Differential Effects of Lower Limb Revascularisation on Organ Injury and the Role of the Amino Acid Taurine

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Lower torso revascularisation following ischaemia results in a systemic inflammatory response. Endothelial barrier function is disrupted by neutrophil-derived proteases and oxidants. Taurine, an amino acid found in large quantities in neutrophils, is a powerful endogeneous anti-oxidant. The aims of this study were to investigate the systemic effects of reperfusion following lower limb revascularisation and to evaluate the role of taurine administration in preventing this injury. A rat model of aortic occlusion (30 min) followed by 2 h of reperfusion was used. Animals were randomised to one of three groups (n = 10 per group): control; ischaemia reperfusion untreated (IR) and taurine-treated. Taurine (4% solution) was administrated orally for 48 h prior to the experiment. Neutrophil infiltration and microvascular permeability were assessed by measuring tissue myeloperoxidase activity and wet/dry weights respectively in lung, liver, kidney, and in cardiac and skeletal muscle. Statistical analysis was by means of analysis of variance (ANOVA). Reperfusion resulted in pulmonary and renal microvascular injury as assessed by organ oedema. Hepatic tissue, skeletal and cardiac muscle were unaffected by lower limb revascularisation. Taurine was effective in preventing neutrophil-mediated pulmonary but not renal microvascular injury. These data suggest that, whilst reperfusion-induced pulmonary injury is predominantly neutrophil-mediated, agents other than neutrophil-derived oxidative metabolites, capable of independently causing organ injury through direct endothelial damage, are produced during reperfusion.

Introduction

Reperfusion injury following an ischaemic insult is characterised by a systemic inflammatory response resulting in widespread endothelial damage and microvascular leakage.[1] Patients undergoing aortic reconstructive surgery suffer a prolonged ischaemic insult followed by reperfusion of a large mass of tissue. These patients have been shown to suffer transient organ dysfunction affecting particularly the lung, gut and kidney.[2,3] Lung microvascular injury resulting from reperfusion has been shown to be predominantly neutrophil-dependent.[4–7] However, the occurrence and pathogenesis of reperfusion-induced injury in other organs, such as kidney, heart, liver and skeletal muscle has not been as well established.

Hypochlorous acid (HOCl) produced from hydrogen peroxide (H_2O_2), is one of the most toxic of the oxygen-derived metabolites released by activated neutrophils producing cell and tissue destruction by lipid peroxidation.[8,9] Taurine is an amino acid found in high concentrations in human leucocytes, specifically in polymorphonuclear leucocytes. One of its main roles *in vivo* is to protect blood cells and tissues against attack by chlorinated oxidants, particularly HOCl.[10] Green *et al.* have demonstrated that the concentration of intracellular taurine decreases approximately 80% with the conversion of resting neutrophils into activated cells.[11] This suggests that the biological role of taurine is linked to the release of oxidants accompanying activation of resting neutrophils. In addition to its intracellular site of action, taurine is released into the extracellular medium by neutrophils *in vitro* and is found in blood plasma.[12] Concentrations of taurine inside leucocytes are up to 500 times the concentrations in plasma, favouring loss of taurine across a concentration gradient into the extracellular medium.[10] Extracellular taurine may help to protect blood cells and the tissues against attack by chlorinated oxidants produced outside the leucocytes. Based on these known endogenous antioxidant properties, taurine has already been used with some success in the experimental setting. Animal studies have shown it to be effective in preventing bleomycin-induced

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lung fibrosis and nitrous oxide related lung injury, the early stages of which are thought to be mediated by O_2-derived free radicals.[13-18]

The aims of the present study were to investigate the systemic effects of reperfusion, to evaluate the role of the neutrophil in mediating this injury and, to examine the role of the naturally occurring endogenous antioxidant, taurine, in preventing this injury.

Methods

Animal preparation

Animal experiments were carried out in accordance with the principles of laboratory animal care. Thirty-three adult male Sprague-Dawley rats weighing 400–500 g were anaesthetised with inhalational halothane (IIG, Dublin, Ireland). An external jugular venous catheter was inserted for fluid, drug and heparin administration. Core temperature was monitored for the duration of the experiment using a rectal temperature probe.

Aortic occlusion and reperfusion

Prior to the experiment, animals were randomised to one of three groups: control; ischaemia reperfusion untreated and taurine-treated animals. All animals were fed standard rat chow prior to the experiment and control and IR animals were given water to drink *ad libitum*. In the taurine group, taurine (4% weight/volume) was given in drinking water for 48 h prior to the experiment. Anaesthetised animals underwent a midline laparotomy. Following systemic heparinisation (400 units heparin/kg Leo Laboratories) the infrarenal aorta was exposed. In control animals the aorta was exposed but not clamped and a saline infusion was commenced after 30 min, lasting 125 min. In the ischaemia reperfusion (IR) group and taurine-treated group the aorta was clamped for 30 min using a microvascular clamp. A saline infusion (1 ml/h) was commenced 5 min prior to aortic unclamping and continued throughout the 2 h period of reperfusion. At the end of the experiment the animals were killed with an overdose of anaesthetic. After sternotomy the left main bronchus was clamped. Bronchoalveolar lavage (BAL) of the right lung was performed with 2 ml of saline containing 0.07M EDTA and repeated three times. The combined lavage 3–4 ml) returned was centrifuged at 1500 rpm for 20 min at

4°C, frozen at –20°C and subsequently assessed for protein concentration.

Myeloperoxidase assay

Neutrophil infiltration in lung, heart, skeletal muscle, kidney and liver was assessed by measuring tissue myeloperoxidase activity. Myeloperoxidase (MPO) is a haem-containing enzyme within the azurophil granules of neutrophils. Its measurement has been shown to be a simple quantitative method of detecting leucosequestration. The right ventricle was cannulated using a 25 gauge needle (Jelco, Critikon Ltd.) and the right pulmonary hilum clamped. The pulmonary vasculature of the left lung was flushed using 50 ml of saline to clear the lung of intravascular neutrophils. After weighing, the left lung was homogenised in 10 ml of 0.5% hexadecyltrimethyl ammonium bromide (HTAB) in 50 mM potassium phosphate buffer at a pH of 6. A similar protocol was used to assess MPO activity in the left ventricle, intercostal muscle, left kidney and right lobe of the liver. The homogenates were freeze-thawed twice and centrifuged at 12 000 g for 15 min. The resultant supernatent was assayed spectrophotometrically for myeloperoxidase activity by incubating 0.1 ml of the supernatent with 2.9 ml of a solution containing 2.9 ml of O-dioniside dihydrochloride in 90 ml distilled water, 10 ml of 50 mM potassium phosphate buffer (pH 6.0) and hydrogen peroxide. The change in absorbance with time, at 460 nm was then recorded (Phillips, CPU 8720, UV/VIS Scanning Spectrophotometer). One unit of MPO was defined as that degrading 1 micromole peroxide/min at 25°C.

Wet-to-dry lung weight ratio

The wet-to-dry ratio of lung, heart, liver, kidney and skeletal muscle was calculated after weighing the freshly harvested organ, and heating it at 60°C in a gravity convection oven (Gallen-Kamp, Model IH-150) over a 72 h period after which the weight had become constant.

Bronchoalveolar lavage

Lung lavage fluid protein content was measured spectophotometrically using the Lowry method.[19]

Neutrophil counts in bronchoalveolar lavage fluid was carried out by Diff-Quick staining and consecutive counting of neutrophils per high power field.

Neutrophil respiratory burst activity

Blood samples were drawn from the central venous line prior to aortic cross clamping, at the end of the cross clamp period, and at 5, 30 and 60 min following clamp removal. Blood was drawn into heparinised tubes and assayed within 4 h. The Respiratory Burst was assessed using a BURSTTEST (Orpegen, Heidelberg, Germany). This method allows the determination of intracellular leucocyte oxidative and enzymatic activity using Dihydrorhodamin 123 as a fluorogenic substrate, measured flow cytometrically (FACSCAN, Becton-Dickinson).[20] One-hundred microlitres of whole blood was incubated alone or with 10 μl of rat serum opsonised *Escherichia Coli* for 10 min. Ten microlitres of the fluorogenic substrate, dihydrorhodamin 123 (DHR 123) was then added and incubated for a further 10 min. Erythrocytes were lysed by addition of lysing solution for 20 min. Following centrifugation the cell pellets were washed and suspended in 100 μl DNA staining solution. The analysis of PMN respiratory burst activity was performed on a FACScan cytofluorometer (Becton Dickinson, CA, U.S.A.) detecting mean channel fluorescence. A minimum of 5000 cells were collected and analysed using the software Lysis II.

Statistics

Results were expressed as mean ± s.e.m. in text and figures. Statistical analysis was carried out using analysis of variance for comparison of multiple means with post-hoc Scheffe test analysis. Significance was accepted for $p < 0.05$.

Results

Myeloperoxidase activity

Ischaemia followed by reperfusion resulted in a significant increase in pulmonary leucosequestration as indicated by an increase in MPO activity from 3.4 ± 0.44 units/g in control animals to 7.29 ± 0.84 units/g in the IR group ($p < 0.01$). This increase was

significantly attenuated by treatment with taurine (4.37 ± 0.64 units/g) ($p < 0.01$ ANOVA) (Fig. 1). Reperfusion had no effect on kidney MPO activity. Values obtained were similar in control (0.528 ± 0.06 units/g) and IR animals ($0.552 \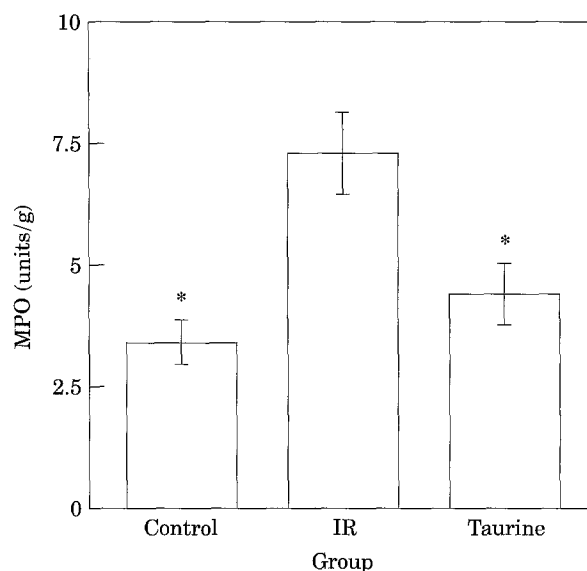pm 0.07$ units/g). MPO activity in taurine-treated animals were lower (0.437 ± 0.06 units/g) but this was not statistically significant (Fig. 2). Reperfusion had no effect on liver MPO activity. Values obtained were similar in control (0.412 ± 0.03 units/g) and IR animals (0.42 ± 0.07 units/g). MPO activity in taurine-treated animals was

Fig. 1. Effect of ischaemia reperfusion on pulmonary myeloperoxidase activity. Data is expressed as mean ± s.e.m. *$p < 0.01$ vs. Control/taurine (ANOVA).

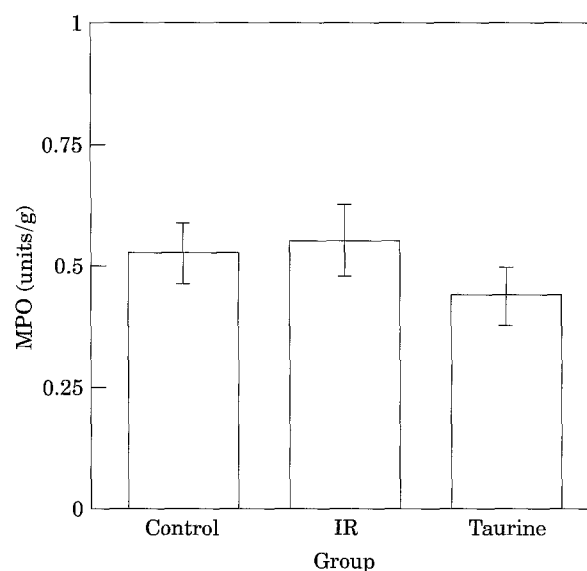Fig. 2. Effect of ischaemia reperfusion on kidney myeloperoxidase activity. Data is expressed as mean ± s.e.m.

lower (0.385 ± 0.08 units/g) but this was not statistically significant (Fig. 3). Lower torso revascularisation resulted in a significant increase in MPO activity in intercostal muscle in IR animals (2.25 ± 0.4 units/g) compared to controls (0.86 ± 0.14 units/g) ($p < 0.05$ ANOVA) (Fig. 3). This was prevented by prior administration of taurine (0.868 ± 0.2 units/g) ($p < 0.05$ ANOVA). Ischaemia reperfusion had no effect on left ventricular neutrophil infiltration (Control 0.465 ± 0.08 units/g; IR 0.49 ± 0.06 units/g). MPO activity was significantly lower in the taurine treated group (0.244 ± 0.05 units/g) ($p < 0.05$ ANOVA) (Fig. 3).

Organ water content (wet/dry ratio)

Wet to dry lung weight ratios followed a similar trend to the previous results indicating a higher amount of extravascular lung water in untreated IR animals (3.66 ± 0.08) in comparison to control (3.29 ± 0.14) and taurine treated animals (3.29 ± 0.1) ($p < 0.05$ ANOVA) (Fig. 4). Ischaemia reperfusion resulted in significant renal oedema in both the untreated IR group (2.99 ± 0.08) and the taurine treated group (3.01 ± 0.08) compared to controls (2.7 ± 0.06) ($p < 0.01$ ANOVA) (Fig. 5). Ischaemia reperfusion had no effect on liver wet/dry ratio, (Control 1.9 ± 0.04; IR

1.95 ± 0.04). Oedema in the taurine treated group was less severe (Taurine 1.83 ± 0.01) but this was not statistically significant (Fig. 6). Skeletal muscle wet/ dry weight ratio was similar in control (2.6 ± 0.1) and IR animals (2.56 ± 0.1). Oedema was less marked in the taurine treated animals (2.25 ± 0.21) but this was not statistically significant (Fig. 6). Left ventricular muscle wet/dry weight ratio was similar in control (3.28 ± 0.18) and IR animals (3.14 ± 0.17). Oedema was less marked in the taurine treated animals (2.94 ± 0.13) but this was not statistically significant (Fig. 6).

Pulmonary permeability

Pulmonary permeability as assessed by BAL protein and neutrophil counts was significantly higher in untreated animals compared to control and taurine treated groups.

BAL neutrophil counts were 1.50 ± 0.098/mm^3 in IR animals, 0.491 ± 0.060/mm^3 in controls ($p < 0.02$ vs. IR) and 0.533 ± 0.1/mm^3 ($p < 0.02$ vs. IR) in taurine treated animals (Fig. 7). Similarly, treatment with taurine returned BAL fluid protein content (124 ± 5.59 µg/ml) towards control levels (117 ± 6.3 µg/ml) compared to the IR group (150 ± 8.88 µg/ml; $p < 0.01$ vs. control and taurine) (Fig. 8).
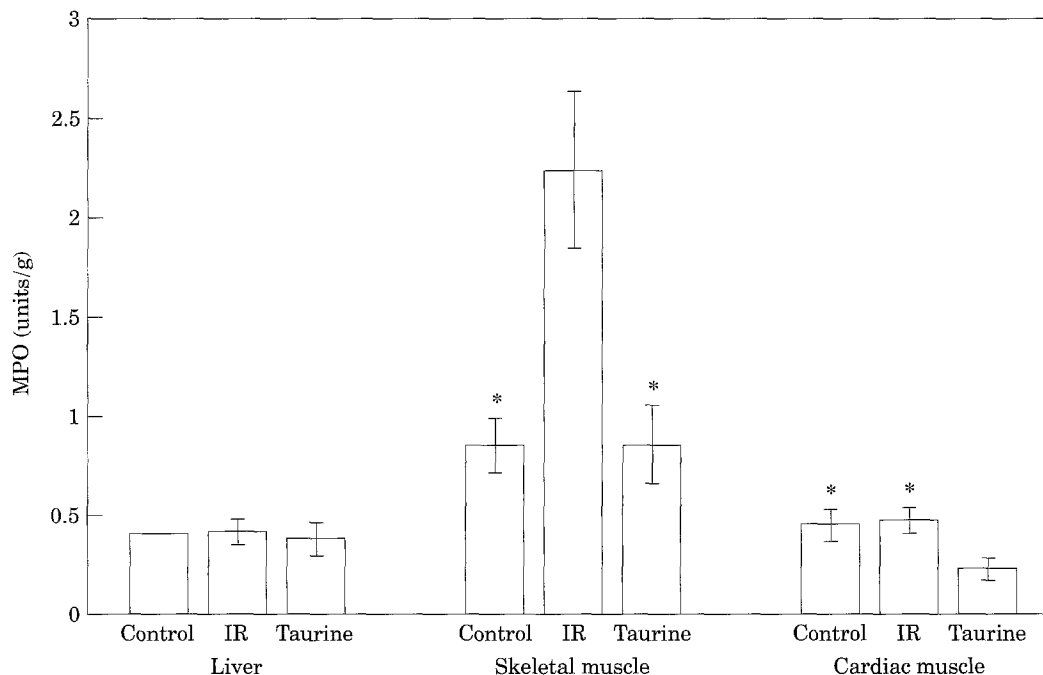

Fig. 3. Effect of ischaemia reperfusion on liver, cardiac muscle and skeletal muscle myeloperoxidase activity. Data is expressed as mean ± s.e.m. *$p < 0.05$ vs. IR/taurine (ANOVA).

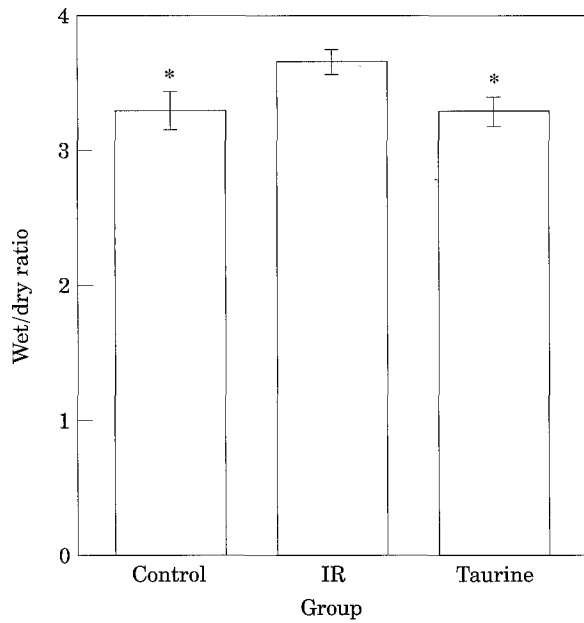**Fig. 4.** Effect of ischaemia-reperfusion on pulmonary wet/dry lung weight ratio. Data is expressed as mean ± s.e.m. $^{*}p < 0.05$ vs. IR (ANOVA).
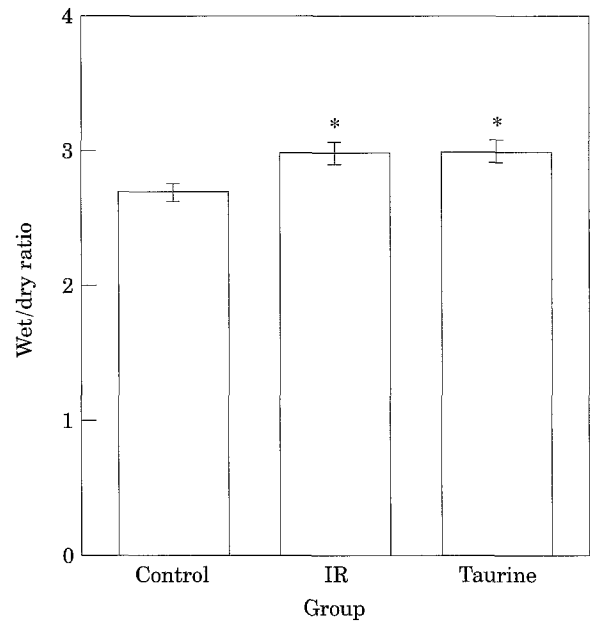
Fig. 5. Effect of ischaemia-reperfusion on kidney wet/dry weight ratio. Data is expressed as mean ± s.e.m. $^{*}p < 0.05$ vs. Control (ANOVA).
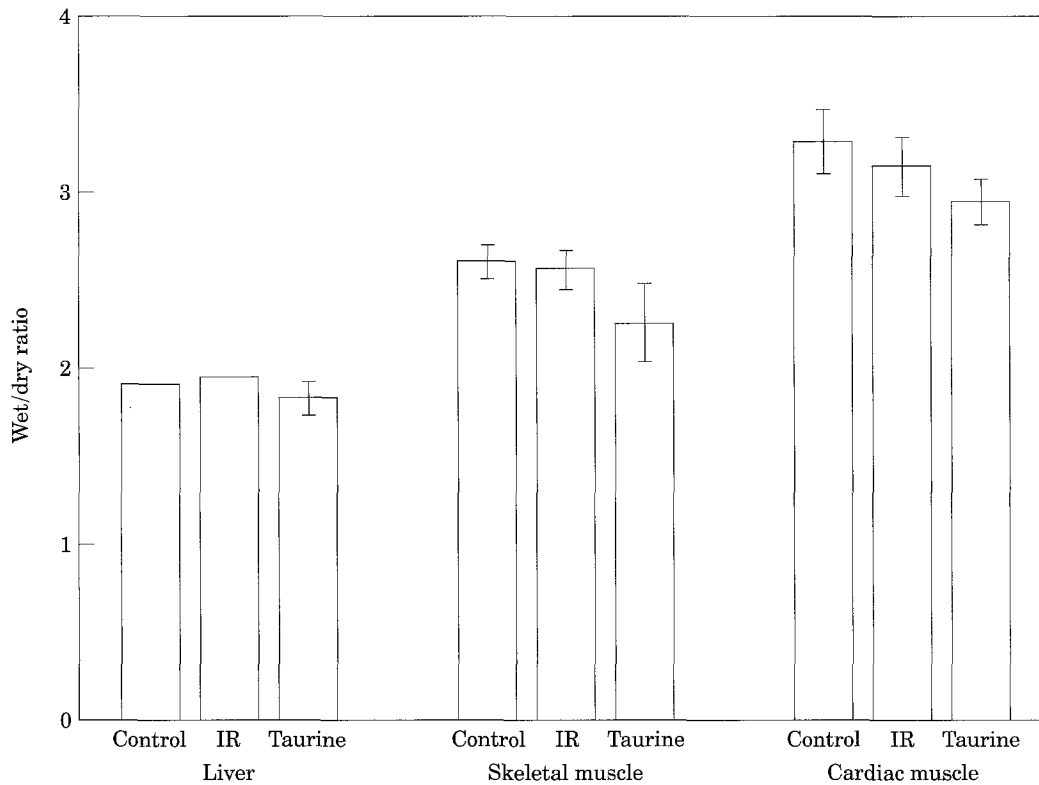

Fig. 6. Effect of ischaemia-reperfusion on liver, skeletal muscle and cardiac muscle wet/dry weight ratio. Data is expressed as mean ± s.e.m.

Neutrophil respiratory burst activity

Baseline PMN respiratory burst activity was similar at the start of the experiment (control 21.12 ± 2.48 mean channel fluorescence; IR 22.34 ± 1.62; taurine 18.73 ± 4.87 mcf). Reperfusion resulted in an increase in PMN respiratory burst activity at 5 min

(28.27 ± 2.29 mcf) and 40 min (28.1 ± 1.73) in the IR group compared to the control group (19.9 ± 0.44 at 5 min; 21.51 ± 2.1 at 40 min). Animals treated with taurine exhibited a more marked and significant increase in neutrophil respiratory burst activity at 30 min in response to reperfusion (31.16 ± 9.3 mcf at 5 min; 39.89 ± 15 mcf at 40 min, $p < 0.05$ ANOVA). (Fig. 9).

Discussion
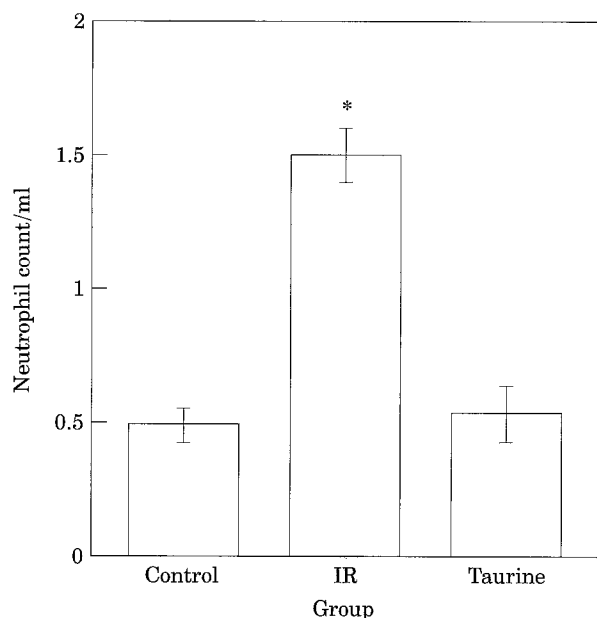
There is widespread experimental data to support a major role for neutrophils in the initiation and promotion of oxidative damage in the lung following lower torso reperfusion injury.[4-7] However, the effect of reperfusion on other organs such as the heart, kidney and liver has been less intensely studied. Neutrophils stimulated by mediators of reperfusion undergo a "respiratory burst" which results in increased production of short-lived oxidant species such as superoxide anion (O_2^-).[9] Superoxide anion is rapidly metabolised to hydrogen peroxide by the action of superoxide dimutase. In the presence of chloride ions, MPO converts hydrogen peroxide to

Fig. 7. Effect of ischaemia-reperfusion on bronchoalveolar lavage neutrophil counts. Data is expressed as mean ± s.e.m. $*p < 0.02$ vs. Control/taurine (ANOVA).

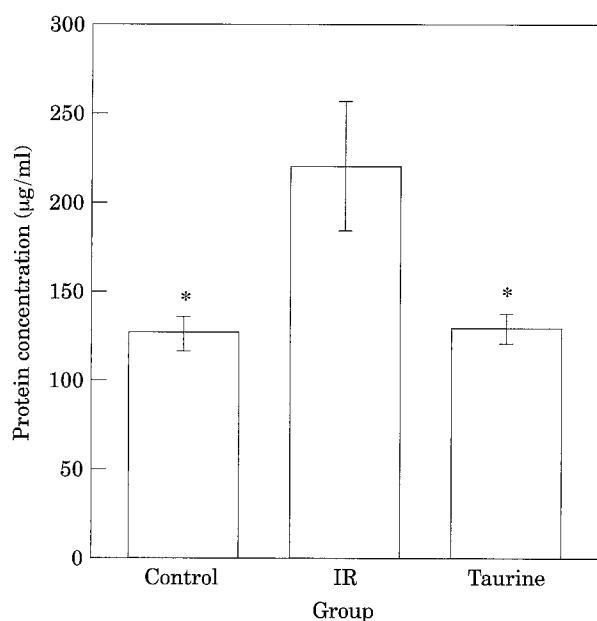Fig. 8. Effect of ischaemia-reperfusion on bronchoalveolar lavage protein content. Data is expressed as mean ± s.e.m. $*p < 0.01$ vs. IR (ANOVA).
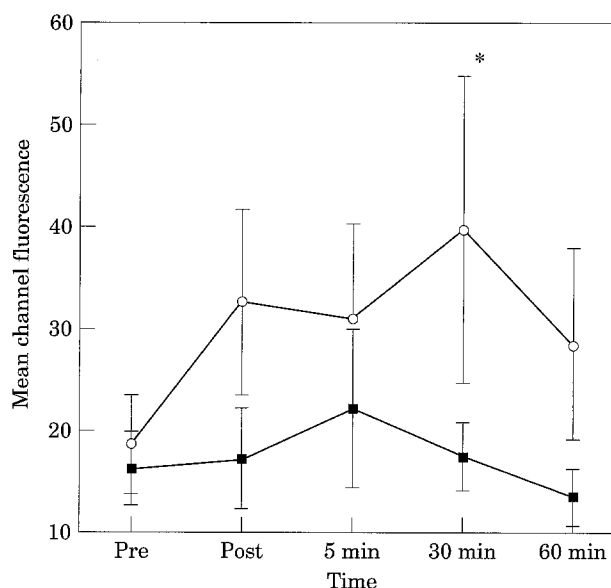
Fig. 9. Effect of ischaemia-reperfusion on peripheral blood neutrophil activation state assessed by neutrophil respiratory burst activity. Time points at which blood samples were taken are indicated on the x-axis: Pre = Prior to application of aortic cross clamp; Post = post-aortic cross-clamping; 5 min; 30 min; 60 min = 5 min, 30 min, 60 min post release of cross clamp. The y-axis documents neutrophil respiratory burst activity as Mean Channel Fluorescence (MCF). Data is expressed as mean ± s.e.m. $*p < 0.05$ vs. Baseline taurine-treated at 30 min (ANOVA). (○) taurine treated; (■) untreated IR.

hypochlorous acid, the most potent and destructive of the neutrophil-derived oxidants.

In the current study reperfusion resulted in significant neutrophil activation, as assessed by respiratory burst activity, in animals subjected to ischaemia reperfusion compared to control animals. This phenomenon, previously described by our group, has also been demonstrated by Freischlag et al. in an animal model of lower limb ischaemia and revascularisation.[21,22] The role of neutrophils in mediating reperfusion lung injury has been well established in animal and human studies.[6] Neutrophil depletion and the use of monoclonal antibodies to neutrophil adhesion receptors have been shown to prevent this type of injury.[23,24] Taurine (2-aminoethanesulphonic acid) is a naturally occurring sulphur-containing amino acid present in high concentrations in many tissues prone to oxidant attack, including lung. This amino acid has anti-oxidant and membrane-stabilising properties.[18,25] Taurine interacts uniquely with neutral phospholipids of biological membranes, stabilising the cell membrane by altering membrane cation binding characteristics and preventing excessive calcium influxes. Exogenous administration of taurine has previously been shown to protect against oxidant-induced lung damage. Giri et al. have demonstrated that administration of taurine in combination with niacin prevented bleomycin-induced injury.[18] Gordon et al. have also demonstrated a protective effect for taurine in NO_2 induced lung injury.[16,17] NO_2 has been shown to mediate lung injury through the process of lipid peroxidation. In addition to its known membrane-stabilising properties taurine acts as a trap for the hypochlorous acid produced by the MPO-hydrogen peroxide-chloride system of monocytes and neutrophils. The reaction of taurine with HOCl forms the oxidant taurine-chloramine, which is less reactive and much less toxic than HOCl but is also bacteriocidal.[26] In the current study animals pre-treated with taurine showed a more exaggerated neutrophil response to reperfusion than the untreated group, but did not have any features of pulmonary microvascular injury. The finding of enhanced neutrophil activation is in keeping with previous studies from this department which have shown that taurine possesses potent antimicrobial properties as assessed by its ability to increase neutrophil phagocytic ability and respiratory burst activity.[27]

Lower torso revascularisation in this study resulted in significant renal oedema. However, unlike the lung there was no significant change in myeloperoxidase activity in the kidney. Since tissue MPO activity is an indicator of neutrophil number, this finding suggests that neutrophils do not play a significant role in

mediating renal endothelial damage. Our results correlate with those of Augustin et al. who demonstrated similar findings in a rat model of supradiaphragmatic aortic cross-clamping.[28] Further support for this hypothesis comes from the work of Paller et al. who failed to protect against renal ischaemia-reperfusion injury by inducing neutropenia.[29] There is evidence to suggest that the injury induced by ischaemia and reperfusion of the kidney is due to an imbalance between vasodilating and vasoconstricting agents rather than a direct neutrophil-mediated effect.[30,31] However, the finding of evidence of endothelial injury in the absence of neutrophil infiltration may also imply that some other agent generated during reperfusion may be capable of inducing endothelial injury independent of the neutrophil. This is supported by the finding that taurine, which prevented the pulmonary injury associated with reperfusion, failed to prevent the renal oedema resulting from the same injury. Paterson et al. and others have previously suggested the existence of such a plasma factor, which is capable of producing an inflammatory reaction independently of neutrophils.[32] Results from our own laboratory which demonstrate direct endothelial and neutrophil activation by plasma harvested during the reperfusion phase of aortic surgery support this theory.[33]

The liver is relatively immune to reperfusion injury due to its high ischaemic tolerance and large array of free radical scavenging systems.[5,28] The absence of mesenteric ischaemia and resultant endotoxaemia in our model of infrarenal aortic cross-clamping probably explains the absence of hepatic tissue damage in this experiment. Hind limb tourniquet iscahemia has been shown to induce skeletal muscle oedema, microcirculatory thrombosis, and neutrophil accumulation in the small capillaries of the affected limb.[34,35] Clinically this is associated with severe limb swelling and muscle dysfunction. We were interested to investigate the effect of lower limb ischaemia and revascularisation on more remote skeletal muscle function. The muscle chosen was the intercostal muscle given its importance in postoperative respiratory mechanics. Reperfusion resulted in significant neutrophil infiltration which was not reflected in increased tissue oedema, but was prevented by prior administration of taurine. The finding of neutrophils in skeletal muscle remote from the site of injury has not been previously reported. However, given the lack of muscle oedema, this neutrophil aggregation does not appear to be clinically significant.

The role of neutrophils in causing postischaemic damage to the heart has been well established in experimental models of coronary artery occlusion

followed by reperfusion.[36–39] Intravenous taurine administration prior to coronary artery bypass grafting in patients with stable angina has been shown to reduce lipid peroxidation and decrease cell damage at the time of revascularisation.[40] Its mechanism of action in this situation has not been fully elucidated, but may relate to its ability to scavenge free radicals released at the time of revascularisation. The myocardial dysfunction seen in patients undergoing aortic reconstruction surgery is generally attributed to the profound haemodynamic changes associated with aortic clamping and de-clamping,[41–45] and to the generation of negative inotropic agents from the site of reperfusion.[46] The current study was designed to investigate the hypothesis that systemic neutrophil activation induced by lower limb ischaemia and revascularisation would cause neutrophil-mediated myocardial damage. Lower torso revascularisation did not increase myocardial oedema nor did it cause significant neutrophil infiltration. However, MPO levels in the taurine-treated group were significantly lower than those in either the control or ischaemia reperfusion groups, indicating perhaps, that general anaesthesia alone may induce some degree of myocardial neutrophil influx, which can be blocked by taurine.

The results of this study indicate that the lower torso revascularisation produces significant pulmonary and renal microvascular injury. The pulmonary but not the renal injury is predominantly neutrophil-dependent. The disproportionate injury suffered by the lung and sparing of other organs, in this model may be explained by the filter role played by the lung in removing more activated leucocytes from the circulation, preventing neutrophil-mediated injury in other vital organs. Taurine provided protection against the lung, but not the kidney injury. These data suggest that agents other than neutrophil-derived oxidants and proteases are produced during reperfusion, which are capable of independently causing significant organ injury.

Further studies are required to elucidate the mechanism of action of taurine in limiting neutrophil sequestration while paradoxically increasing oxidative activity. The effect of taurine on such humoral mediators as thromboxane and leukotriene B4 also needs to be examined. McLoughlin et al. have demonstrated that manipulation of the MPO-H_2O_2-halide system *in vitro* by the addition of taurine can reduce levels of LTB4.[47] We have examined the effect of taurine on neutrophil CD11b expression and have found that it to have no effect (unpublished data). This is in keeping with previous *in vitro* studies from this department

which demonstrated that taurine has no effect on leucocyte adherence to endothelial cells.[48]

Taurine is a non-toxic substance and normal constituent of the human diet. This study suggests that this agent administered prior to an ischaemic insult may be potentially useful in minimising the systemic inflammatory response and organ dysfunction that results from reperfusion.

References

1 WELBOURN CRB, GOLDMAN G, PETERSON IS, VALERI CR, SHEPRO D, HECHTMAN HB. Pathophysiology of ischaemia reperfusion injury: central role of the neutrophil. *Br J Surg* 1991; **78**: 651–655.

2 FANTINI GA, CONTE MS. Pulmonary failure following lower torso ischemia: Clinical evidence for a remote effect of reperfusion. *Am Surg* 1995; **61**: 316–319.

3 PATERSON IS, CORSON J, McCOLLUM CN et al. Multiple organ dysfunction following abdominal aortic aneurysm repair. *Br J Surg* 1992; **76**: 358.

4 PATERSON IS, KLAUSNER JM, PUGATCH R et al. Noncardiogenic pulmonary edema after abdominal aortic aneurysm surgery. *Ann Surg* 1989; **108**: 231–236.

5 SIMPSON R, ALON R, KOBZIK L, VALERI R, SHEPRO D, HECHTMAN HB. Neutrophil and non-neutrophil-mediated injury in intestinal ischaemia-reperfusion. *Ann Surg* 1993; **218**: 444–454.

6 KLAUSNER JM, ANNER H, PATERSON IS et al. Lower torso ischemia-induced lung injury is leukocyte dependent. *Ann Surg* 1983; **208**: 761–767.

7 ANNER H, KAUFMAN RP, KOBZIK L, VALERI CR, SHEPRO D, HECHTMAN HB. Pulmonary hypertension and leukosequestration after lower torso ischemia. *Ann Surg* 1987; **206**: 642–648.

8 BABIOR BM. Oxygen-dependent microbial killing by phagocytes. *N Engl J Med* 1978; **298**: 659–668.

9 WEISS SJ. Tissue destruction by neutrophils. *N Engl J Med* 1989; **320**: 365–376.

10 LEARN DB, FRIED VA, THOMAS EL. Taurine and hypotaurine content of human leukocytes. *J Leuk Biol* 1990; **48**: 174–182.

11 GREEN TR, FELLMAN JH, EICHER AL, PRATT KL. Antioxidant role and subcellular location of hypertaurine and taurine in human neutrophils. *Biochimica et Biophysica Acta* 1991; **1073**: 91–97.

12 VINTON NE, LAIDLAW SA, AMENT ME, KOPPLE JD. Taurine concentrations in plasma and blood cells of patients undergoing long-term parenteral nutrition. *Am J Clin Nutr* 1986; **44**: 398–404.

13 WANG Q, GIRI SN, HYDE DM, LI C. Amelioration of bleomycin-induced pulmonary fibrosis in hamsters by combined treatment with taurine and niacin. *Biochem Pharmacol* 1991; **42**: 1115–1122.

14 WANG Q, HOLLINGER MA, GIRI SN. Attenuation of amiodarone-induced lung fibrosis and phospholipidosis in hamsters by taurine and/or niacin treatment. *Pharmacol Experiment Therapeutic* 1992; **262**: 127–132.

15 WANG Q, HYDE DM, GIRI SN. Abatement of bleomycin-induced increases in vascular permeability, inflammatory cell infiltration and fibrotic lesions in hamster lungs by combined treatment with taurine and niacin. *Lab Invest* 1992; **67**: 234–242.

16 GORDON RE, HELLER RF, DEL VALLE JR, HELLER RF. Membrane perturbations and mediation of gap junction formation in response to taurine treatment in normal and injured alveolar epithelia. *Exp Lung Res* 1989; **15**: 895–908.

17 GORDON RE, SHAKED AA, SOLANO DF. Taurine protects hamster bronchioles from acute NO_2-induced alterations. A histologic, ultrastructural and freeze-fracture study. *Am J Pathol* 1986; **125**: 585–600.

18 Giri SN Wang Q. Taurine and niacin offer a novel therapeutic modality in prevention of chemically-induced pulmonary fibrosis in hamsters. *Advan Exp Med Biol* 1992; **315**: 329–340.

19 Lowry OH, Rosenrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265–275.

20 Smith JA, Weidemann MJ. Further characterisation of the neutrophil oxidative burst by flow cytometry. *J Immunol Meth* 1993; **162**: 261–268.

21 Kelly CJ, Abdih H, Bouchier-Hayes D *et al*. Nitric oxide (Endothelium-derived Relaxing Factor) attenuates revascularisation-induced lung injury. *J Surg Res* 1994; **57**: 39–43.

22 Freischlag JA, Hanna D. Superoxide anion release (02-) after ischaemia and reperfusion. *J Surg Res* 1991; **50**: 565–568.

23 Horgan MJ, Wright SD, Malik AB. Antibody against lekocyte integrin CD18 prevents reperfusion-induced lung vascular injury. *Am J Physiol* 1990; **259**: 315–319.

24 Vedder NB, Winn RK, Rice CL, Chi EY, Arfors KE, Harlan JM. Inhibition of leukocyte adherence by anti CD18 monoclonal antibody attenuates reperfusion injury in the rabbit ear. *Proc Natl Acad Sci USA* 1990; **87**: 2643–2646.

25 Huxtable RJ, Sebring LA. Towards a unifying theory for the actions of taurine. *TIPS* 1986; **7**: 481–485.

26 Zgliczynski JM, Stelmaszynska T, Domanski J, Ostrowski W. Chloramines as intermediates of oxidation reaction of amino acids by myeloperoxidase. *Biochimica et Biophysica Acta* 1971; **235**: 419–424.

27 Watson RWG, Redmond HP, Bouchier Hayes D. Taurine upregulates antimicrobial function of human inflammatory cells through a calcium-dependent mechanism. *Surg Forum* 1994; **XLV**: 679–681.

28 Augustin AJ, Lutz J. Intestinal, hepatic and renal production of thiobarbituric acid reactive substances and myeloperoxidase activity after temporary aortic occlusion and reperfusion. *Life Sci* 1991; **49**: 961–968.

29 Paller MS. Effect of neutrophil depletion on ischemic renal injury in the rat. *J Lab Clin Med* 1989; **113**: 379–386.

30 Klausner JM, Paterson IS, Kobzik L *et al*. Vasodilating prostaglandins attenuate ischemic renal injury only if thromboxane is inhibited. *Ann Surg* 1989; **209**: 219–224.

31 Thornton MA, Winn R, Alpers CE, Zager RA. An evaluation of the neutrophil as a mediator of *in vivo* renal ischemic reperfusion injury. *Am J Pathol* 1989; **135**: 509–511.

32 Paterson IS, Smith FCT, Tsang GMK, Hamer JD, Shearman CP. Reperfusion plasma contains a neutrophil activator. *Ann Vasc Surg* 1993; **7**: 68–75.

33 Barry MC, Wang J, Kelly C *et al*. Plasma factors augment neutrophil and endothelial cell activation during aortic surgery. *Br J Surg* 1995; **82**: 562 (Abstr.).

34 Carden DL, Smith JK, Korthuis RJ. Neutrophil-mediated microvascular dysfunction in postischaemic canine skeletal muscle. Role of granulocyte adherence. *Circ Res* 1990; **66**: 7436–7444.

35 Welbourn CRB, Goldman G, Paterson IS, Valeri CR, Shepro D, Hechtman HB. Neutrophil elastase and oxygen radicals: synergism in lung injury after hindlimb ischemia. *Am J Physiol* 1991: **260** (Heart Circ Physiol 29): H1852–H1856.

36 Byrne JG, Appleyard RF, Lee CC *et al*. Controlled reperfusion of the regionally ischemic myocardium with leukocyte-depleted blood reduces stunning, no-reflow phenomenon, and infarct size. *J Thorac Cardiovasc Surg* 1992; **103**: 66–71.

37 Romson JL, Hook BG, Kunkel SL, Abrams GD, Schork MA, Lucchesi BR. Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. *Circulation* 1983; **67**: 1016–1023.

38 Ricevuti G, Mazzone A, Pasotti D, De Servi S, Specchia G. Role of granulocytes in endothelial injury in coronary heart disease in humans. *Atherosclerosis* 1991; **91**: 1–14.

39 Williams FM, Kus M, Tanda K, Williams TJ. Effect of duration of ischaemia on reduction of myocardial infarct size by inhibition of neutrophil accumulation using an anti-CD18 monoclonal antibody. *Br J Pharmacol* 1994; **111**: 1123–1128.

40 Milei J, Ferreira R, Llesuy S, Forcada P, Covarrubias J, Boveris A. Reduction of reperfusion injury with preoperative rapid intravenous infusion of taurine during myocardial revascularisation. *Am Heart J* 1992; **123**: 339–345.

41 Huval WV, Lelcuk S, Allen P, Mannick JA, Shepro D, Hechtman HB. Determinents of cardiovascular stability during abdominal aortic aneurysmectomy (AAA). *Ann Surg* 1984; **199**: 216–222.

42 Silverstein PR, Caldera DL, Cullen DJ, Davison JK, Darling RC, Emerson CW. Avoiding the haemodynamic consequences of aortic cross-clamping and unclamping. *Anesthesiology* 1979; **50**: 462–466.

43 Damask MC, Weissman C, Rodriguez J, Askanazi J, Rosenbaum SH, Hyman AI. Abdominal aortic cross-clamping. Metabolic and hemodynamic consequences. *Arch Surg* 1984; **119**: 1332–1337.

44 Carroll RM, Laravuso RB, Schauble JF. Left ventricular function during aortic surgery. *Arch Surg* 1976; **111**: 740–743.

45 Dunn E, Prager RL, Fry W, Kirsh M. The effect of abdominal aortic cross-clamping on myocardial function. *J Surg Res* 1977; **22**: 463–468.

46 Mathieson MA, Dunham BM, Huval WV *et al*. Ischemia of the limb stimulates thromboxane production and myocardial depression. *Surg Gyn Obs* 1983; **157**: 500–504.

47 McLoughlin DM, Stapleton PP, Bloomfield FJ. Influence of taurine and a substituted taurine on the respiratory burst pathway in the inflammatory response. *Biochemical Society Transactions* 1991; **19**: 73–78.

48 Finnegan N, Redmond HP, Watson RGW *et al*. Synergy between taurine and interleukin-2 suppresses endothelial cell injury. (Abstr.) *Br J Surg* 1994; **81**: 1799.