

Continuous infusion of *N*-acetylcysteine reduces liver warm ischaemia–reperfusion injury

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Background: *N*-acetylcysteine (NAC) may modulate the initial phase (less than 2 h) of liver warm ischaemia–reperfusion (IR) injury but its effect on the late phase remains unclear. The present study investigated the role of NAC during the early and late phases in a rabbit lobar IR model.

Methods: Liver ischaemia was induced by inflow occlusion to the median and left liver lobes for 60 min, followed by 7 h of reperfusion. In the NAC group ($n = 6$), NAC was administered intravenously at 150 mg per kg over the 15 min before reperfusion and maintained at 10 mg per kg per h during reperfusion. In the IR group ($n = 6$), 20 ml 5 per cent dextrose was infused over the 15 min before reperfusion and continued at a rate of 10 ml/h. Animals in a sham operation group ($n = 6$) underwent laparotomy but no liver ischaemia. All animals were killed at the end of the experiment.

Results: Intracellular tissue oxygenation was improved after the second hour of reperfusion in animals treated with NAC compared with that in the IR group ($P = 0.023$). Hepatic microcirculation improved after 5 h of reperfusion ($P = 0.036$) and liver injury was reduced after 5 h, as indicated by alanine aminotransferase activity ($P = 0.007$) and indocyanine green clearance (uptake, $P = 0.001$; excretion, $P = 0.032$).

Conclusion: The main protective effect of NAC becomes apparent 5 h after hepatic ischaemic injury.

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Introduction

Liver ischaemia–reperfusion (IR) injury occurs during major liver surgery and transplantation or following haemorrhagic shock and subsequent fluid resuscitation^{1–3}. Extracorporeal circulation used in cardiac or vascular surgery is also associated with low-flow IR of the liver⁴. When the degree of injury is severe it may result in both liver failure¹ and remote organ failure in the lungs, heart and the systemic circulation^{5,6}. The pathophysiology of liver IR injury involves the activation of many metabolic pathways and the release of mediators that induce liver injury⁷. There is growing evidence that there are two distinct phases of liver injury after warm ischaemia and reperfusion^{8–10}. The initial phase (within 2 h of starting reperfusion) is characterized by Kupffer cell-induced oxidative stress⁸. The late phase (after 4 h) results mainly from the accumulation of neutrophils and is associated with more extensive injury¹⁰. Main contributors to the liver

damage are reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are released in both phases of reperfusion and produce significant oxidative stress¹¹. Mammals have a complex antioxidant system to protect themselves from such stress. One of the most important components of the intracellular antioxidant system is glutathione, a powerful active radical scavenger that is depleted during severe liver IR injury¹².

N-acetylcysteine (NAC) is a thiol-containing compound used in the management of fulminant liver failure after paracetamol overdose^{13,14}. One mechanism by which NAC acts is by entering cells and undergoing hydrolysis to cysteine, a glutathione precursor capable of rapidly replenishing depleted intracellular reduced glutathione concentrations¹⁵. NAC also scavenges several ROS and RNS directly¹⁶. Experimental studies in which NAC has been used to reduce liver warm IR injury have produced conflicting results^{17,18} and the use of antioxidants for liver

IR still requires study of the mechanism and timing of any beneficial effects.

The present study evaluated the effect of NAC administration on liver function in both the early phase and initial part of the late phase of liver warm IR injury. A rabbit lobar IR model was used in which several markers of liver function were monitored continuously or at intervals for 7 h after the ischaemic injury.

Materials and methods

The study was conducted under a licence granted by the Home Office in accordance with the Animals (Scientific Procedures) Act 1986. New Zealand White rabbits (mean(s.d.) weight 3.8(0.5) kg; $n = 18$) were used. Anaesthesia was induced by intramuscular injection of 0.5 ml/kg Hypnorm™ (fentanyl citrate and fluanisone; Janssen Animal Health, High Wycombe, UK). Following tracheostomy, anaesthesia was maintained with 0.5–3 per cent isoflurane through an anaesthetic circuit.

Body temperature was maintained at 37–38.5°C by a warming blanket (Harvard Apparatus, Southmattick, Massachusetts, USA). Haemoglobin saturation and heart rate were recorded continuously by a pulse oximeter (Ohmeda® Biox 3740 pulse oximeter; Ohmeda, Louisville, Colorado, USA). A radio-opaque 20-G catheter was inserted into the right femoral artery for monitoring of arterial blood pressure and collection of blood samples. Ear marginal veins were cannulated with radio-opaque catheters (22 G) for the administration of anaesthetics, fluids and medication.

Laparotomy was performed through a midline incision. Lobar ischaemia was induced by clamping the vascular pedicles of the median and left lobes of the liver, using an atraumatic microvascular clip. This method produces a severe ischaemic insult without mesenteric venous hypertension¹⁹. After 60 min of ischaemia, the vascular clip was removed and reperfusion was allowed for 7 h. At the end of the experiment the animals were killed by exsanguination.

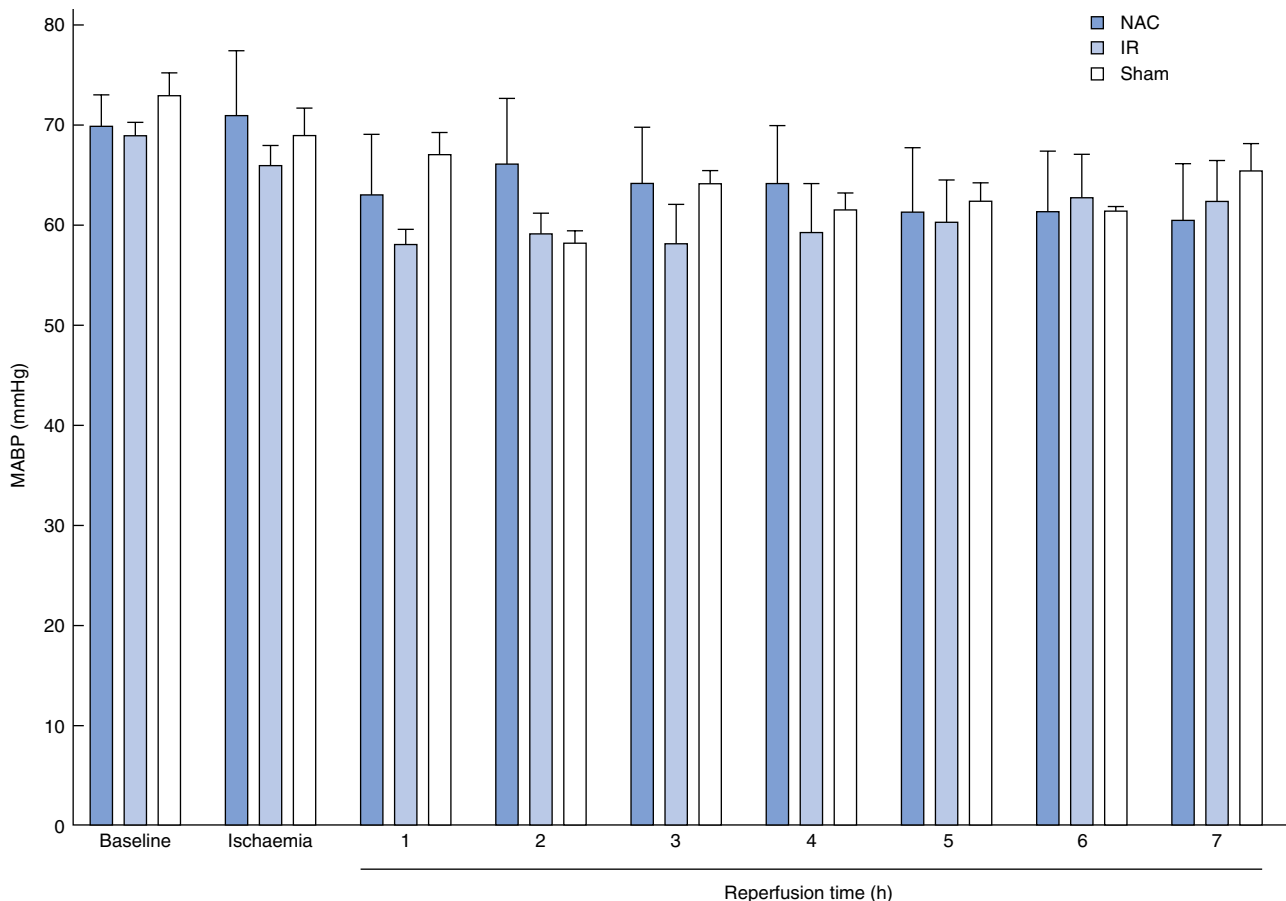


Fig. 1 Mean arterial blood pressure (MABP) during ischaemia and reperfusion. Values are mean(s.d.). NAC, *N*-acetylcysteine; IR, ischaemia–reperfusion. There were no significant differences between groups

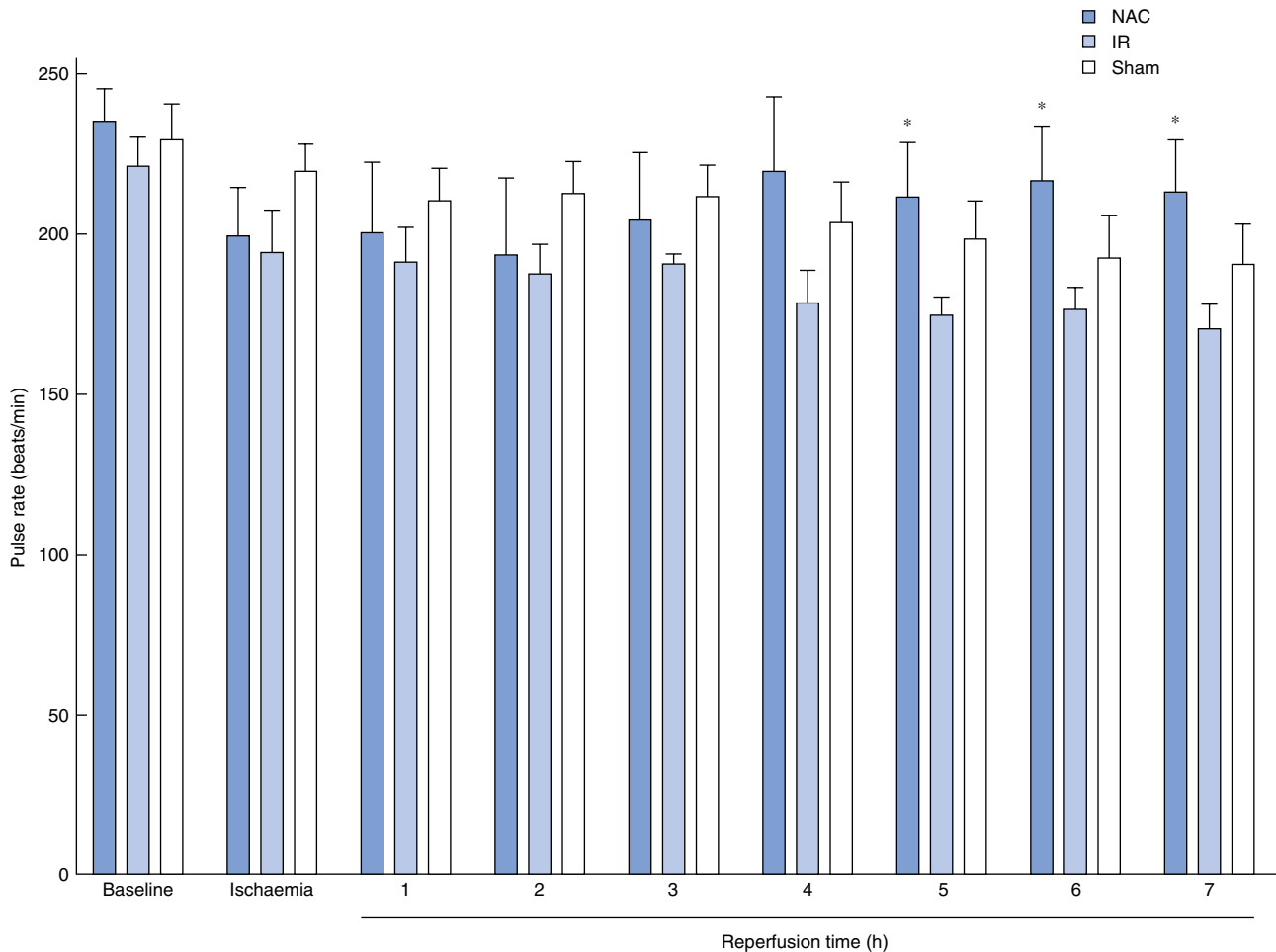


Fig. 2 Pulse rate during ischaemia and reperfusion. Values are mean(s.d.). NAC, *N*-acetylcysteine; IR, ischaemia–reperfusion. * $P < 0.050$ versus IR group (one-way ANOVA with Bonferroni adjustment for multiple comparisons)

Three groups of animals were used. In the NAC group 150 mg/kg NAC (Parvolex®; Medeva Pharma, Ashton-under-Lyne, UK) in 20 ml 5 per cent dextrose was infused intravenously through the ear vein over the 15 min before reperfusion and maintained at 10 mg per kg per h in 5 per cent dextrose (10 ml/h) during the 7-h reperfusion period. In the IR group 20 ml 5 per cent dextrose was infused intravenously 15 min before reperfusion and continued at a dose of 10 ml/h during the reperfusion period. Animals in the sham operation group underwent laparotomy but no liver ischaemia. In all groups 0.9 per cent sodium chloride was administered intravenously at 10 ml per kg per h to compensate for intraoperative fluid loss.

Laparotomy and baseline measurements were completed over 1 h. In the sham group monitoring continued for the equivalent of the ischaemia and reperfusion

periods in treated animals (total monitoring period 9 h).

Portal flow was monitored continuously using a perivascular transonic flow probe 3 mm in diameter (Transonic® Medical Flowmeter system, HT207; Transonic Medical System, Ithaca, New York, USA).

Arterial blood samples (1 ml each) were taken before the induction of liver ischaemia (baseline), at the end of ischaemia (60 min), and 2, 5 and 7 h after reperfusion for measurement of alanine aminotransferase (ALT) activity. Serum was separated from the samples and stored at -20°C until assayed. Measurements were made using an automated clinical chemistry analyser (Hitachi® 747; Roche Diagnostics, Lewes, UK).

Hepatic microcirculation was measured by a surface laser Doppler flowmeter (LDF) (DTR4; Moor Instruments, Axminster, UK)²⁰. The LDF probe was placed on a fixed

site on the median lobe of the liver and was held in place by a retort holder.

Intracellular hepatic tissue oxygenation was measured by near-infrared spectroscopy (NIRS). This measures changes in concentrations of oxyhaemoglobin, deoxyhaemoglobin, cytochrome oxidase²¹ and the synthetic dye indocyanine green (ICG)^{22,23}. For continuous monitoring of hepatic tissue cytochrome oxidase, NIRS probes were positioned flat on the surface of the left lobe of the liver 10 mm apart. A flexible probe holder was used to ensure satisfactory contact with the liver surface with fixed interprobe spacing. NIRS measurements during ischaemia and reperfusion were expressed relative to baseline values before vascular occlusion.

A bolus of 0.5 mg/kg ICG (Cardiogreen®, 90 per cent dye content; Sigma Chemical Company, Poole, UK) was given after 7 h of reperfusion. ICG was dissolved in sterile water (50 mg per 10 ml) and administered via the marginal

ear vein over 20 s. Continuous measurement of hepatic ICG by NIRS produced a concentration–time curve. This curve was analysed to produce two exponential rate constants: α , which represented hepatic ICG uptake from the plasma to the hepatocytes, and β , which represented hepatic ICG excretion from the liver by cytoplasmic transport and biliary excretion²⁴.

Data collection and statistical analysis

Data from the pulse oximeter, blood pressure and portal flow monitor, LDF and NIRS were collected continuously on a laptop computer. The data were averaged for 2 min before the induction of ischaemia (baseline), at the end of ischaemia and at the end of each hour of a 7-h reperfusion period. Changes in hepatic tissue oxygenation at the end of each period were calculated relative to baseline. Values are expressed as mean(s.d.). Student's *t* test for paired samples

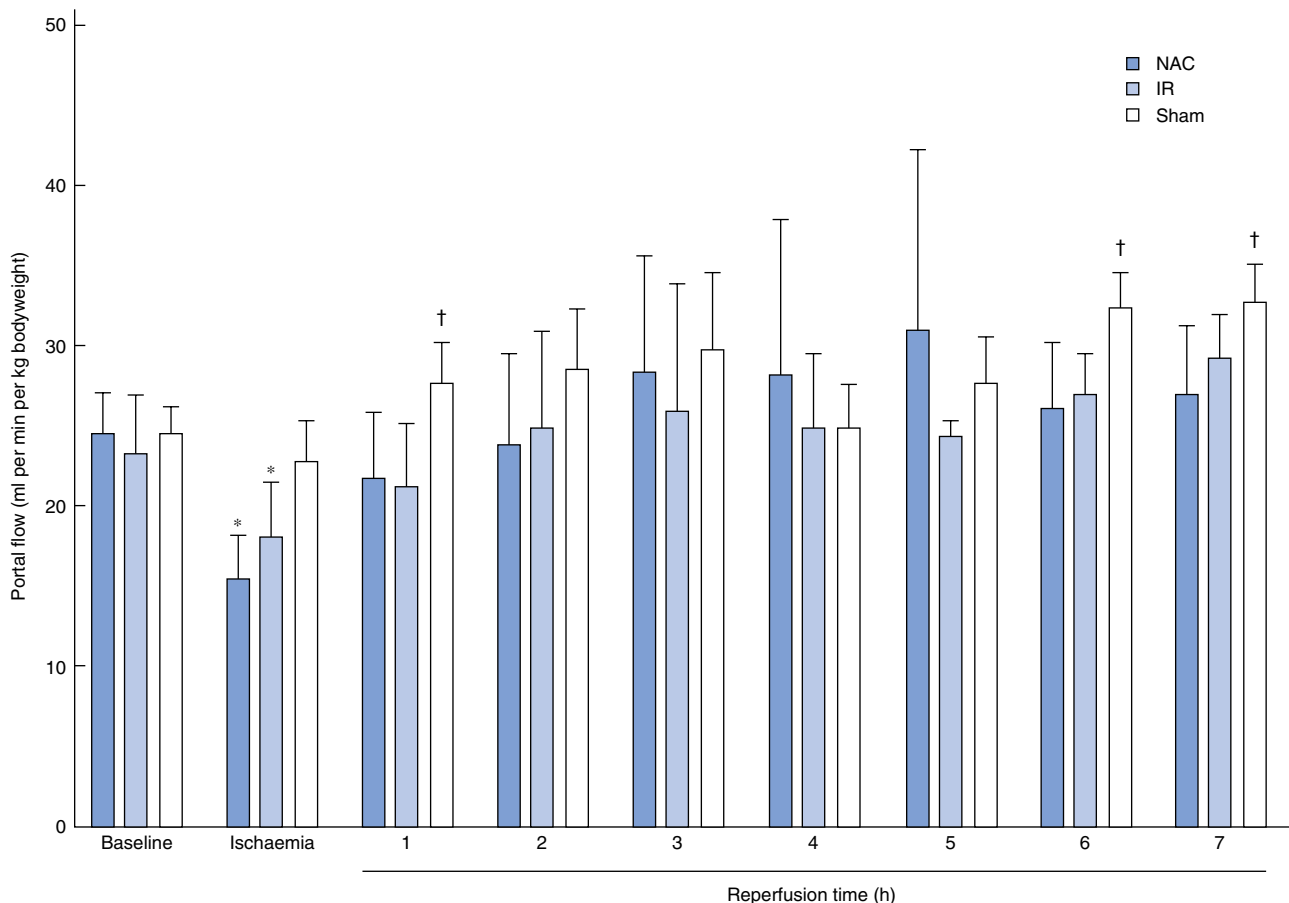


Fig. 3 Portal flow during ischaemia and reperfusion. Values are mean(s.d.). NAC, *N*-acetylcysteine; IR, ischaemia–reperfusion. **P* < 0.050 versus baseline (Student's *t* test for paired samples); †*P* < 0.050 versus NAC and I/R groups (one-way ANOVA with Bonferroni adjustment for multiple comparisons)

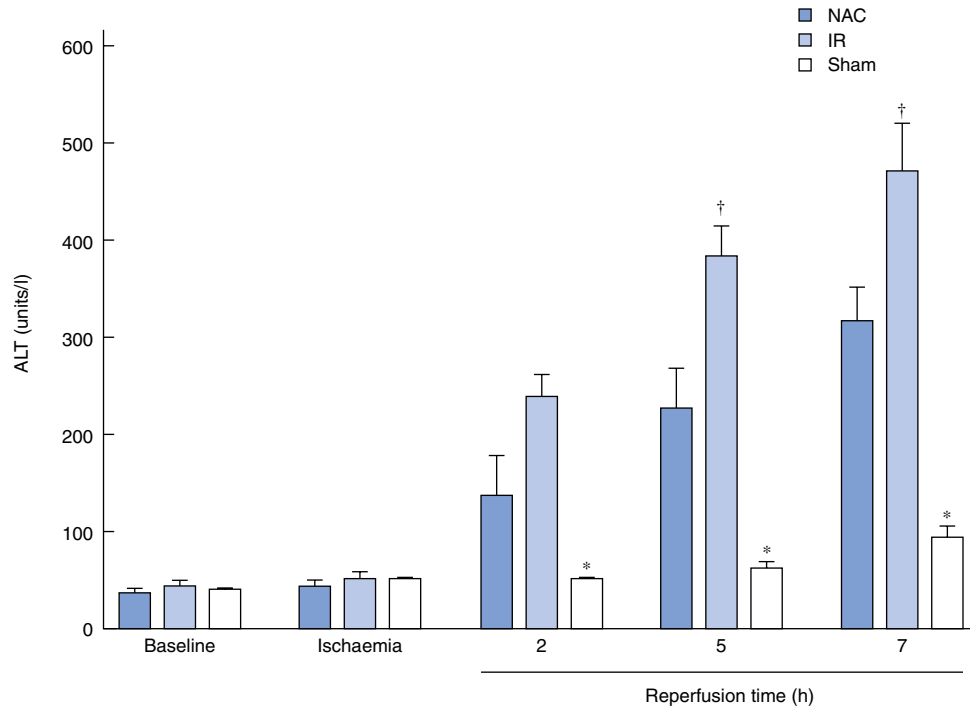


Fig. 4 Serum alanine aminotransferase (ALT) activity during ischaemia and reperfusion. Values are mean(s.d.). NAC, *N*-acetylcysteine; IR, ischaemia–reperfusion. * $P < 0.050$, sham group *versus* baseline (Student's *t* test for paired samples); † $P < 0.050$, *versus* NAC group, (one-way ANOVA with Bonferroni adjustment for multiple comparisons)

and one-way ANOVA with Bonferroni adjustment for multiple comparisons were used for statistical analysis. $P < 0.050$ was considered statistically significant.

Results

Systemic haemodynamic variables

In all three groups systemic mean arterial blood pressure fell in the reperfusion period compared with baseline. These changes were not significantly different between groups (*Fig. 1*). In all groups the pulse rate slowed during the course of the experiment in comparison to baseline measurements. The pulse rate stayed closer to baseline in the NAC group than in the IR group; this difference was significant after 5 h ($P = 0.026$), 6 h ($P = 0.025$) and 7 h ($P = 0.016$) of reperfusion (*Fig. 2*).

Hepatic haemodynamic variables

There were no differences in portal blood flow between the groups at baseline. During the period of ischaemia the portal flow was reduced in both the NAC ($P = 0.003$) and IR ($P = 0.001$) groups, compared with baseline values. The reduction started 1 min after the induction of ischaemia and

was maximal by 10 min. During the rest of the experiment, the portal flow fluctuated in all three groups. In the sham group there was an increase in portal flow rate. Portal flow was significantly greater in the sham group in comparison to that in the NAC and IR groups after 1 h ($P = 0.037$), 6 h ($P = 0.025$) and 7 h ($P = 0.033$) of reperfusion (*Fig. 3*).

Liver function tests

Mean serum ALT activity at baseline was within the normal range, with no significant differences between the three groups (*Fig. 4*). After reperfusion, the mean ALT activity in the NAC and IR groups was significantly higher than that in the sham-operated group. The serum ALT activity was lower in the NAC group than in the I/R group after 5 h ($P = 0.007$) and 7 h ($P = 0.021$) of reperfusion (*Fig. 4*). By the end of the experiment ALT activity had increased twofold in the sham-operated group compared with baseline values (*Fig. 4*).

Hepatic microcirculation

There was no significant change in the hepatic microcirculation over the 9-h sham operation. Following IR, flow

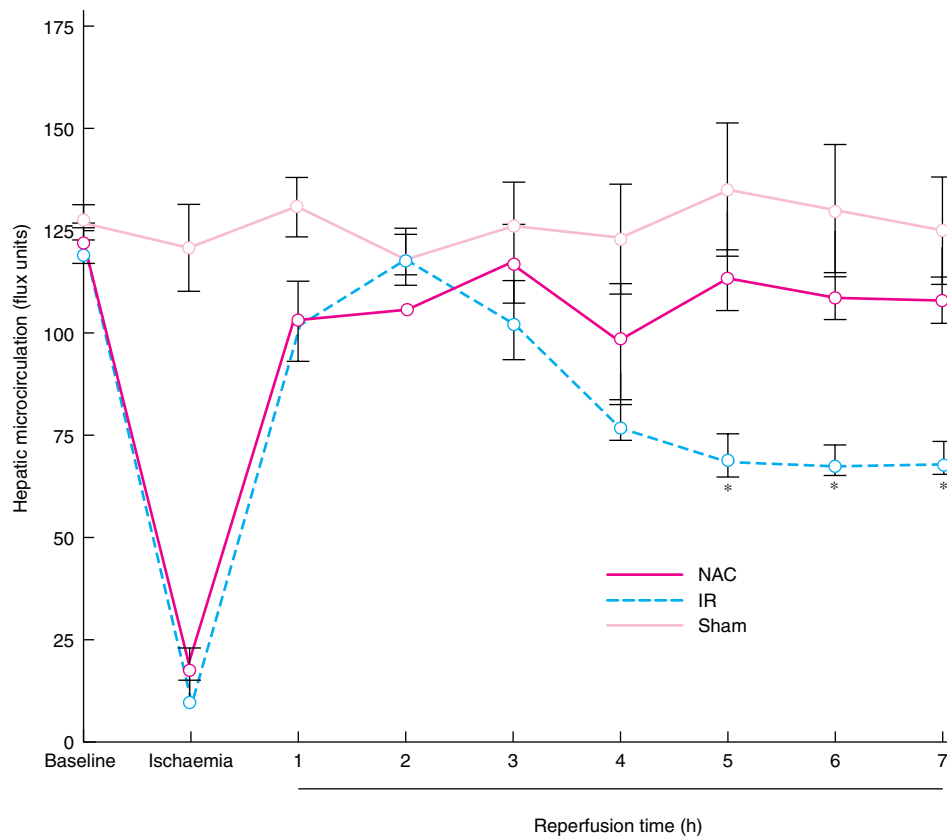


Fig. 5 Hepatic microcirculation during ischaemia and reperfusion. Values are mean(s.d.). NAC, N-acetylcysteine; IR, ischaemia–reperfusion. * $P < 0.050$ versus NAC group (one-way ANOVA with Bonferroni adjustment for multiple comparisons). There were no significant differences in the hepatic microcirculation during the experiment in the sham-operated group (Student's t test for paired samples)

in the microcirculation was reduced in the NAC and IR groups. NAC treatment was associated with an increased flow in the hepatic microcirculation after 3 h of reperfusion compared with that in the IR group, but this was not statistically significant until the fifth hour ($P = 0.036$) (Fig. 5).

Hepatic tissue oxygenation

During ischaemia there was a significant decrease in cytochrome oxidase, which was maximal at 5–10 min after induction of ischaemia (Fig. 6). After reperfusion, cytochrome oxidase levels in the IR group returned towards baseline values during the first hour, but subsequently decreased. In the NAC group, levels also fell with ischaemia but were significantly higher than levels in the IR group after 2 h ($P = 0.023$), 6 h ($P = 0.005$) and 7 h ($P = 0.004$) of reperfusion (Fig. 6). Levels in the NAC group were similar to those in the sham group after 6 and 7 h (Fig. 6).

Indocyanine green clearance

ICG uptake (α) and excretion (β) rates in NAC and IR groups were reduced by IR, in comparison to values in the sham group (Table 1). NAC produced a significant improvement in both ICG uptake and excretion at 7 h after ischaemic injury compared with rates in the IR group (Table 1).

Table 1 Hepatic indocyanine green uptake and excretion rates

	α (per min)	β (per min)
N-acetylcysteine	2.245(0.32)*	0.024(0.007)†
Ischaemia–reperfusion	0.848(0.394)	0.005(0.001)
Sham	2.565(0.488)	0.064(0.019)

Values are mean(s.d.) of six animals in each group. α , Hepatic indocyanine green (ICG) uptake rate; β , hepatic ICG excretion rate. * $P = 0.001$, † $P = 0.032$ versus ischaemia–reperfusion group (one-way ANOVA with Bonferroni adjustment for multiple comparisons).

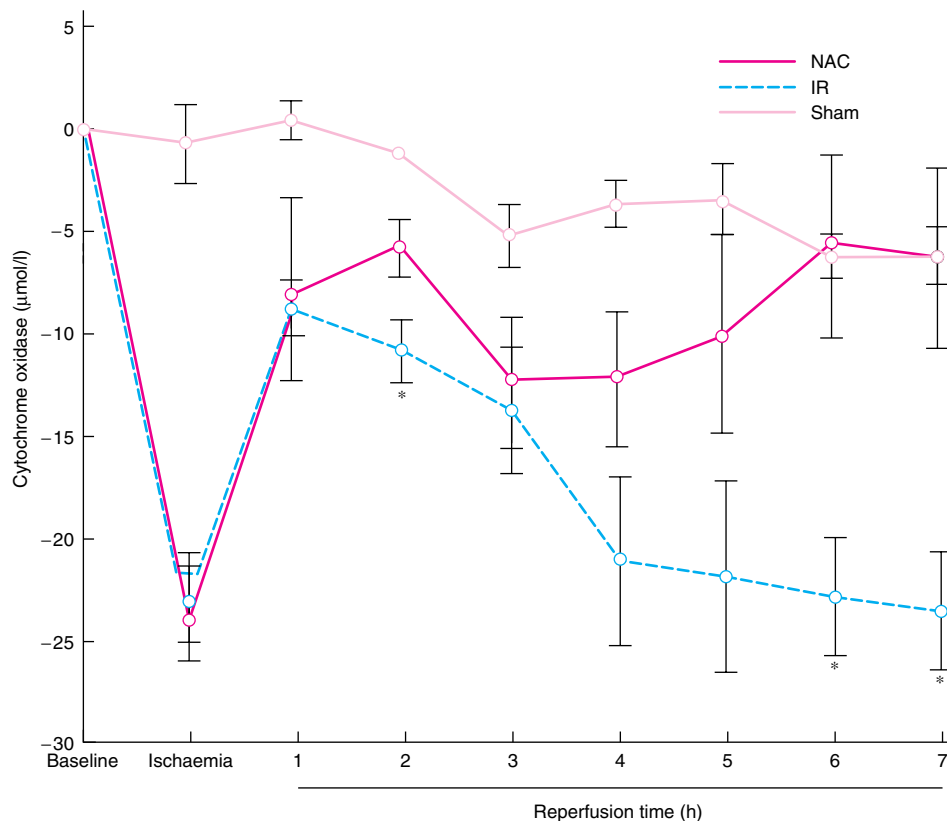


Fig. 6 Changes in cytochrome oxidase levels during ischaemia and reperfusion. Values are mean(s.d.). NAC, *N*-acetylcysteine; IR, ischaemia–reperfusion. * $P < 0.050$ versus NAC group (one-way ANOVA with Bonferroni adjustment for multiple comparisons)

Discussion

The late phase of liver warm IR injury is considered to start at 4 h after reperfusion and is mainly due to release of ROS and proteases from activated neutrophils. This produces more extensive hepatocellular injury than the early phase^{10,25}.

The present study investigated continuous infusion of NAC in a liver lobar IR model, which allowed hepatic function and haemodynamics to be evaluated in both the early and initial late phase of liver IR injury. Liver microcirculation and intracellular oxygenation were continuously recorded during a reperfusion period of 7 h. An inhaled agent (isoflurane), which is mainly metabolized in the lungs rather than the liver, was used for maintenance of anaesthesia, to avoid cumulative effects or hepatotoxicity. The sham group allowed the effects of prolonged anaesthesia and surgical trauma alone to be evaluated. The duration of general anaesthesia was similar to that for major human liver surgery (liver resection and transplantation).

In the sham-operated control group, arterial blood pressure dropped, ALT activity was significantly increased and hepatic intracellular oxygenation was significantly decreased during the period of anaesthesia compared with baseline values. This suggests that the anaesthetic agent and operative trauma had an effect on liver function during the lengthy surgical procedures, although there was no effect on the hepatic microcirculation; thus minor changes in the systemic circulation did not influence hepatic parenchymal perfusion.

Analysis of changes in the microcirculation and intracellular oxygenation during the 7-h reperfusion period suggests that the initial part of the late phase starts from the third hour after reperfusion and reaches a peak at the sixth hour. A previous study¹⁰ suggested that the late phase starts 6 h after reperfusion, based on the measurement of hepatocellular necrosis and neutrophil infiltration. Histological changes indicative of liver injury are likely, however, to be detected later than changes in the hepatic microcirculation or tissue oxygenation.

Portal flow values were calculated with respect to bodyweight to eliminate variations related to animal size. There was a significant decrease in portal flow during ischaemia in the IR and NAC groups. This decrease may result in a reduced metabolic need of the liver. Similar findings have been reported in previous studies²². During reperfusion there was fluctuation in portal flow in all three groups. In the sham group, the trend in portal flow was for a gradual increase during reperfusion, which might have been an effect of isoflurane, which increases hepatic blood flow and oxygen supply despite lowering systemic arterial pressure^{26,27}.

During inflow occlusion in the IR group the hepatic microcirculation dropped acutely almost to zero. There was a short-term recovery during the first 2 h of reperfusion, followed by a further decrease. Mechanisms that might contribute to this impairment of sinusoidal perfusion include sinusoidal endothelial cell swelling with luminal narrowing²⁸ and sinusoidal vasoconstriction mediated by an altered endothelin–nitric oxide balance^{4,29,30}. The increased expression of adhesion molecules, with subsequent leucocyte and endothelial cell interaction, may also play a crucial role in these changes in the microcirculation, especially during the late phase of reperfusion injury³¹. It has been reported that the changes in microcirculation during the immediate reperfusion period in liver transplantation correspond directly with the maximum postoperative enzyme release from the liver³², and are of value in assessing and predicting the degree of liver injury⁴.

Cytochrome oxidase is the terminal electron carrier of the mitochondrial respiratory chain that catalyses the reduction of oxygen to water with the concomitant synthesis of adenosine triphosphate through the oxidative phosphorylation process³³. In hepatocytes, approximately 90 per cent of the oxygen is consumed by mitochondrial cytochrome oxidase³³. Changes in cytochrome oxidase levels are indicative of the level of intracellular oxygenation and the mitochondrial redox state^{34,35}. The degree of impairment of the hepatic mitochondrial redox state determines the survival rate of patients after haemorrhagic shock and resuscitation³⁶. In the present study, during ischaemia there was a significant fall in cytochrome oxidase levels in the IR group, reflecting severe cellular hypoxia. Levels returned towards baseline during the first hour of reperfusion but subsequently decreased and by 7 h had reached levels similar to those observed during ischaemia. This further decline in cytochrome oxidase indicates persistent tissue and cellular hypoxia, which correlates

well with the changes in hepatic microcirculation during the late phase of reperfusion injury.

ICG uptake is related to liver blood flow and excretion is related to hepatocellular injury²². Direct measurement of ICG by NIRS may avoid the inaccuracies inherent in assessing liver function from peripheral blood clearance of ICG³⁷. In the present study IR produced a significant reduction in both the uptake and excretion of ICG compared with levels in the sham-operated group.

The effect of NAC on liver IR injury was evaluated using a dose of NAC similar to that used for patients with fulminant hepatic failure due to paracetamol overdose³⁸. The administration of NAC maintained the pulse rate close to baseline levels, reduced liver injury (as indicated by ALT serum activity), and improved flow in the microcirculation and intracellular tissue oxygenation. All these effects were obvious after 5 h of reperfusion. NAC also improved ICG clearance at 7 h after reperfusion. In another experimental study, in which a rat lobar liver IR model was used, no benefit was shown with NAC administration in warm IR injury¹⁸, although a shorter ischaemic period was used (45 min) and the NAC (300 mg/kg) was administered intramuscularly before ischaemia, which may have resulted in a less reliable systemic distribution or a shorter-term effect. The effect of NAC on the hepatic microcirculation in the late phase of reperfusion has not been reported previously. Previous experiments were limited to the first 1–2 h after reperfusion and the results were conflicting^{17,18}.

The effect of NAC on cytochrome oxidase level in the altered parenchymal microcirculation with IR was examined, with clear evidence of effects on the delivery and metabolism of oxygen in the hepatic parenchyma. This study did not assess the delayed effect of NAC (at 24 or 48 h), as the model used was not appropriate for these longer periods.

The mechanism underlying the protective effect of NAC in the late phase of warm IR injury was not established in this study. However, NAC is a precursor of glutathione regeneration¹⁵, is a direct scavenger of free radicals¹⁶, inhibits inducible nitric oxide synthase expression³⁹, and inhibits the expression of intercellular adhesion molecule 1 and vascular cell adhesion molecule 1⁴⁰.

Further studies are required to assess the effect of NAC at later time points and to elucidate the underlying mechanisms of action. Use of other thiols such as buccillamine⁴¹ or a combination of NAC with other antioxidants such as melatonin⁴² may further reduce liver IR injury. A large randomized clinical trial will be required to demonstrate a clear benefit of NAC administration in human liver warm IR.

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References

- Huguet C, Gavelli A, Bona S. Hepatic resection with ischemia of the liver exceeding one hour. *J Am Coll Surg* 1994; **178**: 454–458.
- Henderson JM. Liver transplantation and rejection: an overview. *Hepatogastroenterology* 1999; **46**(Suppl 2): 1482–1484.
- Vedder NB, Fouty BW, Winn RK, Harlan JM, Rice CL. Role of neutrophils in generalized reperfusion injury associated with resuscitation from shock. *Surgery* 1989; **106**: 509–516.
- Pannen BH. New insights into the regulation of hepatic blood flow after ischemia and reperfusion. *Anesth Analg* 2002; **94**: 1448–1457.
- Matuschak GM. Liver–lung interactions in critical illness. *New Horiz* 1994; **2**: 488–504.
- Lichtman SN, Lemasters JJ. Role of cytokines and cytokine-producing cells in reperfusion injury to the liver. *Semin Liver Dis* 1999; **19**: 171–187.
- Lentsch AB, Kato A, Yoshidome H, McMasters KM, Edwards MJ. Inflammatory mechanisms and therapeutic strategies for warm hepatic ischemia/reperfusion injury. *Hepatology* 2000; **32**: 169–173.
- Jaeschke H, Farhood A. Neutrophil and Kupffer cell-induced oxidant stress and ischemia–reperfusion injury in rat liver. *Am J Physiol* 1991; **260**: G355–G362.
- Colletti LM, Remick DG, Burtch GD, Kunkel RM, Strieter RM, Campell DA Jr. Role of tumor necrosis factor- α in the pathophysiologic alterations after hepatic ischemia/reperfusion injury in the rat. *J Clin Invest* 1990; **85**: 1936–1943.
- Jaeschke H, Farhood A, Smith CW. Neutrophils contribute to ischemia/reperfusion injury in rat liver *in vivo*. *FASEB J* 1990; **4**: 3355–3359.
- Jaeschke H. Reactive oxygen and mechanisms of inflammatory liver injury. *J Gastroenterol Hepatol* 2000; **15**: 718–724.
- Liu P, Fisher MA, Farhood A, Smith CW, Jaeschke H. Beneficial effects of extracellular glutathione against endotoxin-induced liver injury during ischemia and reperfusion. *Circ Shock* 1994; **43**: 64–70.
- Prescott LF, Illingworth RN, Critchley JAJH, Stewart MJ, Adam RD, Proudfoot AT. Intravenous *N*-acetylcysteine: the treatment of choice for paracetamol poisoning. *BMJ* 1979; **ii**: 1097–1100.
- Chyka PA, Butler AY, Holliman BJ, Herman MI. Utility of acetylcysteine in treating poisonings and adverse drug reactions. *Drug Saf* 2000; **22**: 123–148.
- Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*. Oxford University Press: Oxford, 1999; 840–842.
- Cotgreave IA. *N*-acetylcysteine: pharmacological considerations and experimental and clinical applications. *Adv Pharmacol* 1997; **38**: 205–227.
- Koeppel TA, Thies JC, Lehmann T, Gebhard MM, Herfald C, Otto G *et al*. Improvement of hepatic microhemodynamics by *N*-acetylcysteine after warm ischemia. *Eur Surg Res* 1996; **28**: 270–277.
- Chavez-Cartaya R, Jamieson NV, Ramirez P, Marin J, Pino-Chavez G. Free radical scavengers to prevent reperfusion injury following experimental warm liver ischaemia. Is there a real physiological benefit? *Transpl Int* 1999; **12**: 213–221.
- Koo A, Komatsu H, Tao G, Inoue M, Guth PH, Kaplowitz N. Contribution of no-reflow phenomenon to hepatic injury after ischemia–reperfusion: evidence for a role for superoxide anion. *Hepatology* 1992; **15**: 507–514.
- Seifalian AM, Mallet SV, Rolles K, Davidson BR. Hepatic microcirculation during human orthotopic liver transplantation. *Br J Surg* 1997; **84**: 1391–1395.
- El Desoky AE, Seifalian AM, Davidson BR. Effect of graded hypoxia on hepatic tissue oxygenation measured by near infrared spectroscopy. *J Hepatol* 1999; **31**: 71–76.
- El Desoky A, Seifalian AM, Cope M, Delpy DT, Davidson BR. Experimental study of liver dysfunction evaluated by direct indocyanine green clearance using near infrared spectroscopy. *Br J Surg* 1999; **86**: 1005–1011.
- El Desoky AE, Delpy DT, Davidson BR, Seifalian AM. Assessment of hepatic ischaemia reperfusion injury by measuring intracellular tissue oxygenation using near infrared spectroscopy. *Liver* 2001; **21**: 37–44.
- Shinohara H, Tanaka A, Kitai T, Yanabu N, Inomoto T, Satoh S *et al*. Direct measurement of hepatic indocyanine green clearance with near-infrared spectroscopy: separate evaluation of uptake and removal. *Hepatology* 1996; **23**: 137–144.
- Harbrecht BG, Billiar TR, Curran RD, Stadler J, Simmons RL. Hepatocyte injury by activated neutrophils *in vitro* is mediated by proteases. *Ann Surg* 1993; **218**: 120–128.
- Bernard JM, Doursout MF, Wouters P, Hartley CJ, Cohen M, Merin RG *et al*. Effects of enflurane and isoflurane on hepatic and renal circulations in chronically instrumented dogs. *Anesthesiology* 1991; **74**: 298–302.
- Gatecel C, Losser MR, Payen D. The postoperative effects of halothane *versus* isoflurane on hepatic artery and portal vein blood flow in humans. *Anesth Analg* 2003; **96**: 740–745.
- Vollmar B, Glasz J, Leiderer R, Post S, Menger MD. Hepatic microcirculatory perfusion failure is a determinant of liver dysfunction in warm ischemia–reperfusion. *Am J Pathol* 1994; **145**: 1421–1431.

- 29 Pannen BH, Al Adili F, Bauer M, Clemens MG, Geiger KK. Role of endothelins and nitric oxide in hepatic reperfusion injury in the rat. *Hepatology* 1998; **27**: 755–764.
- 30 Scommotau S, Uhlmann D, Loffler BM, Breu V, Spiegel HU. Involvement of endothelin/nitric oxide balance in hepatic ischemia/reperfusion injury. *Langenbecks Arch Surg* 1999; **384**: 65–70.
- 31 Vollmar B, Glasz J, Menger MD, Messmer K. Leukocytes contribute to hepatic ischemia/reperfusion injury via intercellular adhesion molecule-1-mediated venular adherence. *Surgery* 1995; **117**: 195–200.
- 32 Klar E, Bredt M, Kraus T, Angelescu M, Mehrabi A, Senninger N *et al.* Early assessment of reperfusion injury by intraoperative quantification of hepatic microcirculation in patients. *Transplant Proc* 1997; **29**: 362–363.
- 33 Capaldi RA. Structure and function of cytochrome *c* oxidase. *Annu Rev Biochem* 1990; **59**: 569–596.
- 34 Seifalian AM, El Desoky H, Delpy DT, Davidson BR. Effect of graded hypoxia on the rat hepatic tissue oxygenation and energy metabolism monitored by near-infrared and ³¹P nuclear magnetic resonance spectroscopy. *FASEB J* 2001; **15**: 2642–2648.
- 35 Koti RS, Seifalian AM, McBride AG, Yang W, Davidson BR. The relationship of hepatic tissue oxygenation with nitric oxide metabolism in ischemic preconditioning of the liver. *FASEB J* 2002; **16**: 1654–1656.
- 36 Nakatani T, Spolter L, Kobayashi K. Arterial ketone body ratio as a parameter of hepatic mitochondrial redox state during and after hemorrhagic shock. *World J Surg* 1995; **19**: 592–596.
- 37 Ott P, Keiding S, Johnsen AH, Bass L. Hepatic removal of two fractions of indocyanine green after bolus injection in anesthetized pigs. *Am J Physiol* 1994; **266**: G1108–G1122.
- 38 Harrison PM, Wendon JA, Gimson AE, Alexander GJ, Williams R. Improvement by acetylcysteine of hemodynamics and oxygen transport in fulminant hepatic failure. *N Engl J Med* 1991; **324**: 1852–1857.
- 39 Hur GM, Ryu YS, Yun HY, Jeon BH, Kim YM, Seok JH *et al.* Hepatic ischemia/reperfusion in rats induces *iNOS* gene transcription by activation of NF-kappaB. *Biochem Biophys Res Commun* 1999; **261**: 917–922.
- 40 Weigand MA, Plachky J, Thies JC, Spies-Martin D, Otto G, Martin E *et al.* *N*-acetylcysteine attenuates the increase in alpha-glutathione *S*-transferase and circulating ICAM-1 and VCAM-1 after reperfusion in humans undergoing liver transplantation. *Transplantation* 2001; **72**: 694–698.
- 41 Amersi F, Nelson SK, Shen XD, Kato H, Melinek J, Kupiec-Weglinski JW *et al.* Bucillamine, a thiol antioxidant, prevents transplantation-associated reperfusion injury. *Proc Natl Acad Sci U S A* 2002; **99**: 8915–8920.
- 42 Sener G, Tosun O, Sehirli O, Kacmaz A, Arbak S, Ersoy Y *et al.* Melatonin and *N*-acetylcysteine have beneficial effects during hepatic ischemia and reperfusion. *Life Sci* 2003; **72**: 2707–2718.