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Hydrogen sulphide as a novel therapy to ameliorate cyclosporine nephrotoxicity

Gwyn Lee, MRCS, BMBS, BA (Hons), Sarah A. Hosgood, PhD, BSc, Meeta S. Patel, BSc (Hons), MSc, and Michael L. Nicholson, MD, DSc, FRCS*

Department of Infection, Immunity and Inflammation, Transplant Group, The University of Leicester, Leicester General Hospital, Leicester, United Kingdom

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ABSTRACT

Background: Calcineurin inhibitors have significant nephrotoxic side effects, which can exacerbate ischemia–reperfusion injury in renal transplantation. Novel therapeutic agents such as hydrogen sulphide (H_2S) may reduce these harmful effects. This study investigated the effects of H_2S on cyclosporine (CsA) induced nephrotoxicity.

Materials and methods: Porcine kidneys were subjected to 15 min of warm ischemia and 2 h of static cold storage. They were reperfused for 3 h with oxygenated normothermic autologous whole blood on an isolated organ reperfusion apparatus. Kidneys were treated with CsA during reperfusion ($n = 6$) or cyclosporine and 0.25 mmol/L of H_2S infused 10 min before and 20 min after reperfusion ($n = 6$). These were compared with untreated controls ($n = 7$).

Results: CsA caused a significant reduction in renal blood flow during reperfusion, which was reversed by H_2S (area under the curve renal blood flow CsA 257 ± 93 *versus* control 477 ± 206 *versus* CsA + H_2S 478 ± 271 mL/min/100 g.h; $P = 0.024$). Urine output was higher after 2 h of reperfusion in the CsA + H_2S group (CsA + H_2S 305 ± 218 *versus* CsA 78 ± 180 *versus* control 210 ± 45 mL; $P = 0.034$). CsA treatment was associated with an increase in tubular injury, which was not reversed by H_2S (area under the curve fractional excretion of sodium, control 77 ± 53 *versus* CsA 100 ± 61 *versus* CsA + H_2S 111 ± 57%.h; $P = 0.003$). Histologic evaluation showed significant vacuolation and glomerular shrinkage in the CsA group. These were significantly reduced by H_2S ($P = 0.005$, 0.002).

Conclusions: H_2S reversed the vasoconstriction changes associated with CsA treatment during reperfusion.

1. Introduction

Cyclosporine (CsA) is widely used as maintenance immunosuppression after kidney transplantation [1] and remains the archetype of this crucial class of immunosuppressant drugs. Calcineurin inhibition downregulates interleukin (IL) 2 secretion and signal transduction. This in turn reduces the activation and proliferation of T-helper cells, a leukocyte subset crucial for cell-mediated rejection. The benefits of CsA as an effective immunosuppressant are countered by the nephrotoxic sequence, which occurs after its administration. It begins with initially reversible renal and systemic vasoconstriction [1] but also causes an endothelin-mediated dysregulation of afferent and efferent glomerular arteriolar tone. This interferes

* *Corresponding author*. Department of Infection, Immunity and Inflammation, Transplant Group, The University of Leicester, Leicester General Hospital, Gwendolen Rd, LE5 4PW, Leicester, United Kingdom. Tel.: +44 0116 252 2951; fax: +44 0116 252 5030.
E-mail address: gwynlee@hotmail.co.uk (G. Lee).

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with tubuloglomerular feedback, which in turn reduces glomerular filtration rate (GFR) [2]. Eventually this process culminates in interstitial fibrosis and tubular atrophy, the final common pathway of chronic allograft nephropathy [3].

Reperfusion injury associated with organ transplantation is unavoidable; it initiates immunogenic inflammation, oxidative damage, cell edema, and lysis. There is a refractory medullary vasoconstriction that follows renal reperfusion injury and this leads to a disproportionate ischemic insult, which contributes to tubular injury, cortical necrosis, and subsequent fibrosis. The vasoconstriction caused by CsA is central to the severity of its nephrotoxicity during reperfusion [4,5]. It may be possible to counteract the nephrotoxic effects of CsA during reperfusion with the coadministration of another therapy.

Hydrogen sulphide (H_2S) is well known as a toxic foul smelling product of putrefaction; however, it has recently been characterized as an endogenous biologically active gaseous signaling molecule, or gasotransmitter [6,7]. It is synthesized from amino acid substrates by enzymes including cystathionine β-synthase and cystathionine γ-lyase, although it is 3-mercaptopyruvate sulfurtransferase, which predominates in the kidney [8]. The exact mechanisms by which these enzymes are regulated are yet to be described, but redox signaling and product inhibition are thought to play a role [9]. H_2S has a number of effects that are beneficial in renal reperfusion injury [10]. These include antithrombotic [11], anti-inflammatory, antioxidant, and antiapoptotic properties but it is the ability of H_2S to vasodilate [8] that is most likely to counteract the effects of CsA in renal reperfusion injury.

The aim of this study was to investigate the immediate effects of CsA and coadministration of H_2S in a porcine *ex vivo* transplant reperfusion injury model.

2. Materials and methods

Experiments were conducted so as to comply with the Home Office Animals (Scientific Procedures) Act 1986. Under the Schedule 1 method, female large white pigs (60–70 kg) were euthanized by electrocution and exsanguination. Females were selected for ease of husbandry. The blood was collected from a neck incision into a sterilized bottle containing 25,000 U of heparin (Leo Laboratories, Dublin, Republic of Ireland). Immediately postmortem, the abdomen was opened in the midline and the retroperitoneal space exposed. Kidneys were harvested and subjected to a standard period of 15 min of warm ischemia in the abdominal cavity and then immersed in iced Soltran preservation solution (Baxter, Newbury, United Kingdom). They were flushed with 500 mL of Soltran at 100 cm H_2O pressure. Kidneys were stored for 2 h on ice and then reperfused on an isolated organ perfusion system for 3 h (Fig. 1A).

2.1. Experimental design

Kidneys were divided into three groups as follows: control ($n = 7$), CsA ($n = 6$) or cyclosporine + hydrogen sulphide (CsA + H_2S, $n = 6$). Kidneys in the control group were reperfused in an identical way to the treatment groups but without the addition of CsA or H_2S.

2.2. Cyclosporine

To achieve a therapeutic level of 250–300 $ng.mL^{-1}$ of CsA, 0.5 mL (25 mg) of the Sandimmune injection (Novartis Pharmaceuticals Ltd, Basel, Switzerland) was dispersed in 100 mL of normal saline to give a 0.25 $mg.mL^{-1}$ solution. One milliliter of this was added to the reperfusate (1 L) 15 min before reperfusion of the kidney. Prereperfusion CsA levels were then determined for each kidney by the automated Siemens (Camberley, United Kingdom) Xpand Dimension CsA method. This is a spectrophotometric enzyme-linked antibody assay which uses magnetic beads coated with CsA to bind to and then remove unligated CsA antibody [12].

2.3. Hydrogen sulphide

Sodium sulfide ([Na_2S]; Sigma–Aldrich, St Louis, MO) was used as the H_2S donor. Immediately before the experiment, 0.25 mmol/L of Na_2S was dissolved in 60 mL of Ringer lactate (Baxter) and infused into the venous reservoir of the isolated organ perfusion system for 10 min before and 20 min after initiation of reperfusion.

2.4. Reperfusion

The organ perfusion apparatus was based on a paediatric cardiopulmonary bypass system (Bio-Console 550; Medtronic, Watford, United Kingdom) as previously described [13]. The system was primed with equal parts of whole blood and Ringer's lactate. This was allowed to equilibrate to 38°C and achieve maximal oxygenation. One thousand micromoles of creatinine (Sigma–Aldrich) was added as was 375 mg cefuroxime (Flynn Pharmaceuticals, Stevenage, United Kingdom) and nutrients (Synthamin; Baxter, Deerfield IL). To prepare kidneys for *ex vivo* normothermic perfusion, the renal artery and ureter were cannulated with trimmed silastic urethral catheters. The arterial feed was inserted into the arterial cannula. During reperfusion, a normothermic oxygenated blood-based perfusate was pumped into the renal artery at set mean arterial pressure of 80 mm Hg, an optimal pressure determined from previous work [14]. The mean arterial pressure remains constant throughout reperfusion allowing the kidney to regulate its own blood flow according to the level of intrarenal resistance (IRR; Fig. 1B).

Urine produced during reperfusion was carried to a urinometer and measured hourly. Nutrients, glucose, and bicarbonate (Polyfusor; Fresenius Kabi, Runcorn, UK) were continuously infused by peristaltic pumps, and Ringer lactate was replenished to the reservoir to make up losses as urine and evaporation.

2.5. Data collection

Blood samples were taken before reperfusion and at 1, 2, and 3 h for biochemical analysis. Arterial and venous blood gas and acid and/or base parameters were also determined at 1 and 3 h. Renal blood flow (RBF) was measured and IRR