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Hunterian Lecture

Aortic aneurysms, inflammatory pathways and nitric oxide

RD Sayers

Department of Surgery, Leicester General Hospital, Leicester, UK

The Leicester experience of treatment of aortic aneurysms indicates that workload is increasing. Despite an increase in elective repairs, the number of ruptured abdominal aortic aneurysms is also increasing. The mortality of ruptured abdominal aortic aneurysm remains static despite advances in critical care medicine. Multi-organ failure is the commonest cause of death following ruptured abdominal aortic aneurysm and the systemic inflammatory response syndrome, ischaemia-reperfusion injury and activation of inflammatory pathways are important precursors. Organ failure, reperfusion injury and inflammatory pathway activation can be studied at a cellular and biochemical level in animal models of aortic cross-clamping. The nitric oxide response is an important component of the inflammatory response and augmentation of the NO response may protect against renal injury caused by aortic cross-clamping during aortic aneurysm repair.

Key words: Abdominal aortic aneurysm – Inflammatory pathways – Nitric oxide – Systemic inflammatory response syndrome

John Hunter was born in East Kilbride near Glasgow in 1723. He has become known, quite rightly, as the father of scientific surgery because he sought to explain what he saw clinically by careful scientific study and experimentation. In addition, he became a renowned teacher of anatomy and surgery and an avid collector of anatomical and pathological specimens, many of which are housed today in the Hunterian Museum at The Royal College of Surgeons of England. His early adult life was spent preparing dissection specimens at his brother William's Covent Garden school of anatomy. In 1756, he was appointed house surgeon at St George's Hospital, London and he maintained a life-long association with that famous teaching hospital and medical school. During his life, many honours were bestowed upon him including a Fellowship of the Royal Society (a rare honour among surgeons) and, of course, membership of The College of Surgeons in London. He wrote widely on many diverse subjects including teeth and human dentition, disorders of the stomach and digestion, venereal disease, blood, inflammation and gunshot wounds (a treatise based upon his experiences in Portugal with the army) and, of course, the treatment of popliteal aneurysms. It is perhaps for this last work on popliteal aneurysm for which he is best remembered.

John Hunter and popliteal aneurysms

John Hunter performed his famous operation for the treatment of popliteal aneurysm at St George's Hospital in December 1785. The patient was a 45-year-old London coachman who had a 3-year history of popliteal aneurysm and claudication in the leg. He presented with pain behind the knee, which was thought to be due to rapid expansion

Correspondence to: RD Sayers, Department of Surgery, Leicester General Hospital, Gwendolen Road, Leicester LE5 4PW, UK

of the aneurysm. The traditional treatment was amputation of the leg because attempts at limb salvage usually led to death of the patient either from gangrene or from exsanguinating haemorrhage if the ligatures cut through the arterial wall or if infection developed. However, on this occasion, Hunter decided to attempt ligation of the aneurysm and reasoned that the patient's history of claudication might have led to the development of a collateral circulation. In addition, he reasoned that others had failed because they attempted ligation of the aneurysm at its neck where the artery was often diseased and the ligatures were prone to cutting through. Therefore, he exposed the superficial femoral artery in the subsartorial canal proximal to the diseased neck of the aneurysm and ligated it with 4 loosely applied ligatures. The operation was a success; the patient recovered and returned to work. Hunter went on to treat popliteal aneurysms in 4 more patients and was successful in 3 out of 4. Two years later, Hunter was able to audit his original work when his first patient was re-admitted to St George's Hospital and subsequently died from a fever. Hunter was present at the postmortem examination and was able to inspect his operative work and declared it to be intact. The original specimen is preserved in the Hunterian Museum at The Royal College of Surgeons in London and shows both the popliteal aneurysm and one of the ligatures applied to the femoral artery.

In his later life, Hunter sought to explain the difference between syphilis and gonorrhoea and went to the extreme lengths of inoculating himself with syphilis. Unfortunately, he developed syphilitic aortitis and had frequent angina attacks. He died in 1793 from a fatal angina attack during a St George's Hospital board meeting when told the name of his successor.

The history of aortic surgery

Following the work of John Hunter, other famous surgeons pioneered the development of surgery of aortic aneurysm, often in the Hunterian tradition. In 1817, Sir Astley Cooper, a former President of The Royal College of Surgeons of England, attempted ligation of the aortic bifurcation in a case of ruptured iliac artery aneurysm. In 1921, Vaughan attempted to treat a ruptured aortic aneurysm by partial ligation of the infrarenal aortic neck and Matas attempted complete ligation of the aortic neck for ruptured aortic aneurysm in 1923. Prior to this, Carrel and Guthrie had published pioneering work on vascular suture and anastomotic techniques, many of which are still in use in vascular and transplant surgery today. The first successful repair of an aortic aneurysm is attributed to Charles Dubost in 1951, during which he restored arterial continuity with aortic homograft and, following this, Voorhees developed the prosthetic aortic graft in 1952, which led to the modern era of aortic aneurysm repair.

Modern aortic surgery

There is no doubt that elective abdominal aortic aneurysm (AAA) repair is a very successful operation particularly as it involves major abdominal surgery in elderly patients who often have co-existing co-morbidities. In the largest published meta-analysis of elective aneurysm repair, Eickleboom confirmed what many of the smaller studies had shown; namely that the mortality is low (between 3–10%) and that cardiac events account for most of the deaths.[1] Despite this success rate, numerous problems still exist for aortic surgeons including controversies surrounding the most appropriate technique (open *versus* endovascular repair), resources, training, infection (particular MRSA), screening, and of course rupture. Although many of these problems are being investigated and addressed, the problems of treating ruptured AAA remain a major concern in vascular surgery.

Ruptured aortic aneurysm

The UK statistics show that ruptured AAA accounts for 1.4% of deaths in men over the age of 65 years and 0.5% of deaths in women. Overall, ruptured AAA causes 10,000 deaths in England and Wales per annum and the incidence of ruptured AAA is increasing.[2]

Aortic aneurysms in Leicestershire

The issue for most vascular units is whether these national and international data are relevant to their own unit. In order to answer this question, we have performed several clinical studies on AAA repair in Leicester to investigate work-load, outcome and mortality.

Initially, we studied all aortic aneurysm repairs in Leicestershire between 1979–1991. At this time, there were 3 acute hospitals in Leicester providing vascular services for a population of approximately 950,000. Patients were identified from OPCS and ICD-9 codes. During the study period, 727 patients underwent aortic aneurysm repair (410 elective and 317 ruptured). Analysis of the data showed that there had been a significant increase in total AAA work-load (Fig. 1) with similar significant increases in both elective and rupture AAA work-load (Fig. 2). Despite this, the mortality rates of ruptured AAA remained high with no significant decrease (Fig. 3).[3]

In order to investigate the causes of death of patients undergoing surgery for ruptured AAA, we reviewed all AAA repairs at Leicester Royal Infirmary between

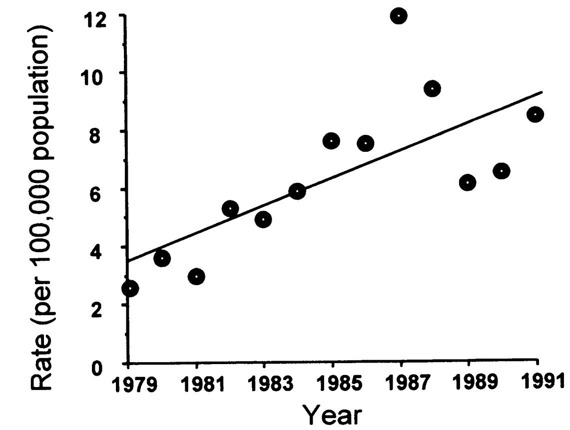

Figure 1 Total AAA workload in Leicestershire 1979–1991 ($P <$ 0.004).

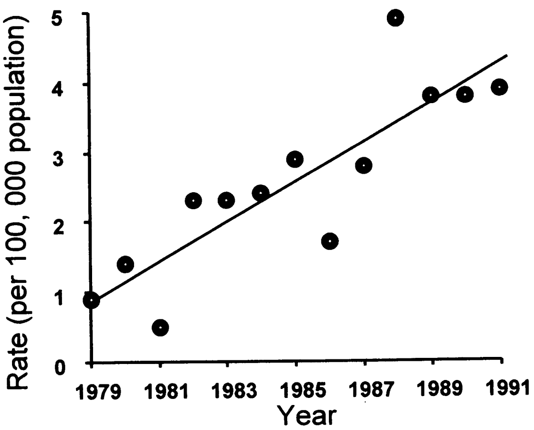

Figure 2 Ruptured AAA workload in Leicestershire 1979–1991 ($P < 0.0002$).

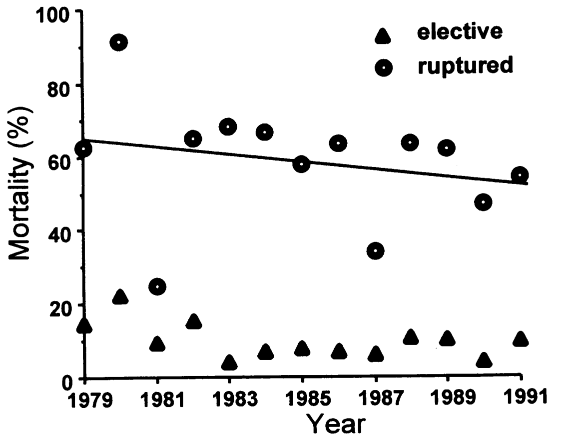

Figure 3 AAA mortality in Leicestershire 1979–1991 ($P > 0.05$).

1981–1993. This hospital contains the only accident and emergency department in Leicester and, therefore, admits the majority of patients with ruptured AAA. This study identified 671 patients who underwent AAA repair (213 elective, 80 urgent [symptomatic, non-ruptured] and 278 ruptured). Patients with rupture were divided into 2 groups (1981–1987 and 1988–1993). During this time, several advances in treatment had occurred with the development of protocols for ruptured AAA admission and repair, intensive care unit (ITU) guidelines for organ support, a consultant vascular surgical rota, guidelines for seniority of anaesthetic staff, and protocols relating to supply of blood and coagulation products. Despite this, the mortality of rupture between 1981–1987 was 52% and did not decrease significantly between 1988–1993 (54%). Although some patients died intra-operatively from cardiac collapse or failure to control bleeding, many patients died post-operatively from single or multiple organ failure.[4]

In order to investigate the significance of postoperative organ failure in patients with ruptured AAA, we studied patients who developed acute renal failure (ARF) after ruptured AAA repair. We chose to study ARF as a marker of organ failure because there is a regional dialysis unit in Leicester which receives referrals for all patients with ARF after ruptured AAA repair. Between 1984–1996, 65 patients with ARF following ruptured AAA repair were referred for dialysis. The overall mortality was 75%, which shows the significance of developing a single organ system failure in these patients. The independent variables, which predicted death in these patients, included pre-operative vascular disease (usually ischaemic heart disease), the requirement for further surgery (usually a second laparotomy to control bleeding caused by failure of coagulation), and the failure of additional organ systems (usually cardiac, respiratory or haematological).[5]

Thus, the data from Leicester show that there has been a significant increase in AAA work-load and that, despite an increase in elective repairs, there has also been a significant increase in the number of patients undergoing surgery for ruptured AAA. Despite advances in surgery and critical care medicine, the mortality of ruptured AAA has remained static and many of these patients die postoperatively from multiple organ failure.

Postoperative organ failure and the inflammatory response

The advances in cellular and molecular biology over the last decade have led to a greater understanding of the pathophysiology of organ failure. All surgical disease and treatment leads to the development of an inflammatory response, which has 4 main components at the tissue/microcirculation level: (i) vasodilatation; (ii) increased vascular permeability; (iii) cellular adhesion and activation; and (iv) coagulation.

This results in activation of white cells that migrate into tissues and act as a defence against harmful agents. It is a 'normal' beneficial response that is essential for survival and, in a surgical context, is also an important aspect of tissue repair and wound healing. The cellular events involved include up-regulation of adhesion molecules, which facilitate endothelial-white cell interaction, and subsequent migration of neutrophils into the tissues. Neutrophils undergo a respiratory burst and release lysosomal enzymes and free radicals, which are involved in neutrophil killing of invading micro-organisms. The effectors of the inflammatory response are cytokines such as tumour necrosis factor-α (TNF-α) and various interleukins (ILs). Cytokines are produced by a variety of cells involved in the inflammatory response including macrophages, neutrophils and endothelial cells and they have a variety of effects including up-regulation of adhesion molecule expression, chemotaxis, and activation of other inflammatory pathways (coagulation, complement, kinins and fibrinolysis). This local response is tightly controlled by the production of anti-inflammatory cytokines (IL-10) and endogenous antagonists.[6]

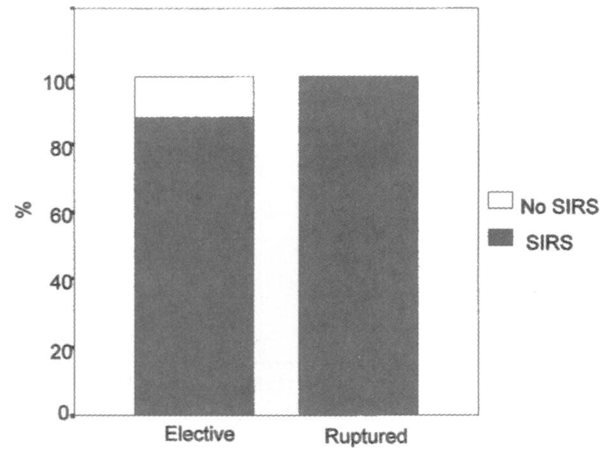

Figure 4 Development of SIRS post AAA repair (elective, $n = 49$; ruptured, $n == 20$).

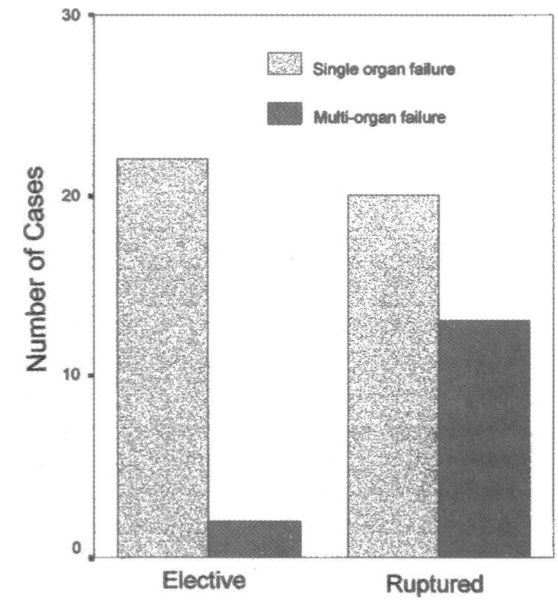

Figure 5 Development of organ failure after AAA repair.

The systemic inflammatory response syndrome (SIRS)

If the response escapes these local control mechanisms, systemic inflammation may result which is characterised by the systemic inflammatory response syndrome (SIRS).[7] The concept of SIRS came about in studies of patients with surgical diseases such as burns, trauma and pancreatitis that had features of a septic-like picture, but in whom no organisms could be identified. It is now known that these patients were not septic but had systemic inflammation (SIRS). The major feature of SIRS is high levels of circulating cytokines and activated neutrophils in the systemic circulation. SIRS is defined clinically by changes in temperature, heart rate, respiratory rate, and white cell count. The importance of SIRS is that it can produce organ dysfunction, which may progress to single, or multiple organ failure (*e.g.* non-cardiogenic pulmonary oedema,[8] acute renal failure, myocardial depression and failure of coagulation). Our own on-going studies of aortic aneurysm and SIRS ($n = 49$ elective, 20 ruptured) have shown that both elective and ruptured AAAs may develop SIRS, but it is more common and more severe in the ruptured group (Fig. 4). Similarly, both groups of patients may develop organ failure, but elective AAAs tend to develop single organ failure which is transient and can be managed by ITU support whereas ruptured AAAs are more likely to progress to multiple organ failure (Fig. 5; M Bown, unpublished data).

The cause of SIRS following AAA repair is probably due to ischaemia-reperfusion injury.[9] This is the process where application of an aortic cross-clamp causes distal ischaemia in the lower limbs. During this process, lack of oxygen leads to inability to replenish ATP stores with the result that AMP and hypoxanthine accumulate.[10,11] In addition, the enzyme xanthine dehydrogenase is converted to xanthine oxidase. When the cross-clamp is removed and the ischaemic lower limbs are reperfused with oxygen-rich blood, hypoxanthine and oxygen react to produce superoxide and other free radicals, the reaction being driven by xanthine oxidase. The end result of this process is the production of toxic free radicals, which exert local and

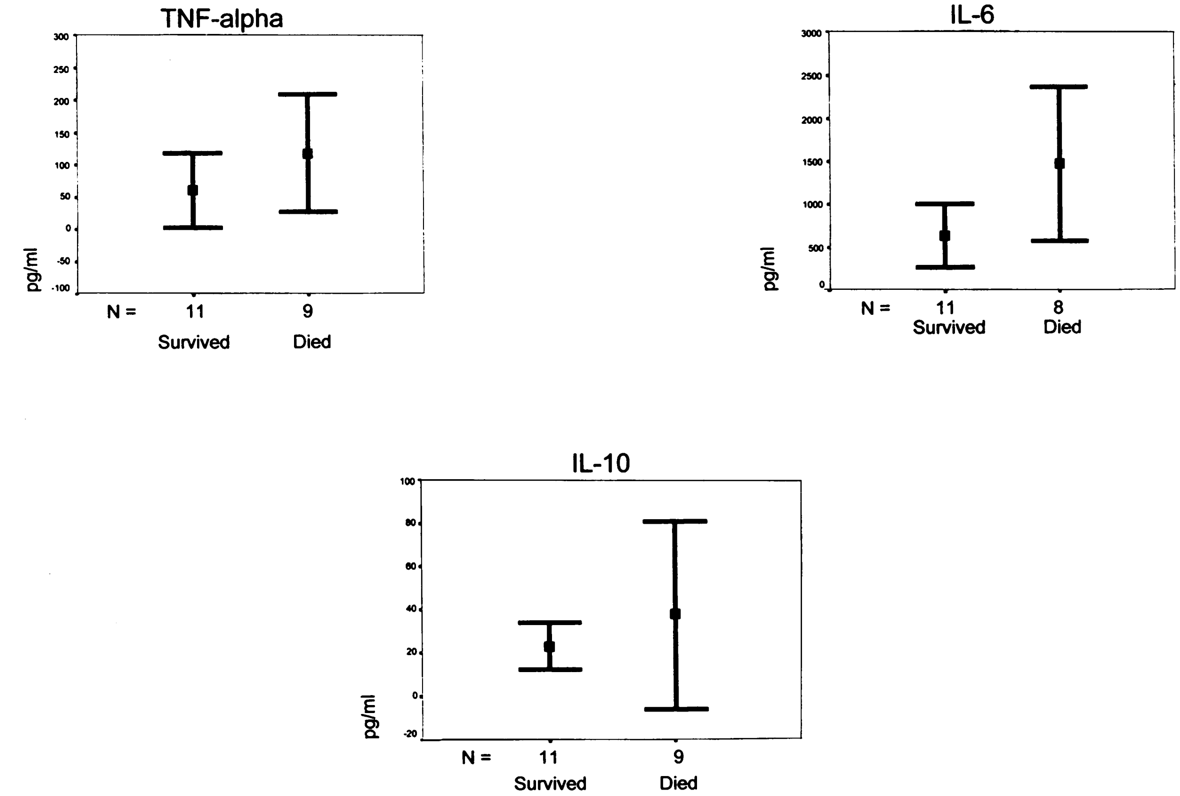

Figure 6 Cytokine profiles after ruptured AAA repair.

distant effects, particularly in the lungs and kidneys, by causing endothelial cell injury mainly by lipid peroxidation of the endothelial cell membrane.[12] Ischaemia-reperfusion injury causes wide-spread changes in the microcirculation and the pathways involved are similar to those already described for inflammation.[13,14] Thus there is an interaction between damaged endothelial cells and neutrophils facilitated by adhesion molecules, which leads to neutrophil infiltration into the tissues.[15] There are changes to the microcirculation vessels with increased production of thromboxane and endothelin (vasoconstrictors) and decreased production of prostacyclin (a vasodilator). This response is driven and mediated by a cytokine response with increased production of TNF-α, IL-6 and IL-10 although, at present, it is not possible to determine whether the cytokine response predicts survival (Fig. 6; M Bown, unpublished data).

Thus both elective and ruptured AAAs develop SIRS and this response is caused by ischaemia-reperfusion injury. In patients with ruptured AAA, there are the additional factors of hypotension, acidosis, blood transfusion and hypothermia (the so-called second hit or second insult phenomena), which cause further wide-spread activation of the inflammatory pathways and probably account for the progression to multiple organ failure.

Experimental models of AAA repair

An alternative to clinical studies of AAA repair is the use of animal models. We have developed a rodent model of aortic cross-clamping to study activation of inflammatory pathways and ischaemia-reperfusion injury following AAA repair. The model involves a laparotomy and exposure of the infrarenal aorta, aortic cross-clamping for a variable ischaemic period and then release of the clamp for a variable reperfusion period. We have used this model to study reperfusion injury to the lungs and kidney, the role of nitric oxide in the inflammatory response and pharmacological manipulation of the nitric oxide pathway in an attempt to prevent lung and renal injury.

Lung injury

Using the rodent model, we have investigated lung injury in several ways:

1. Lung wet/dry weight ratio: a marker of lung water and non-cardiogenic pulmonary oedema.
2. Broncho-alveolar lavage: to measure pulmonary neutrophil infiltration into alveoli.

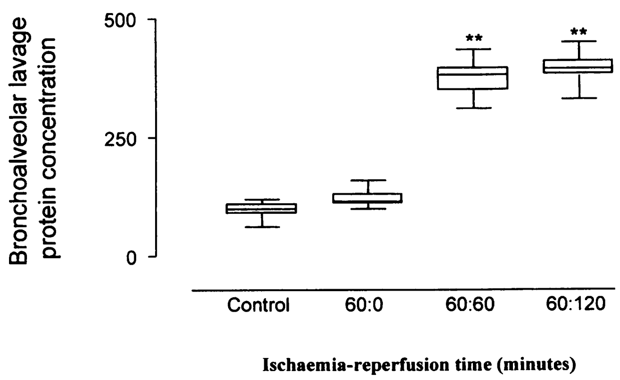

Figure 7 Broncho-alveolar lavage protein concentration (mg/ml) post ischaemia-reperfusion injury (** $P < 0.01$ *versus* control, Mann Whitney U test).

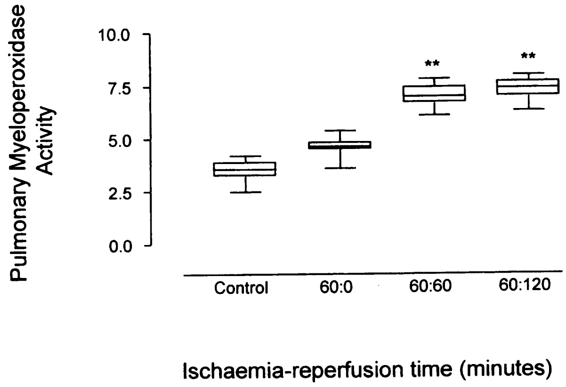

Figure 8 Pulmonary myeloperoxidase activity (units/g) post ischaemia-reperfusion injury (** $P < 0.01$ *versus* control, Mann Whitney U test).

3. Broncho-alveolar lavage: to measure protein leakage into alveoli.
4, Myeloperoxidase activity: an enzyme involved in the neutrophil respiratory burst.
5. TNF-α levels

Using this model, we have shown that aortic cross-clamping and ischaemia-reperfusion injury leads to activation of inflammatory pathways and lung injury. There were significant increases in lung water, neutrophil and protein leakage (Fig. 7) into alveoli, pulmonary myeloperoxidase (Fig. 8) and serum TNF-α.[16]

Renal injury

We have used a similar model to study renal ischaemia-reperfusion injury following aortic cross-clamping. Renal injury was investigated by measurement of glomerular filtration rate (GFR) using a technetium clearance technique. We have been able to follow the renal injury in survival experiments where animals were allowed to recover and GFR measured at 24 h post-injury and again 7 days later. In addition, a left nephrectomy was performed during the initial laparotomy in order to measure nitric oxide activity and this was compared to nitric oxide production in the right kidney on day 7.

The results show that there is significant renal injury with a decrease in GFR at 24 h and this injury persists on day 7 (Fig. 9).[17]

Nitric oxide and inflammation

Using this model, we have investigated nitric oxide production in the lungs and kidneys following aortic cross-clamping, ischaemia-reperfusion injury and activation of inflammatory pathways. Nitric oxide (NO) is an endothelial-derived vasodilator, which acts on smooth muscle cells in the vessel wall and also has antiplatelet and anti-neutrophil effects. It is formed during the conversion of L-arginine to L-citrulline and the reaction is catalysed by an enzyme system known as nitric oxide synthase (NOS). Nitric oxide has potential benefits in an inflammatory response because its vasodilator and antiplatelet properties may help prevent poor microcirculatory flow and platelet plugging, which contribute to inflammatory ischaemia-reperfusion injury. However, nitric oxide can also react with some of the toxic free radicals produced by reperfusion injury and form peroxynitrite – a further toxic free radical that may cause additional tissue injury.[18] Thus, the role of NO in inflammatory reperfusion injury is unclear and worthy of further investigation.

Nitric oxide is difficult to measure directly because it has a very short half-life (few seconds). An alternative is to measure nitric oxide synthase, the enzyme responsible for NO production.[19] Nitric oxide synthase is not one enzyme but 3 iso-enzymes. Endothelial NOS (eNOS) and neuronal NOS (nNOS) are constitutive isoforms, which are released under basal physiological conditions.

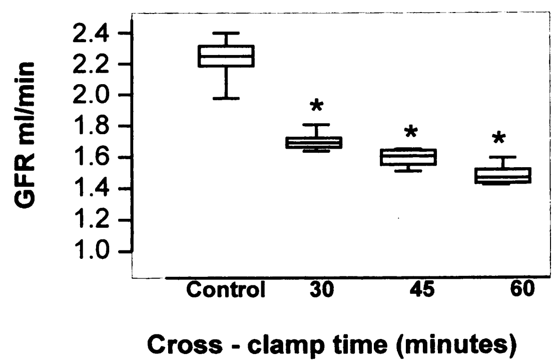

Figure 9 Glomerular filtration rate (ml/min) on day 7 post ischaemia-reperfusion injury. (* $P < 0.05$ *versus* control, Mann Whitney U test).

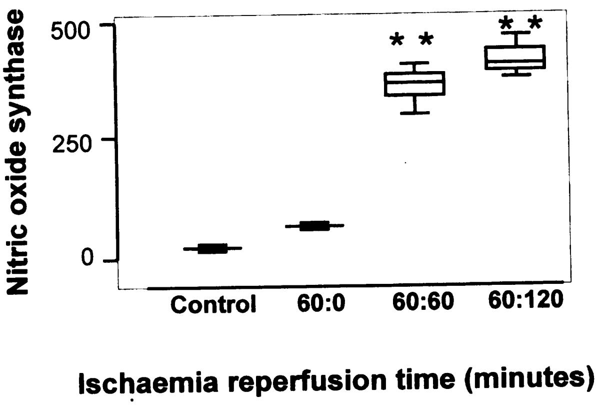

Figure 10 Lung nitric oxide synthase activity (picomoles L-citrulline/mg protein/45 min) post ischaemia-reperfusion injury. (**$P < 0.01$ *versus* control, Mann Whitney U test).

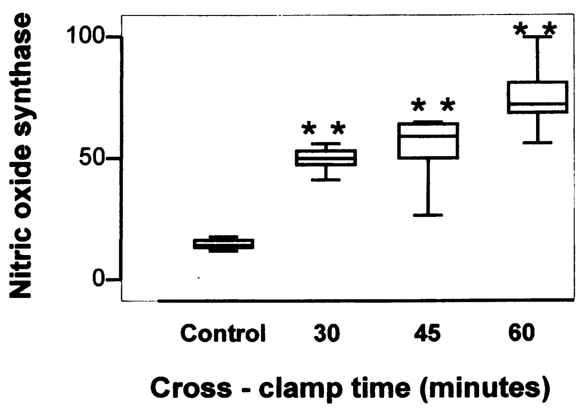

Figure 11 Renal nitric oxide synthase activity on day 7 (picomoles L-citrulline/mg protein/45 min) post ischaemia-reperfusion injury. (**$P < 0.01$ *versus* control, Mann Whitney U test).

Inducible NOS (iNOS) is unregulated or down-regulated under pathological conditions such as ischaemia-reperfusion injury. Total NOS activity can be measured by a radioactive citrulline assay whereby radioactive arginine is converted to citrulline. The amount of citrulline produced is directly proportional to the activity of NOS. Using this method, we have shown that reperfusion injury produces a significant increase in total pulmonary NOS (Fig. 10) and renal NOS (Fig. 11). We also investigated the expression of iNOS using Western blotting and showed strong iNOS expression in renal tissue on day 7.

Pharmacological manipulation of the NO response

Although we were able to show that aortic cross-clamping and ischaemia-reperfusion injury produced significant increases in pulmonary and renal injury, it was not clear whether this was a beneficial or harmful response. In order to investigate this further, we studied pharmacological manipulation of the NO response to attempt to ameliorate the injury. Agents used to increase the production of NO included L-arginine (the substrate for NOS) and NOC-18 (a nitric oxide donor). Agents used to decrease the production of NO included L-NMMA (an inhibitor of all isoforms of NOS) and 1400W (a selective iNOS inhibitor). In addition, the effects of corticosteroids (non-selective inhibition of inflammation) were also studied. These agents were used in the animal model of aortic cross-clamping and were given via the inferior vena cava 5 min before application of the cross-clamp. The use of clinically available NO donors such as glyceryl trinitrate (GTN) and sodium nitroprusside was deliberately avoided because of their effects on blood pressure, which might affect renal perfusion. Normal saline was used as a control.

The results showed that pharmacological manipulation of the NO response did not affect any of the markers of lung injury (lung water, protein or white cell leakage into alveoli or pulmonary myeloperoxidase). However, steroids did significantly protect against lung injury.

Manipulation of the NO response did have a significant affect on renal injury. L-NMMA caused a significant decrease in GFR at 24 h by inhibiting the NO response,

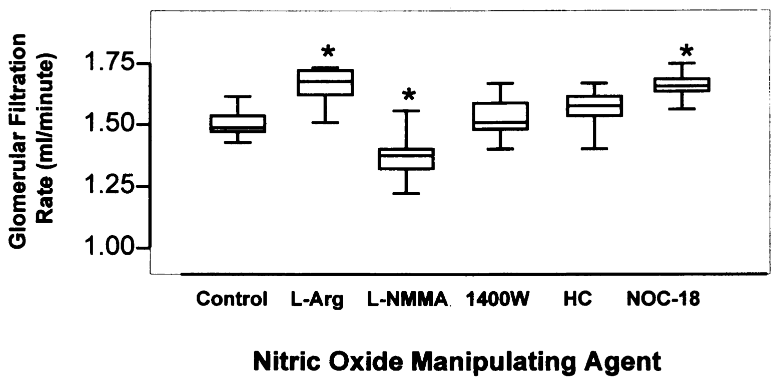

Figure 12 Effects of nitric oxide manipulating agents on glomerular filtration rate (ml/min) on day 7 post ischaemia-reperfusion injury. (**$P < 0.01$ *versus* control, Mann Whitney U test).

but at day 7 both L-arginine and NOC-18 led to a significant increase in GFR (Fig. 12). Thus, augmenting the NO response appeared to protect against renal injury caused by aortic cross-clamping, ischaemia-reperfusion injury and activation of inflammatory pathways.[20]

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