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The effects of arterial pressure during normothermic kidney perfusion

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ABSTRACT

Background: *Ex vivo* normothermic perfusion (EVNP) can reverse some of the detrimental effects of ischemic injury. However, in kidneys with warm and cold ischemic injury the optimal perfusion pressure remains undetermined. The aim of this study was to evaluate the effects of two different arterial pressures during EVNP.

Methods: Porcine kidneys underwent static cold storage for 23 h followed by 1 h of EVNP using leukocyte depleted blood at a mean arterial pressure of either 55 or 75 mm Hg. After this, kidneys were reperfused for 3 h to assess renal function and injury. This was compared with a control group that underwent 24 h cold storage.

Results: During EVNP, kidneys perfused at 75 mm Hg had a higher renal blood flow, increased oxygen consumption (median 59.9 mL/min/g (range 30.1–78.6] *versus* 31.8 [8.2–53.8] mL/min/g; $P = 0.026$), and produced more urine ($P = 0.002$) than kidneys perfused at 55 mm Hg. During *ex vivo* reperfusion, renal blood flow was significantly higher in the 75 mm Hg and 55 mm Hg groups compared with the control (area under the curve median 75 mm Hg 462 [228–745], 55 mm Hg 454 [254–923] *versus* control 262 [215–442] mL/min/100g.h; $P = 0.040$). There was a significant loss of renal function and increase in tubular injury in the 55 mm Hg group kidneys ($P = 0.001$, 0.007). Levels of endothelin 1 were significantly reduced in the 75 mm Hg group ($P = 0.026$).

Conclusions: A mean arterial pressure of 75 mm Hg during EVNP resulted in less tubular damage and less endothelial injury during *ex vivo* reperfusion compared with kidneys perfused at 55 mm Hg.

1. Introduction

Ex vivo normothermic perfusion (EVNP) has recently been introduced into clinical practice as a new technique of kidney preservation [1,2]. The aim of EVNP is to restore function and replenish energy substrates in the kidney for a brief period immediately before transplantation. This technique has been shown to reverse some of the detrimental effects of hypothermic preservation [3,4].

EVNP relies on preserving the kidneys under a controlled condition. Kidneys are perfused with a blood-based solution depleted of leukocytes and with protective agents to create an ideal environment to prevent damage [3]. Kidneys are warmed to near body temperature at a set arterial pressure. The system previously described by Hosgood *et al.* [3] uses pediatric cardiopulmonary bypass technology, which delivers a continuous rather than a pulsatile blood flow to the kidney. Many different normothermic systems have been

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described and conditions vary significantly. Despite the success of the technique, further optimization is needed to refine EVNP for kidneys with greater ischemic injury such as those from donation after circulatory death (DCD) donors. In the United Kingdom, there is a national program of controlled DCD donors. Although the warm ischemic time is relatively short (range11–14 min), the addition of cold ischemia increases the severity of ischemia–reperfusion (I/R) injury and likelihood of early graft dysfunction [5–8]. To prevent injury during EVNP, it is conceivable that lower perfusion pressures may be more ideal than higher perfusion pressures to prevent the risk of further damage and to optimize recovery. The aim of this study was to assess the effects of two different arterial pressures during EVNP in an isolated porcine kidney model.

2. Methods

2.1. Organ retrieval

Under the Home Office (Schedule One) regulations, kidneys were retrieved after 10 min of warm ischemia from female large white pigs weighing 60–70 kg ($n = 9$). They were flushed with 400 mL of cold hyperosmolar citrate solution (Soltran; Baxter Healthcare, Thetford, UK) at a hydrostatic pressure of 100 cm^2 H_20. Kidneys were then preserved by static cold storage for 24 h (control) or 23 h static cold storage followed by 1 h of EVNP at a mean arterial pressure (MAP) of 55 mm Hg (55 mm Hg) or 75 mm Hg (75 mm Hg) ($n = 6$ kidneys per group). At the time of retrieval, approximately 2 L of autologous blood was collected in a sterile blood container containing 25,000 units of heparin (Multiparin; CP Pharmaceuticals, Wrexham, UK). The blood was then transferred into CPDA-1 blood bags (Baxter Healthcare) and stored at 4°C.

2.2. Ex vivo normothermic perfusion

EVNP was carried out using an isolated organ perfusion system (Medtronic, Watford, UK) based on pediatric cardiopulmonary bypass technology as previously described [3]. The vein, artery, and ureter were cannulated; kidneys placed in a organ chamber and perfused for 1 h at a mean temperature of 38°C and a MAP of 55 mm Hg ($n = 6$) or 75 mm Hg ($n = 6$). A priming solution was added to the system which composed of Ringer solution, 10% mannitol (Baxter Healthcare), antibiotics (325 mg cefuroxime (Flynn Pharma Ltd Ireland), sodium bicarbonate 8.4% (Fresenius Kabi, Cheshire, UK), and dexamethasone 8 mg (Organon Laboratories, Cambridge, UK)). Nutrients (Lipid-Peri, B. Braun, Sheffield, UK) containing 100 IU insulin (Novo Nordisk, Denmark), sodium bicarbonate 8.4%, and multivitamins (Cernevit; Baxter Healthcare) were added as an infusion at a rate of 20 mL/h. Glucose (5%) (Baxter Healthcare) containing a vasodilator sodium nitroprusside (25 mg) (Sigma–Aldrich) was also added at a rate of 7 m/h. Autologous blood (500 mL) was passed through a white cell filter to deplete leukocytes and added to the system. The blood-based solution was oxygenated with 95% O_2/5% CO_2 at 0.2 L/min.

2.3. Ex vivo reperfusion

After preservation, all kidneys were flushed with Ringer lactate solution (4°C) before being reperfused with whole autologous blood to assess the effects of renal function and injury. Autologous blood was diluted in priming solution consisting of 500 mL Ringer solution, 5 g D-Mannitol, 2.4 g Urea, 8.4% sodium bicarbonate, and antibiotics (325 mg cefuroxime). To accurately assess renal function, creatinine was added to the circuit to achieve a circulating creatinine concentration of 1000 μmol/L. Infusion fluids included nutrients (B. Braun) (containing 8.4% sodium bicarbonate and 100 U insulin) and 5% glucose (Baxter Healthcare). Kidneys were reperfused for 3 h at 38°C at a MAP of 85 mm Hg.

2.4. Outcome measures

The renal blood flow (RBF), mean arterial pressure (MAP), urine output, and temperature were recorded continuously. Intrarenal resistance (IRR) was calculated by MAP divided by the RBF. A blood gas analyzer (OPTI Medical CCA-TS, Roswell, GA) was used to record PCO_2, PO_2, and pH, Base Excess, HCO_3^- for acid-base homeostasis. Oxygen consumption was calculated by the (PO_2 [arterial]−PO_2 [venous] × RBF/g).

Serum and urine samples were obtained hourly for biochemical analysis and whole blood was collected before and after *ex vivo* reperfusion for hematological analysis. Creatinine clearance was calculated by urine creatinine × urine output (mL/min)/plasma creatinine. To assess tubular injury, the fractional excretion of Na^+ was calculated by (urine Na^+ × urine output (mL/min)/creatinine clearance × plasma Na^+). Urine samples were collected at the end of the 3 h *ex vivo* reperfusion period and stored at−80°C until analyzed for the urinary biomarkers neutrophil gelatinase-associated lipocalin (NGAL) and endothelin 1 (ET-1).

2.5. Endothelin 1

To assess levels of endothelial injury, the levels of ET-1 were quantified in after *ex vivo* reperfusion urine samples using an enzyme immunometric assay (Enzo Life Sciences, Exeter, UK). Samples and standards were added in duplicate to a 96-well plate. Wells were precoated with an affinity purified monoclonal antibody specific for ET-1. After 1 h incubation at room temperature, the plate was washed with Tris buffered saline to remove any unbound nonspecific binding. To capture the bound ET-1, Horseradish peroxidase (HRP)-labeled monoclonal antibody specific to ET-1 was added to the wells and the plate was incubated for 30 min at room temperature. After washing to remove any unbound HRP-labeled antibody, a 3,3′,5,5′-tetramethylbenzidine substrate was added, which reacted to the HRP to generate a blue color. After 30 min, a solution containing hydrochloric acid was added to stop the reaction, and the optical density was read immediately using a spectrophotometer at a wavelength of 450 nm.

2.6. Acute kidney injury NGAL

Levels of NGAL were quantified in urine samples collected after perfusion using a porcine NGAL enzyme-linked