], in this model a central lobular deposition of fat is...
Table 2  Peak integration of $^1$H-NMR bile spectra

Values are medians (range).

<table>
<thead>
<tr>
<th>Peak integration</th>
<th>Baseline</th>
<th>Ischaemia</th>
<th>Time post-reperfusion (h)</th>
<th>2</th>
<th>5</th>
<th>6</th>
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<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NAC group</td>
<td>16.14 (7.95–17.00)</td>
<td>17.22 (16.69–17.75)</td>
<td>17.86 (12.99–20.92)</td>
<td>12.81 (10.67–21.08)</td>
<td>15.00 (10.88–18.16)</td>
<td></td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.465</td>
<td>0.564</td>
<td>0.117</td>
<td>0.564</td>
<td>0.248</td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>1.12 (0.64–1.72)</td>
<td>1.18 (0.94–1.61)</td>
<td>0.65 (0.54–1.17)</td>
<td>0.51 (0.26–0.76)</td>
<td>0.73 (0.37–1.13)</td>
<td></td>
</tr>
<tr>
<td>NAC group</td>
<td>1.12 (0.78–1.25)</td>
<td>0.70 (0.69–0.71)</td>
<td>1.07 (0.60–1.24)</td>
<td>1.18 (0.86–1.73)</td>
<td>1.92 (1.65–2.50)</td>
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<tr>
<td>$P$ value</td>
<td>0.754</td>
<td>0.083</td>
<td>0.175</td>
<td>0.021</td>
<td>0.021</td>
<td></td>
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<tr>
<td>Pyruvate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>0.90 (0.60–1.53)</td>
<td>1.05 (0.46–1.70)</td>
<td>0.65 (0.62–1.37)</td>
<td>0.57 (0.07–1.05)</td>
<td>0.69 (0.63–1.53)</td>
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<tr>
<td>NAC group</td>
<td>1.45 (1.05–1.52)</td>
<td>1.76 (1.70–1.82)</td>
<td>1.81 (1.53–2.33)</td>
<td>1.25 (1.15–1.92)</td>
<td>1.60 (0.64–2.45)</td>
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</tr>
<tr>
<td>$P$ value</td>
<td>0.175</td>
<td>0.105</td>
<td>0.009</td>
<td>0.021</td>
<td>0.248</td>
<td></td>
</tr>
<tr>
<td>PC head group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>4.33 (1.35–5.63)</td>
<td>8.70 (6.31–10.12)</td>
<td>5.68 (2.61–8.84)</td>
<td>4.86 (1.57–13.75)</td>
<td>3.25 (1.83–8.40)</td>
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<tr>
<td>NAC group</td>
<td>2.08 (1.31–3.47)</td>
<td>2.57 (2.37–2.79)</td>
<td>4.52 (2.01–8.88)</td>
<td>2.59 (2.08–3.76)</td>
<td>2.64 (1.83–3.36)</td>
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</tr>
<tr>
<td>$P$ value</td>
<td>0.175</td>
<td>0.047</td>
<td>0.347</td>
<td>0.248</td>
<td>0.772</td>
<td></td>
</tr>
</tbody>
</table>

Figure 7  $^1$H-NMR spectra of acetate levels following warm I/R

$^1$H-NMR spectra of acetate peaks in representative samples from the control (A) and NAC (B) groups at 6 h of reperfusion.

observed, similar to that found in the majority of human fatty livers, such as in diabetes, obesity and alcoholism [9]. Liver steatosis reduces flow in the hepatic microcirculation and mitochondrial ATP generation [11]. Fat accumulation in the cytoplasm of hepatocytes causes compression or even complete occlusion of the sinusoidal spaces with shunting of a proportion of THBF (total hepatic blood flow) [26]. Our group has demonstrated previously [14] in an experimental model that, in moderate steatosis, THBF was reduced by 30% and flow in the hepatic microcirculation was 50% of normal controls.

A rabbit model was used instead of a rat model, since the size of rabbits is more convenient for the placement of probes, bile flow measurement and blood uptake at different time points. The period of hepatic inflow ischaemia (1 h) is also similar to the warm ischaemia time during human liver resection and transplantation. In this model, partial ischaemia was obtained by interrupting the blood flow to the median and left lobes of the liver. Maintaining splanchnic blood flow through the right and caudate lobe minimizes portal vein stasis and intestinal venous congestion, reducing the risk of portal bacteremia and haemodynamic instability [27,28].

Several studies on liver I/R injury have focused on the initial reperfusion phase [29–31], which is characterized by oxidative stress induced by Kupffer cells and occurs in the first 2 h [7]. In the present study, the observation
time was extended to 6 h as a distinct late phase of warm I/R injury develops 4 h after reperfusion and is primarily caused by activated neutrophils with release of ROS and proteases [6,32].

NAC was chosen as an antioxidant to modulate hepatic I/R injury as it is routinely administered to patients with acute liver failure secondary to acetaminophen (paracetamol) overdose [33], and has been used in other clinical conditions where oxidant damage was the putative or known mechanism of injury, such as ARDS (acute respiratory distress syndrome) [34] and I/R after cardiac surgery [35]. Recent work from our group [20] has also shown beneficial effects of NAC in normal liver following 1 h of lobar ischaemia. The dose of NAC administered is that used in clinical practice to treat patients with acute liver failure [33]. The beneficial effect of NAC was demonstrated by improvement in liver microcirculation, bile production and composition, which was associated with reduced hepatocellular injury.

Several studies have validated the use of LDF to measure changes in hepatic microcirculation [27,36,37]. During partial inflow occlusion, parenchymal perfusion was greatly reduced but was still recordable. This low level of perfusion has also been reported in other studies and can be caused by a random wandering motion of red cells and breathing movements [38]. In the NAC group, hepatic microcirculation returned to baseline values at the end of reperfusion, suggesting that NAC reversed the perfusion failure of the late phase of I/R injury. This correlates well with the return of portal blood flow rates towards baseline at the end of the reperfusion period in the NAC group.

In order to adequately investigate hepatic injury and function, changes in serum ALT activity, and bile flow and composition, were studied. ALT is relatively liver specific and reflects the loss of membrane integrity and release of cytoplasmic content into the circulation [39]. Bile production is a reliable marker of liver function [40]. The NAC group had decreased hepatocellular injury as indicated by lower ALT values following reperfusion and improved biliary excretion, an energy-dependent process, resulting in increased bile drainage volumes. A comparable rise in ALT values has been observed during reperfusion in steatotic animals subject to a similar ischaemic insult [18].

This is the first study utilizing \(^1\)H-NMR of bile to study the effect of NAC on steatotic livers in a controlled experimental model of liver I/R. The use of spin-echo spectra allowed the interference from broad lipid signals to be reduced and enabled us to more clearly identify and comparatively quantify bile constituents. Continuous bile flow was recorded in both the control and NAC groups, and \(^1\)H-NMR spectroscopy revealed fluctuations in the concentration of several bile constituents. More specifically, NAC administration enhanced the biliary excretion of acetate, pyruvate and lactate.

Acetate is produced as a result of fatty acid \(\beta\)-oxidation in the liver [41], and this reaction requires ATP at its initiation. Following I/R injury, ATP is consumed to clear excess lactate and pyruvate and may not be readily available for fatty acid oxidation. The I/R-induced decrease or depletion in ATP may result in a decreased ability to utilize \(\beta\)-oxidation causing acetate production to falter. The higher bile acetate levels in the NAC-treated livers would suggest improved ATP production and energetics. The increased excretion of pyruvate and lactate in the bile of NAC-treated livers is difficult to interpret. Steatotic livers have lower ATP levels after stress compared with normal livers and mitochondrial injury has been proposed as one of the causes of reduced hepatocellular ATP stores in steatosis [9]. The main source of PC in hepatocytes is the cell membrane. The increase following ischaemia correlates well with the theory of cellular breakdown and membrane lipid peroxidation [42]. Increased PC has also been observed by other workers in association with poor graft function [43].

In the present study, NAC administration significantly decreased the oxidation of DHR 123 to RH. The formation of RH is partially peroxynitrite dependent, although other oxidants, such as \(\text{H}_2\text{O}_2\) in the presence of horseradish peroxidase and hypochlorous acid, can oxidize DHR 123 to RH [44]. ROS and peroxynitrite can cause cellular injury and necrosis through several mechanisms, including peroxidation of membrane lipids, protein denaturation and DNA damage [44]. Oxidation of DHR 123 to RH has been reported in endotoxaemia, haemorrhagic shock and splanchnic I/R [23]. Although the protective effect of NAC in DHR oxidation has been demonstrated in \textit{in vitro} studies [45] and in splanchnic artery occlusion [46], the present study is the first to report a decrease in DHR oxidation by NAC infusion in warm I/R in steatotic livers.

In conclusion, the administration of NAC, prior to and during reperfusion, reduced the extent of I/R injury in the steatotic liver. This was apparent during the late phase of reperfusion and was demonstrated by increased portal blood flow and liver parenchymal perfusion. Bile excretion was increased and acute liver injury was reduced. This improvement was associated with reduced levels of ROS and RNS. A trial of NAC infusion in patients with hepatic steatosis undergoing liver resection or in patients undergoing liver transplantation with steatotic grafts is required to confirm these benefits in clinical practice.

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