An initial evaluation of post-cardiopulmonary bypass acute kidney injury in swine

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Abstract

Objective: Acute kidney injury (AKI) post-cardiac surgery is associated with mortality rates approaching 20%. The development of effective treatments is hindered by the poor homology between rodent models, the mainstay of research into AKI, and that which occurs in humans. This pilot study aims to characterise post-cardiopulmonary bypass (CPB) AKI in an animal model with potentially greater homology to cardiac surgery patients.

Methods and results: Adult pigs, weighing 50—75 kg, underwent 2.5 h of CPB. Pigs undergoing saphenous vein grafting procedures served as controls. Pre-CPB measures of porcine renal function were within normal ranges for adult humans. The effect of CPB on renal function; a 25% reduction in $^{51}$Cr-EDTA clearance ($p = 0.068$), and a 33% reduction in creatinine clearance ($p = 0.043$), was similar to those reported in clinical studies. CPB resulted in tubular epithelial injury (median NAG/creatinine ratio 2.6 mmol/L (interquartile range (IQR): 0.81—5.43) post-CPB vs 0.48 mmol/L (IQR: 0.37—0.97) pre-CPB, $p = 0.043$) as well as glomerular and/or proximal tubular injury (median albumin/creatinine ratio 6.8 mg mmol/L (IQR: 5.45—13.06) post-CPB vs 1.10 mg mmol/L (IQR: 0.05—2.00) pre-CPB, $p = 0.080$). Tubular injury scores were significantly higher in kidneys post-CPB (median score 2.0 (IQR 1.0—2.0) relative to vein graft controls (median score 1.0 (IQR 1.0—1.0), $p = 0.019$). AKI was associated with endothelial injury and activation, as demonstrated by reduced DBA (dolichos biflorus agglutinin) lectin and increased endothelin-1 and vascular cell adhesion molecule (VCAM) staining.

Conclusions: The porcine model of post-CPB AKI shows significant homology to AKI in cardiac surgical patients. It links functional, urinary and histological measures of kidney injury and may offer novel insights into the mechanisms underlying post-CPB AKI.

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Keywords: Acute kidney injury; Cardiopulmonary bypass; Endothelial activation

1. Introduction

Acute kidney injury (AKI) is one of the most serious complications post-cardiac surgery with mortality rates of 6—19% [1—3]. The pathogenesis is poorly understood and there have been no advances in the treatment of this condition since the development of dialysis over 30 years ago. Where postoperative renal dysfunction is so severe as to require dialysis, mortality rates are as high as 63% [2,3]. Cardiopulmonary bypass (CPB) is a major contributor to AKI post-cardiac surgery [4,5]. Attempts to ameliorate this injury in clinical studies have, with few exceptions [6], been unsuccessful principally because our understanding of the underlying mechanisms is poor [7]. Reno-protective strategies developed in rodent models, the mainstay of research into AKI, have failed to translate into clinical benefits [8] and there is a widely acknowledged need for the development of large animal models of renal dysfunction with closer homology to humans [8,9]. Previous authors have noted significant homology between human and porcine renal anatomy, haemodynamics and function [10,11]. The aim of this pilot study was to investigate the functional and pathological features of post-cardiopulmonary-bypass (CPB) AKI in a large animal model with potentially greater homology to cardiac surgery patients than those currently available.

2. Methods

2.1. Animals

Fourteen adult, female, farm-bred, Large White-Landrace crossbred pigs weighing 50—70 kg were used in this pilot study. The procedures were performed under licence and received care in accordance with the Home Office Guidance...
on the operation of the Animals (Scientific Procedures) Act 1986, published by HMSO, London. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). Because of the Home Office licence restrictions, and the need to reduce animal numbers in what was a pilot study, we were obliged to use two control groups. Firstly, to determine the effect of CPB on renal function and urinary markers of kidney injury, we compared paired pre-CPB versus post-CPB values \((n = 7)\). Secondly, to assess the effect of CPB on histological markers of renal tubular and glomerular injury, we compared pigs undergoing sternotomy with CPB versus a control group of pigs \((n = 7)\) undergoing harvesting of tissues post-saphenous vein to carotid artery interposition grafts \([12]\). Both groups received identical diet and unlimited access to water. Food, but not water, was restricted on the day of surgery.

Anaesthesia and CPB were performed by a modification of our protocol described previously \([13]\). Venous access for the administration of intravenous anaesthetic agents was achieved through the internal jugular vein. Arterial blood pressure was continuously monitored via a 20 G Vygon catheter placed in the right femoral artery at the groin. Urine output was measured via a silastic 14 Fr bladder catheter. Animals received heparin \(300 \text{ IU} \text{ kg}^{-1}\) following sternotomy, venous drainage was established through a two-stage right atrial cannula with arterial return achieved via a 22 Fr ascending aortic cannula (both Medtronic, UK). The CPB circuit, composed of a Jostra Quadrox oxygenator, a VHK4201 hardshell reservoir with standard PVC/silicon tubing (all Maquet Cardiopulmonary AG, Hirrlingen, Germany) was primed with Hartman’s solution \((500 \text{ ml})\), Gelofusin \((500 \text{ ml})\), mannitol \(20\% \ (500 \text{ mg} \text{ kg}^{-1})\) and heparin \((5000 \text{ i.u.})\). Normothermic \((38–39 \degree \text{C})\) in pigs, non-pulsatile CPB was commenced using a Stöckert Multifl ow Roller Pump (Sorin Group GmbH, Munich, Germany) to achieve a target flow rate of \(70–90 \text{ ml} \text{ kg}^{-1} \text{ min}^{-1}\). Mean arterial blood pressure was maintained between 60 and 80 mmHg using metaraminol boluses (one pig required a low-dose norepinephrine infusion), \(\text{PaO}_2\) between 200 and 300 mmHg and, \(\text{PaCO}_2\) between 35 and 45 mmHg. During CPB, the lost mediastinal blood was captured with a pericardial sucker. Total CPB time was \(2.5 \text{ h}\). This corresponds to the normal CPB duration for many adult cardiac surgical procedures. The animals were weaned from CPB, monitored for a further \(1.5 \text{ h}\) and euthanised by an overdose of anaesthesia prior to nephrectomy. Control animals had undergone saphenous-vein-to-carotid interposition grafting \(4 \text{ weeks}\) previously. Pigs undergoing saphenous vein graft harvest underwent identical anaesthetic induction and maintenance to those undergoing CPB. After vein graft harvest and general anaesthesia for approximately \(2 \text{ h}\), the animals were euthanised and urine and kidneys harvested for analysis. All animals received \(500 \text{ ml} \text{ h}^{-1} \ 0.9\% \text{ NaCl} \) saline via infusion pump for the duration of the experiments to maintain hydration.

2.2. Renal function

2.2.1. Glomerular filtration rate (GFR)

\(^{51}\text{Cr}-\text{EDTA}\) clearance was measured for \(90 \text{ min}\) pre-CPB and then for a second \(90 \text{ min}\) post-weaning from CPB using a single bolus \(^{51}\text{Cr}-\text{EDTA}\) injection with clearance determination obtained using the slope—intercept method as previously described \([14,15]\). Briefly, \(4 \text{ ml}\) of blood was extracted as a baseline measurement. Then \(3.7 \text{ MBq}\) of \(^{51}\text{Cr}-\text{EDTA}\) (Amerham, UK) was injected into a central vein. After injection, serial blood samples for analyses \((4 \text{ ml})\) were then obtained over \(90 \text{ min}\) to calculate the plasma clearance curve. The blood was centrifuged \((3000 \times g \text{ for } 10 \text{ min})\) and \(3 \text{ ml}\) of plasma extracted for scintillation counting of \(^{51}\text{Cr}-\text{EDTA}\) (gamma counter 15 min per sample). The radioactivity of the \(^{51}\text{Cr}-\text{EDTA}\) was measured together with a standard sample prepared in combination with that given to the pig. Clearance \((\text{Cl})\) of the marker was expressed as \(\text{Cl} = Q/\text{AUC} \ (\text{ml} \text{ min}^{-1})\), where \(Q = \text{injected activity, AUC} \ (\text{total area under plasma clearance curve}) = A/k_1 + B/k_2\), where \(A\) and \(B\) are the zero time intercepts of the two exponentials and \(k_1\) and \(k_2\) the respective rate constants.

2.2.2. Creatinine clearance

Creatinine clearance was calculated from urine and serum samples taken at the same time points. Urine was collected during two \(90 \text{ min}\) intervals; prior to the commencement of CPB and after weaning from CPB. A blood sample was also taken at the beginning of each period for serum creatinine measurement. Serum and urine creatinine values were determined with a commercial reagent kit (HiCo Creatinine; Boehringer Mannheim GmbH Diagnostica, Lewes, UK). Creatinine clearance was determined by the standard formula: \(\text{creatinine clearance} = (\text{ml min}^{-1}) = [\text{urine creatinine concentration} (\mu\text{mol} \text{ ml}^{-1}) \times \text{urine volume} (\text{ml min}^{-1})]/\text{plasma creatinine concentration} (\mu\text{mol} \text{ ml}^{-1})\). Total solute clearance, free water clearance and fractional excretion of sodium were calculated from urinary and plasma electrolyte and urine output values using accepted formulae \([16,17]\) at similar time points.

2.3. Acute kidney injury

2.3.1. Urine analysis

Ten millilitre aliquots of urine were collected at the same time as listed above in the CPB group and post-procedure only in the vein graft harvest control group. \(\text{N-Acetyl-\beta-glucosaminidase} \ (\text{NAG})\), a widely used and specific marker of renal tubular injury \([18]\), expressed as ratio of urine creatinine concentration, was measured as previously described \([5,19]\). In addition, proteinuria and the urinary \(\alpha-1\)-microalbumin-to-creatinine ratio, a non-specific marker of glomerular and proximal tubular injury, was determined by immunoturbidimetry on the Cobas Mira (Koni Inst., Sweden).

2.3.2. Histological analysis

Post-sacrifice, both kidneys were removed and cut, using a sharp knife, into 0.5-cm thickness slices each extending from cortex to medulla. Alternate slices were fixed in 4% formalin in phosphate-buffered solution \((\text{PBS})\) or snap frozen in liquid nitrogen. Formalin-fixed sections underwent paraffin embedding, were sectioned into \(5-\mu\text{m}\) transverse slices and stained either with haematoxylin and eosin or periodic acid-Schiff. Sections were scored for renal tubular injury and inflammation by an experienced renal pathologist (TT) blinded to the experimental conditions, as described previously \([20]\). The injury of the proximal tubules was
semi-quantitatively analysed, as this is the most vulnerable structural component of the kidney for ischaemic damage. It was possible to distinguish between the proximal and distal tubules, according to at least one of the following morphologic criteria: topographic localisation, tubular size and form, cytoplastic density and position of the nuclei and presence or absence of brush border. The magnitude of tubular injury defined as epithelial swelling, intracytoplasmic vacuolisation, loss of apical brush border or definite acute tubular necrosis (epithelial cell detachment from the underlying tubular basement membrane with or without nuclear staining) was scored into four levels on the basis of morphologic criteria: topographic localisation, tubular size and form, cytoplasmic density and position of the nuclei and presence or absence of brush border. The magnitude of tubular injury was also semi-quantitatively determined by the presence (+1) or absence (0) of tubulointerstitial inflammatory cells. Mean scores from four separate sections were averaged to generate a single score for each animal. In addition, immunocytochemistry (ICC) for inflammatory cell infiltration (MAC 387 for macrophages and MCA 1218 for CD 14), vascular cell adhesion molecule-1 (VCAM (MCA907B); Serotec, Oxford, UK), neutrophil elastase (Dako Laboratories, High Wycombe, Bucks, UK), endothelin-1 and lectin (Sigma, Dorset, UK) was performed using the Vector avidin—biotin complex method (ABC, Vector, Peterborough, UK) or immunofluorescence (Sigma, Dorset, UK) was performed using the Vector avidin—biotin complex method (ABC, Vector, Peterborough, UK) or immunofluorescence, as previously described [12,21]. All image analysis was undertaken with Image-Pro Plus 4 (Cybernetics).

2.4. Statistical analysis

On the basis of a previous study reporting mean $^{51}$Cr-EDTA clearance for adult pigs as (mean ± standard error) 97 ± 6.7 ml min$^{-1}$ [20], we calculated using repeated measures that a pilot study with six animals would have a 90% power to detect a 33% reduction in GFR. Histological and urinary measures of kidney injury were compared between CPB and controls. Datasets were generally non-normally distributed. Values are therefore expressed as median (interquartile range) and non-parametric (Wilcoxon signed-ranks test, paired, Mann—Whitney U test, un-paired) analyses performed throughout. Differences were considered to be statistically significant when $P < 0.05$. All statistical analysis was performed using SPSS version 14.0 (Chicago, IL, USA).

3. Results

3.1. Renal function

We compared post-CPB measures of renal function with pre-CPB values as paired controls. Median prime volume in the CPB circuit was 1500 ml (IQR: 1500—1650). Median total volume infused during the bypass procedure, including the pump prime and maintenance fluid, was 7000 ml (IQR: 6500—8500). One animal died from oxygenator failure. Median flow rate achieved on CPB was 5.41 l min$^{-1}$ (IQR: 5.0—5.95). Median perfusion pressure was 64.5 mmHg (IQR: 61.9—68.5). All pre-CPB measures of porcine renal function were within the normal ranges for healthy human adults (Table 1). CPB resulted in a 23% reduction in $^{51}$Cr-EDTA clearance ($p = 0.068$, $n = 5$) and a 33% reduction in creatinine clearance ($p = 0.043$, $n = 6$, Table 1). Analysis of EDTA clearance curves indicated that the changes were due to a change in clearance rate/half-life, rather than a change in volume of distribution in pigs pre- and post-bypass. There was no significant reduction in fractional sodium excretion post-bypass, with mean values greater than 1, indicating the occurrence of predominantly renal as opposed to pre-renal dysfunction. There were no differences between pre- and post-CPB values for serum creatinine, serum osmolality or free water clearance (Table 1). Reductions in urine output and sodium clearance did not reach statistical significance ($p < 0.1$).

3.2. Acute kidney injury

3.2.1. Urine analysis

To evaluate the effect of CPB on urinary markers of AKI, we compared paired pre- and post-CPB ($n = 6$) urine samples as well as post-CPB values ($n = 6$) versus a post-vein-graft harvest control group ($n = 7$). Total body weights (45—62 kg) were not significantly different between the CPB and vein graft controls. CPB resulted in significant proteinuria (median protein/creatinine ratio 2000 mg mmol$^{-1}$ (IQR: 1360—3528) compared to pre-CPB values (median: 61 mg mmol$^{-1}$ (IQR: 48—225)), $p = 0.043$ and to vein graft controls (median: 57 mg mmol$^{-1}$ (IQR: 48—64), $p = 0.001$, Fig. 1). This included elevated levels of NAG, a specific marker of tubular epithelial injury (median NAG/creatinine ratio 2.60 u mmol$^{-1}$ (IQR: 0.81—5.43) post-CPB versus 0.48 u mmol$^{-1}$ (IQR: 0.37—0.97) pre-CPB, $p = 0.043$), and $\alpha$-1-microglobulin, a non-specific marker of glomerular and/or proximal tubular injury (median albumin/creatinine ratio 6.8 mg mmol$^{-1}$ (IQR: 5.45—13.06)).
post-CPB versus 1.10 mg mmol⁻¹ (IQR: 0.05—2.00) pre-CPB, p = 0.080). Post-CPB values were also significantly higher than vein graft controls for α₁-microalbumin (median albumin/creatinine ratio 0.30 mg mmol⁻¹ (IQR: 0.0—1.30, p = 0.003) and NAG (median NAG/creatinine ratio 0.45 u mmol⁻¹ (IQR: 0.32—0.86), p = 0.030).

3.2.2. Histological analysis

Semi-quantitative assessment of renal tubular damage in haematoxylin and eosin (H&E)-stained sections demonstrated a significant increase in tubular injury scores in post-CPB kidneys, median score 2.0 (IQR: 1.0—2.0) compared to vein graft controls, median score 1.0 (IQR: 1.0—1.0), p = 0.019. Frank acute tubular necrosis was not seen in any section. Commonly proximal tubules in post-bypass kidneys were seen to have undergone severe sublethal injury as evidenced by swelling, loss of brush border and diffuse intracytoplasmic vacuolisation (Fig. 2).

Tubular injury in the CPB group was associated with loss of vascular endothelium as evidenced by reduced or absent DBA lectin staining (Fig. 3). Immunofluorescence demonstrated increased staining for endothelin-1 and VCAM in porcine kidneys post-CPB compared to controls (Figs. 4 and 5). The level of inflammatory cell infiltration was not different between treatment and controls, whether assessed by scoring of H&E sections or with ICC for macrophages (MAC387), neutrophil elastase or CD14 (MCA 1218 for macrophages/granulocytes), data not shown.

4. Discussion

In this pilot study we have characterised an experimental model of AKI. Our data suggests that this has homology to AKI in humans. The main findings of this study are as follows.
Measures of renal function in adult pigs pre-CPB are within the normal range for human adults (Table 1) and are similar to those reported preoperatively in cardiac surgical patients [7,8,22]. CPB elicits reductions in GFR and significant reductions in creatinine clearance despite adequate hydration. The reduction in GFR/creatinine clearance was not evident from serum creatinine measurements or urine output. The 25—33% reduction in GFR/creatinine clearance in this model has clear clinical correlations. The effect size is similar to the reduction in renal function associated with CPB in cardiac surgery patients as reported in our studies [5] and others [6]. In a randomised clinical trial of off-versus on-pump coronary artery bypass grafting on-pump patients experienced a 20% reduction in creatinine clearance postoperatively relative to preoperative values or to postoperative values in off-pump patients [5]. GFR reductions within this range in a clinical setting have prognostic significance; a 25% reduction in GFR corresponds to Stage 1 AKI according to the RIFLE criteria [23] and reductions in estimated GFR greater than 30% are associated with a fivefold increase in procedural mortality after cardiac surgery [1].

- The release of urinary markers of kidney injury is qualitatively similar to those reported in cardiac patients post-CPB [5,22]. These clinical studies report higher quantitative levels of proteinuria and enzyme release; however, this presumably reflects the increased age and mild pre-existing renal impairment in patients undergoing cardiac surgery compared to the health and young age of the animals used in the current study.
- Renal tubular damage was associated with evidence of endothelial cell injury and activation but not neutrophil sequestration. This suggests that CPB elicits an immediate and direct injury to the renal tubules. There was little evidence of a substantial inflammatory component at this time point, as has been implicated in CPB-mediated dysfunction of other organs as well as in current paradigms of AKI [9]. It is possible that our methodology was insufficiently sensitive to detect a difference between treatment and control groups in terms of the level of inflammatory infiltrate. Christianson and colleagues reported small but significant increases in neutrophil sequestration (3.1% vs 2.2%) in pigs’ kidneys post-CPB relative to controls, using radiolabelling [24]. We used several methods to assess inflammatory infiltrate in our model, but we detected no differences between treatment and controls. One explanation is that neutrophil infiltration may be a later development and, as a consequence, not detectable in a non-recovery model at 1.5 h post-CPB. The endothelial injury and activation observed in post-CPB kidneys would certainly promote this. A role for inflammatory cells in the pathogenesis of CPB-induced kidney injury is therefore not discounted by our observations.

There are three main limitations to this study. First, as this was an exploratory study, our animal numbers were restricted and we used pigs undergoing an unrelated surgical procedure as controls. Pigs undergoing sternotomy and cannulation without CPB would undoubtedly have been more appropriate as a control group. Coronary artery bypass grafting via sternotomy without CPB does not result in significant AKI or renal dysfunction however [5], and the absence of sternotomy in the control group is unlikely to have acted as a confounder in our analyses of AKI. Second, as this was a non-recovery model, it does not provide information on the time course of AKI or the rate of recovery. Renal function is often at its lowest at 24 h post-surgery. A recovery model would provide more complete data and is the subject of ongoing studies. Not least it might allow the development of renal histological changes that commonly lag behind changes in function following kidney injury. Third, although there are marked similarities between the porcine model and cardiac surgery patients, in terms of baseline function and the magnitude of the changes in function in response to a clinical event, that is, CPB, it is important to stress that the aetiology of AKI in a clinical setting has a multifactorial aetiology of which CPB is only a part. Other important aetiological factors include preoperative characteristics, notably pre-existing renal disease, as well as postoperative adverse events, such as fluid balance and the use of nephrotoxic medications.
as low cardiac output or sepsis [1–3]. We suggest that the porcine CPB AKI model can be used to explore these interactions. For example, it should be possible to combine this model with porcine models of chronic renal impairment, myocardial infarction and sepsis that are already well described.

These limitations notwithstanding, the model has several potentially useful features. Firstly, CPB is an important contributor to AKI [4,5] and the underlying mechanisms are very poorly understood. This model links functional, histological and urinary measures of kidney injury in response to a clinical stimulus and should enable us to unravel these mechanisms. Our findings suggest that tubular injury and reductions in GFR occur within hours of CPB and prior to significant inflammatory cell infiltration. Endothelial injury and activation were evident at this stage. In rodent models of ischaemic AKI, this is a precursor to inflammatory cell infiltration, vascular congestion and diminished blood flow that are thought then to extend the degree of kidney injury [9–11]. Our findings reinforce the need for improved CPB management algorithms, with emphasis placed on the avoidance of factors likely to exacerbate ischaemic injury, such a low-flow or profound anaemia [25]. These findings also suggest that processes associated with endothelial activation and dysfunction may represent targets for reno-protective strategies in cardiac surgery. Secondly, this model offers a practical step in the development of treatment and prevention strategies for post-CPB AKI. Findings in rodent models of AKI have failed to translate into clinical benefits principally due the poor homology between rodent and human renal anatomy and function, greater tolerance to ischaemia in humans and the difference in the nature of the experimental renal insult versus clinical scenarios associated with kidney injury [10,11]. The porcine model will provide an important tool in the process of translating findings in rodent models to clinical testing and potentially avoid costly

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Fig. 4. Immunofluorescence staining of porcine kidney for endothelin-1. Morphology was determined by DAPI nuclear staining shown in adjacent photomicrograph (×40 magnification). There was increased endothelin-1 expression in post-CPB kidneys (A) compared to controls (B). Dense staining was evident in renal tubule epithelial cells and glomeruli.
negative clinical studies that have characterised research in this field to date [9].

In conclusion, this pilot study has characterised a model of post-CPB acute kidney injury with potential relevance to clinical practice. It has identified pathophysiological processes underlying post-CPB kidney dysfunction. This model may have a useful role in the development of novel renoprotective strategies in cardiac surgery.

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