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Anti-endotoxin Hyperimmune Globulin Attenuates Portal Cytokinaemia, Phagocytic Cell Priming, and Acute Lung Injury after Lower Limb Ischaemia-reperfusion Injury

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Objectives. *Acute limb ischaemia is a common and often lethal clinical event. Reperfusion of an ischaemic limb has been shown to induce a remote gut injury associated with transmigration of endotoxin into the portal and systemic circulation, which in turn has been implicated in the conversion of the sterile inflammatory response to a sepsis syndrome, after lower torso ischaemia-reperfusion injury. This study tests the hypothesis that an anti-endotoxin hyperimmune globulin attenuates ischaemia-reperfusion (I/R) associated sepsis syndrome.*
Design. *Prospective, randomised placebo controlled trial, animal experiment.*
Materials and methods. *Experimental porcine model, bilateral hind limb I/R injury, randomised to receive anti-endotoxin hyperimmune globulin or placebo.*
Results. *Bilateral hind limb I/R injury significantly increased intestinal mucosal acidosis, portal endotoxaemia, plasma cytokine (TNF-alpha, IL-6, IL-8) concentrations, circulating phagocytic cell priming and pulmonary leukosequestration, oedema, and capillary-alveolar protein leak. Conversely, pigs treated with anti-endotoxin hyperimmune globulin (IgG) 20 mg/kg at onset of reperfusion had significantly reduced portal endotoxaemia, early circulating phagocytic cell priming, plasma cytokinaemia and attenuation of acute lung injury.*
Conclusions. *Endotoxin translocation across a hyperpermeable gut barrier, phagocytic cell priming and cytokinaemia are key events of limb I/R injury induced systemic inflammation and acute lung injury. This study shows that an anti-endotoxin hyperimmune globulin attenuates portal endotoxaemia, which may reduce early phagocytic cell activation, cytokinaemia and ultimately acute lung injury.*

Keywords: Ischaemia-reperfusion injury; Anti-endotoxin antibody; Systemic inflammatory response syndrome; Endotoxin; Acute lung injury (ALI); Acute respiratory distress syndrome (ARDS).

Introduction

Acute limb ischaemia is a common and often lethal clinical event.[1] A national audit conducted by the Vascular Surgical Society of Great Britain and Ireland into outcomes in acute limb ischaemia reported an amputation rate of 16 percent and a mortality rate of 22 percent respectively.[2] Mortality rates in these patients remain high after open or endovascular revascularisation due not only to co-existing cardiovascular disease but also as a result of systemic effects attributable to reperfusion of the ischaemic limb.[3,4] Reperfusion of the ischaemic lower limb initiates the systemic inflammatory response syndrome (SIRS), characterised by pro-inflammatory cytokine production, and increased circulating polymorphonuclear (PMN) leucocyte activation.[5–7] After lower limb ischaemia-reperfusion injury (IRI) pulmonary sequestration of activated neutrophils is followed by acute pulmonary microvascular injury, acute lung injury (ALI) which may progress to acute respiratory distress syndrome (ARDS), with a subsequent high mortality.[8–10]

We and others have previously shown that lower limb ischaemia reperfusion injury is associated with increased intestinal permeability and endotoxaemia in association with systemic inflammation and vital organ dysfunction in models of limb ischaemia-reperfusion

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injury,[11] acute limb ischaemia,[12] and after infra-renal and thoracic aortic aneurysm repair.[13,14] It has been postulated that the intestine is a source of inflammatory propagation in critical illness. Endotoxin, a lipopolysaccharide (LPS) component of the gram-negative bacterial cell wall, has been shown to translocate from the intestinal lumen to the portal circulation, thereby providing a potent stimulus to cytokine production and leukocyte activation.[15,16] The precise role of endotoxin in systemic inflammation and sepsis syndrome after vascular surgery remains controversial.[17,20,21]

This study tests the hypothesis that an anti-endotoxin hyperimmune globulin may attenuate systemic inflammation and acute lung injury after lower limb ischaemia-reperfusion injury.

Methods

Experimental Protocol. The studies described herein were performed in accordance with the Animals (Scientific Procedures) Act 1986, and with approval of our institution's Animals Research Committee.

Experimental Model. In these series of experiments we used an established large animal model of lower limb ischaemia-reperfusion injury, as previously described by Harkin, *et al*.[7,18] In brief, male white Landrace pigs (28−35 kg; 6−8 weeks old) were sedated with stresnal (Azaperone BP; i.m. 2−3 mg/kg) prior to induction and maintenance of anaesthesia with Sagatal (sodium pentobarbital; i.v. 20 mg/kg and 8 mg/kg/h respectively), titrated to maintain apnoea. A tracheostomy was created by insertion of a No. 7.5 endo-tracheal tube and mechanical ventilation (tidal volume; 15 ml/kg) was instituted to maintain the arterial partial pressure of CO_2 (Pa_{CO2}) at 30−42 mmHg. Hydration was maintained throughout the experiment by infusion of compound sodium lactate (Hartmann's solution; Baxter Healthcare Ltd., UK; 15 ml/kg/h) and body temperature was maintained at 38.2 ± 0.8 °C. The left carotid artery was catheterized (PE-190, Thomas, Philadelphia, PA, USA) for continuous arterial blood pressure monitoring, arterial blood gas sampling, and systemic blood sampling. A balloon-tip pulmonary artery catheter was guided into the pulmonary artery by pressure wave-form analysis, for central venous (CVP), pulmonary artery (MPAP), and intermittent pulmonary arterial wedge (PAWP) pressure monitoring. Mean pressures were determined using calibrated physiological pressure transducers (Model 1280C) driving an amplifier monitor (Model 7835A; Hewlett-Packard, Andover, MA, USA). The left cephalic vein was catheterized (PE-160; Thomas, Philadelphia, PA, USA) for continuous intravenous infusions of sodium pentobarbital (0.06 mg/kg/min) and Compound Sodium Lactate solution (Hartmann's solution, Baxter, UK). A 14-FG silicon urinary catheter was placed in the bladder for urine sampling. A 7-Fr catheter was advanced through the splenic vein to the portal vein for blood sampling. Tonometer's (Tonometrics Inc., Bethesda, Md, USA) were placed in the gastric antrum and sigmoid colon endo-luminally, and terminal ileum via enterotomy. After assuring haemostasis, the laparotomy was closed. Operative procedures took approximately 60 min, after which subjects were stabilised for 30 min, defined by constant heart rate, arterial pressure, end-tidal CO_2.

Experimental Design. Basal physiological values were recorded after the 30-min recovery period. Blood samples were taken from catheters placed in the systemic, portal, hepatic, inferior vena cava sites. Animals were randomised into three groups ($n = 6$ per group). Two groups underwent bilateral external iliac artery occlusion by application of bulldog arterial clamps for 120 min followed by 150 min of reperfusion on release of bulldog clamps. Absence of flow, and restoration when appropriate, was confirmed using an ultra-sonic flow probe (Transonic Systems Inc, USA) applied to the vessel wall. Six pigs were allocated as sham controls and kept for 270 min with recordings as for the experimental animals (see below).

Treatment. Animals undergoing bilateral hind limb ischaemia were randomised to receive treatment with either Anti-endotoxin hyperimmune globulin (Anti-LPS IgG) at 20 mg/kg body weight, or placebo vehicle administered ($n = 6$ per group) by intravenous infusion over 30 min from the beginning of reperfusion. The endotoxin-core hyperimmune IgG immunoglobulin was prepared from pooled plasma from human donors with high tires of cross-reactive IgG antibodies to endotoxin-core (EndoCAb), using a cold-ethanol process followed by treatment at pH 4 in the presence of pepsin and fresh dried for reconstitution, (*a kind gift from Dr G. Robin Barclay, PhD, formerly Edinburgh & SE Scotland Regional Transfusion Centre, Scottish National Blood Transfusion Service, R&D Laboratories, Edinburgh EH1 1EY, Scotland*).

Blood gas measurements. Arterial blood samples were analysed in an automatic blood gas analyser (1304 pH/blood gas analyser, Instrumentation Laboratory, Warrington, UK) without delay. *Fraction inspired Oxygen (FiO_2)*. This was measured in a ventilatory circuit using an Ohmeda 4700 Oxycap Monitor (Ohmeda, 1315 West Century Drive, Louisville CO 80027, USA). *Arterial-alveolar (A-a) gradient*. This was measured using the formula [(A-a) gradient = fraction inspired $O_2 \times 710 - (\text{arterial } pCO_2/0.8) - \text{arterial } pO_2$]. It is a measure of lung function with a large gradient