Reversal of anemia with allogenic RBC transfusion prevents post-cardiopulmonary bypass acute kidney injury in swine

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ACUTE KIDNEY INJURY IS A COMMON and severe complication of cardiac surgery, where it is associated with a fourfold increase in perioperative mortality (8, 33). There is no effective treatment (19), and primary prevention remains the mainstay of renoprotective strategies (31). Anemia during cardiopulmonary bypass (CPB) has been identified as an important modifiable risk factor for acute kidney injury in observational clinical studies (23), with the risk increasing significantly when hematocrit (Hct) fall below 0.24–0.26 (5, 24, 25, 32). In an apparent paradox, however, reversal of anemia during CPB with red blood cell (RBC) transfusion further increases the likelihood of acute kidney injury as much as twofold (5, 25, 32), an effect attributed to the progressive deterioration in erythrocyte function and the accumulation of proinflammatory mediators in the supernatant during storage, often referred to as the “storage lesion” (34). Because of the correlation between anemia during CPB and RBC transfusion, however, and despite the use of statistical techniques that can adjust for potential confounders, it is impossible to establish the independent role of transfusion beyond that of anemia in the etiology of acute kidney injury from observational studies. Unfortunately, these observational data also create difficulties for prospective randomized trials that seek to answer this question because of ethical concerns over what constitutes a safe lower level of anemia or transfusion rate for patients (36). As a consequence, the clinical evidence to guide the treatment of anemia is poor, the lowest hematocrits tolerated during CPB range from 0.18 to 0.24 (30, 36), and transfusion rates range from 7 to 34% (5, 25, 32).

Methods

Animals

Thirty-nine adult female farm-bred Large-White-Landrace cross-bred pigs weighing 50–70 kg were used. Animals received care in accordance with and under license of the Animals (Scientific Procedures) Act (1986) and conforming to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). The study received local institutional review board approval.

Intervention

Pigs were allocated to the following groups: Sham, neck dissection+500-ml crystalloid infusion; CPB, 2.5 h of CPB+500-ml crystalloid infusion; Sham+transfusion, neck dissection+500 ml of leukocyte-depleted, sucrose-adenosine-glucose-mannitol (SAG-M)-stored porcine RBC units administered over 2 h; or CPB+transfusion, as above.
Anesthesia and CPB were performed by a modification of our protocol described previously (16, 20). Following induction with Ketaset (100 mg/ml ketamine hydrochloride), animals were intubated and anesthetized with halothane (1.5–2.0%) and nitrous oxide (50% in oxygen), with positive pressure ventilation in a circle system. Venous access was achieved through the internal jugular vein. Arterial blood pressure was continuously monitored via a 21-G Vygon catheter placed in the left common carotid artery. Urine output was measured via a urethral silastic 14Fr catheter. CPB venous drainage was established via a 24Fr Smart Cannula (Smartcanca, Lausanne, Switzerland) placed in the right external jugular vein and advanced to the right atrium. Arterial return was achieved via a 14Fr Smart Cannula placed in the right internal carotid artery and advanced to the brachiocephalic trunk. All animals received heparin (300 IU/kg). The CPB circuit was primed with Hartman’s solution (2,000 ml) and heparin (5,000 IU). Normothermic (38–39°C in pigs), nonpulsatile CPB was commenced using a Stöckert Multiflow Roller Pump (Sorin Group, Munich, Germany) to achieve a target flow rate of 80–90 ml·kg⁻¹·min⁻¹ of blood through the hollow fiber-membrane oxygenator apparatus (Dideco D708 Compact-Fl, Sorin Biomedica, Via Crescentio, Italy). Mean arterial blood pressure (MABP) was maintained between 65 and 75 mmHg with small incremental doses of the α-adrenergic agonist metaraminol, percent inspired oxygen (FiO₂) at 50%, and PaO₂/PaCO₂ between 35 and 45 mmHg. Central venous pressure (8–12 mmHg), hydration, and sodium load (500 ml/h, 0.9% NaCl saline) were strictly controlled. In some pigs, cardiac output was measured by a Swan Ganz catheter using the thermodilution technique, based on the Fick principle. This was used to estimate the oxygen delivery for Sham pigs at the end of the intervention period. For CPB pigs, the pump flow rate immediately before discontinuation of CPB was used. Oxygen delivery (DO₂) was calculated as {pump flow or cardiac output × [hematocrit × arterial oxygen saturations (1.342)] + (PaO₂ × 0.0031)}/H11003. Total CPB time was 2.5 h. This represents a prolonged CPB duration, a risk factor for post-cardiac surgery acute kidney injury identified in clinical studies (4, 7) and has previously been shown to result in significant kidney injury in the porcine model (16, 20). Post-CPB animals were recovered, administered intramuscular buprenorphine as required for analgesia, and then reanesthetized and reevaluated after 24 h. Nephrectomy was performed at 24 h before euthanasia.

Allogenic RBC Transfusion

Allogenic blood was harvested from donor pigs (n = 8) in citrate-phosphate-dextrose, Buffy coat removed, leucodepleted, and stored in SAG-M using the Leukotrap WB system (Pall Medical, Portsmouth, UK) and stored at 4°C for a mean of 37 days (range 34–42 days), according to the manufacturer’s instructions. To validate the transfusion model, a comparative analysis was performed between the results for SAG-M-stored pig blood and reference values from human leucodepleted SAG-M-stored RBC obtained from the UK National Health Service Blood and Transplant. Five-milliliter aliquots were removed from six representative bags per group for evaluation of storage-related changes. Biochemical changes in the supernatant were assessed using an ABL 800 Flex blood-gas analyzer (Radiometer, Copenhagen, Denmark) at weekly intervals. Spectrophotometry was used to measure free Hb in the blood units. Briefly, blood in heparinized bottles was centrifuged at 2,000 rpm for 5 min, and the plasma was removed. The plasma was then again centrifuged to remove any residual cells. Plasma Hb was then measured using an Aquarius 7000 Spectrophotometer (Cecil Instruments, Cambridge, UK), and an absorbance of the plasma at wavelengths 560, 576, and 592 nm was determined. Free Hb was expressed as milligrams per deciliter. The hemolysis index = (free Hb × hematocrit)/donor unit Hb. Before transfusion, donor and recipient blood was cross-matched using an agglutination test. Five hundred milliliters of allogenic RBC with average packed cell volume of 37% were transfused over 2 h during the intervention period.

Outcomes

Biochemical markers of acute kidney injury. Creatinine clearance was calculated from urine samples taken over three time periods: 90 min pre-CPB, 90 min post-CPB, and over 90 min at 24 h post-CPB before euthanasia. Serum for creatinine measurement was also collected at the beginning of each period. Serum and urine creatinine values were determined with a commercial reagent kit (Hicò Creatinine, Boehringer Mannheim Diagnostica, Lewes, UK). Creatinine clearance was determined by the standard formula: creatinine clearance (ml/min) = [urine creatinine concentration (μmol/ml) × urine volume (ml/min)]/plasma creatinine concentration (μmol/ml). Total solute clearance, free water clearance, and sodium clearance were calculated using accepted formulas (27) at similar time points. In addition, urinary protein-to-creatinine ratios were determined by immunoturbidimetry on the Cobas Mira (Roche, Basel, Switzerland) at similar time points.

Endothelial function and renal oxygenation. At 24 h post-CPB, renal artery blood flow (T106 Transonic flowmeter, Transonic Systems, Ithaca, NY) and renal cortical microvascular flow (Oxylite p02 E Series, Oxford Optronix, Oxford, UK) were measured. Endothelial dysfunction was determined by the change in blood flow from a baseline value in response to acetylcholine (ACh; 0.1–10 μg·kg⁻¹·min⁻¹). Renal nitric oxide (NO) bioavailability was estimated from urine using a colorimetric nitric oxide assay kit (CaliBiochem, Merck, Nottingham, UK) that is based on the Griess reaction as described previously (28). Outer medullary oxygenation was measured by tissue O₂ sensors (Oxylite p02 E Series, Oxford Optronix). Renal cortical nucleotides were measured using HPLC as previously described (14).

Histological analysis. Formalin-fixed, paraffin-embedded, 5-μm transverse renal sections stained with hematoxylin and eosin were scored for renal tubular injury and inflammation by an experienced renal pathologist (T. Toth) blinded to the experimental conditions, as described previously (9, 16). ICC and immunofluorescence was performed as previously described (3, 17, 22). Selected antibodies included MAC 387 (Abcam, Cambridge), endothelial NO synthase (eNOS), platelet-activating complex-1 (PAC; Santa Cruz Biotechnology, Santa Cruz, CA), inducible NO synthase (iNOS; Thermo Fisher Scientific UK, Loughborough, UK), endothelin-1 (ET-1; Acris Antibodies, Herford, Germany), Dolichos biflorus agglutinin (DBA) lec- tin, platelet endothelial cell adhesion molecule-1 (PECAM-1; R&D Systems, Abingdon, UK), and von Willebrand Factor (vWF; Dako- Cytomation, Glostrup, Denmark). Qualitative differences in staining were confirmed by Western blotting with quantification by densitometry normalized to β-actin values, as previously described (17, 20).

Power Calculation and Statistical Analysis

Creatinine clearance, an index of glomerular filtration rate we have used in clinical studies (1), was our primary end point. On the basis of our previous experimental work (16, 20), we estimated that a study with 24 animals (6/group) with a baseline and two postintervention measurements would have a 90% power to detect a large effect size of 0.7 (equivalent to a difference of 16.5 ml/h in creatinine clearance between groups assuming a within-group SD of 23.5 before adjustment for repeated measures). Additional animals were also allocated to each group to complete the endothelial function experiments. The study was not powered for these or other secondary outcomes, and these analyses must be considered as exploratory. Comparisons among the groups were performed using ANOVA with the Bonferroni correction and unpaired Student t-tests. General linear model ANOVA was used for repeated measures with adjustment for baseline values. Categorical data were compared using Fisher’s exact test. Data were reported throughout as means ± SE or as the geometric mean for nonnormally distributed data. Treatment differences were reported as
Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham</th>
<th>CPB</th>
<th>Transfusion + Sham</th>
<th>Transfusion + CPB</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight*</td>
<td>58.00 (53.07–62.93)</td>
<td>56.21 (52.27–60.15)</td>
<td>55.19 (52.86–57.51)</td>
<td>57.78 (52.52–63.04)</td>
<td>0.644</td>
</tr>
<tr>
<td>Hemoglobin*, g/dl</td>
<td>8.60 (8.0–9.15)</td>
<td>8.57 (7.5–9.62)</td>
<td>8.24 (7.74–8.75)</td>
<td>9.25 (8.42–10.07)</td>
<td>0.117</td>
</tr>
<tr>
<td>Serum creatinine*, μmol/l</td>
<td>136.66 (116.7–156.7)</td>
<td>133.42 (113.9–152.9)</td>
<td>134.88 (118.7–151.0)</td>
<td>133.28 (118.0–148.6)</td>
<td>0.987</td>
</tr>
<tr>
<td>Creatinine clearance, ml/min</td>
<td>118.34 (83.91–129.92)</td>
<td>117.48 (73.83–141.20)</td>
<td>116.39 (108.92–135.97)</td>
<td>113.82 (107.17–130.41)</td>
<td>0.859</td>
</tr>
<tr>
<td>Free water clearance, ml/min</td>
<td>−0.54 (−0.91–0.00)</td>
<td>−0.06 (−0.69–1.82)</td>
<td>1.15 (−1.03–3.17)</td>
<td>−0.60 (−0.82–1.32)</td>
<td>0.689</td>
</tr>
<tr>
<td>Fractional sodium excretion, %</td>
<td>0.22 (0.12–0.49)</td>
<td>0.63 (0.38–1.30)</td>
<td>0.94 (0.36–1.52)</td>
<td>0.50 (0.20–0.70)</td>
<td>0.582</td>
</tr>
<tr>
<td>Albumin creatinine ratio, mg/mmol</td>
<td>0.40 (0.15–0.95)</td>
<td>0.00 (0.00–0.60)</td>
<td>0.50 (0.30–0.98)</td>
<td>0.30 (0.20–0.70)</td>
<td>0.083</td>
</tr>
<tr>
<td>Urine output*, ml/min</td>
<td>1.19 (0.79–1.59)</td>
<td>1.92 (0.79–3.05)</td>
<td>1.62 (1.13–2.10)</td>
<td>2.47 (1.02–3.93)</td>
<td>0.114</td>
</tr>
<tr>
<td>Metaraminol dose, mg</td>
<td>0.0 (0.0–0.0)</td>
<td>1.0 (0.0–2.0)</td>
<td>2.0 (0.0–4.0)</td>
<td>5.0 (0.0–5.0)</td>
<td>0.035†</td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval). P values were derived from the Kruskall-Wallis test, with post hoc comparisons using the Mann-Whitney U-test for nonnormally distributed data. For normally distributed data, *P values were derived from ANOVA. †There were no statistically significant differences in intergroup comparisons with correction for multiple comparisons.

RESULTS

Allogenic RBC Transfusion and CPB

All animals survived the study to recovery, reanesthesia, reevaluation, and euthanasia. Baseline characteristics were similar between groups (Table 1). Porcine RBC units developed a storage lesion that showed qualitative homology to that observed in human RBC units at 35 days of storage (Table 2). Porcine units demonstrated similar potassium and sodium concentrations to human units. The hemolysis index was higher in porcine units, but the mean hemolysis index [mean 0.87 (±0.59)] remained <1, a quality control standard for human red cell storage (3). The mean Hct was lower in porcine units, a reflection of the lower Hct in porcine venous blood [mean 26.3% (±1.3)]. Glucose concentrations and the pH of the storage supernatant were higher, and levels of high-energy phosphates and lactate were lower relative to human units, indicating lower metabolic activity in the porcine units.

CPB was associated with a significantly greater volume of crystalloid (Fig. 1A), although this included the pump-priming volume. CPB resulted in a dilutional anemia relative to baseline values (P = 0.038) and compared with Sham pigs, whereas transfusion in both Sham and CPB pigs increased Hct for up to 24 h postintervention (Fig. 1B). CPB resulted in lower MABP during the intervention period compared with Sham (Fig. 1C). Transfusion increased MABP in CPB pigs. Transfusion only marginally increased MABP in Sham pigs (P = 0.063). There were no differences in MABP between groups postintervention, however (Fig. 1C). At the end of the intervention period, DO2 was significantly higher in CPB-transfusion vs. CPB-only pigs; however, the difference between Sham and Sham-transfusion pigs was not significant (Fig. 1D). CPB resulted in lower venous oxygen saturation (SvO2; Fig. 1E) and higher lactate values, indicating a degree of tissue hypoxia during the intervention period (Fig. 1F). Transfusion reduced SvO2 in Sham pigs and increased SvO2 in CPB pigs (Fig. 1E). Transfusion had no significant effect on arterial lactate (Fig. 1F).

Biochemical Markers of Acute Kidney Injury

The primary end point of the study was postintervention creatinine clearance measured over two postoperative time points. There was a significant interaction between time postintervention and the effects of treatment (P = 0.005), and the estimates for treatment effects at individual time points were therefore reported separately (Fig. 2A). At 1.5 h, neither CPB nor transfusion caused significant reductions in creatinine clearance relative to Sham; however, CPB-transfusion resulted in a significant reduction relative to both Sham and Sham-transfusion pigs. This situation was reversed at 24 h, however. At this time point, there was a significant reduction in creatinine clearance in CPB vs. Sham pigs. Transfusion in CPB

Table 2. Comparison of human and porcine leucodepleted SAG-M-stored RBC

<table>
<thead>
<tr>
<th>Measure</th>
<th>Human, NHSBT Quality Control Data, mean (SE)</th>
<th>Baseline</th>
<th>35 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/dl</td>
<td>11.7 (0.41)</td>
<td>12.1 (0.25)</td>
<td>19.4 (0.07)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>35.7 (1.23)</td>
<td>37.2 (2.66)</td>
<td>58.9 (0.22)</td>
</tr>
<tr>
<td>Hemolysis index, %</td>
<td>0.01 (0.00)</td>
<td>0.07 (0.24)</td>
<td>0.02 (0.00)</td>
</tr>
<tr>
<td>Adenosine triphosphate, μmol/g Hb</td>
<td>3.24 (0.53)</td>
<td>0.01 (0.00)</td>
<td>4.55 (0.08)</td>
</tr>
<tr>
<td>pH</td>
<td>6.8 (0.02)</td>
<td>6.9 (0.02)</td>
<td>6.8 (0.00)</td>
</tr>
<tr>
<td>Sodium, mmol/l</td>
<td>148 (0.85)</td>
<td>118 (0.85)</td>
<td>147 (0.44)</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>29.3 (0.47)</td>
<td>31 (0.47)</td>
<td>27.5 (0.31)</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>0.43 (0.1)</td>
<td>2.3 (0.00)</td>
<td>1.7 (0.11)</td>
</tr>
<tr>
<td>Pco2, mmHg</td>
<td>80.4 (4.1)</td>
<td>37.6 (4.9)</td>
<td>69.7 (0.82)</td>
</tr>
<tr>
<td>Paco2, mmHg</td>
<td>94 (3.8)</td>
<td>256 (8.99)</td>
<td>33.5 (0.60)</td>
</tr>
</tbody>
</table>

Values are least squares means (SE). UK National Health Service Blood and Transplant (NHSBT) quality control data were kindly provided by Drs. Rebecca Cardigan and Stephen Bennett. Hemolysis index = free Hb * hematocrit/unit Hb. SAG-M, sucrose-adenosine-glucose-mannitol.
pigs prevented this reduction (Fig. 2A). Clinical definitions of acute kidney injury compare changes in indices of glomerular filtration with baseline. No groups demonstrated statistically significant overall reductions in creatinine clearance relative to baseline in this study; however, at 24 h a clinical definition of acute kidney injury, a 25% reduction in creatinine clearance from baseline (10), categorized 4/7 pigs in the CPB group as having acute kidney injury, 1/8 in Sham/H11001 transfusion, 0/9 in Sham, and 0/7 in the CPB/H11001 transfusion group (Fisher’s exact test, P = 0.007).

We also evaluated the effects of CPB and transfusion on free water clearance, fractional sodium excretion, and proteinuria (Table 3). There was no interaction between treatment and time postintervention for these outcomes; therefore, data from both postintervention time points were pooled to estimate the overall effect of treatments. There were no differences between the groups for free water clearance, a marker of diuresis (Table 3). CPB+transfusion pigs demonstrated significant reductions in fractional sodium excretion relative to either Sham or CPB pigs but not...
to Sham + transfusion pigs (Table 3). CPB increased urinary protein loss relative to Sham and Sham + transfusion groups. There was no significant difference in protein loss between CPB + transfusion pigs and other groups (Table 3).

**Endothelial Function and Tissue Oxygenation**

CPB decreased global renal blood flow and eliminated the acute hyperemic response to ACh. Transfusion did not prevent these effects (Fig. 2B). Cortical microvascular endothelial responsiveness to ACh was also significantly reduced in the CPB group. This effect again was not prevented by transfusion. Transfusion in Sham pigs significantly reduced cortical microvascular responsiveness, indicative of endothelial dysfunction (Fig. 2C). CPB reduced NO bioavailability relative to Sham pigs. Transfusion had no effect on NO bioavailability in Sham pigs but prevented the decrease in NO bioavailability caused by CPB (Fig. 1D). Analysis of regional oxygenation and cortical nucleotide levels showed that CPB caused a reduction in $P_{O_2}$ at the level of the outer medulla, significant depletion of cortical ATP, and a rise in cortical adenosine (Fig. 1, E and F). Transfusion in Sham pigs also resulted in medullary hypoxia; however, this was less severe than that observed in CPB pigs and was not associated with depletion of cortical ATP or elevated adenosine levels. In contrast, transfusion in CPB pigs attenuated medullary hypoxia relative to CPB only, although not to levels observed in Sham pigs, but prevented the reductions in cortical ATP and adenosine levels observed in CPB-only pigs (Fig. 1, E and F).

**Histological Analysis**

**Tubular injury.** No experimental group demonstrated evidence of acute tubular necrosis (data not shown), as determined using an established scoring system (10). CPB resulted in proximal tubular epithelial cell stress manifest by phenotypic changes causing pseudodilatation of the tubules in association with increased expression of ET-1 (Fig. 3, A and B). Transfusion in Sham pigs also resulted in a significant increase in proximal tubule luminal diameter as well as significant increases in levels of ET-1. Transfusion in CPB pigs prevented these changes, with tubular diameters and levels of ET-1 expression similar to those in Sham pigs (Fig. 3, A and B).

**Vascular endothelium.** CPB caused a reduction in eNOS expression within the vascular endothelium and loss of the endothelial glyocalyx (DBA lectin staining) (Fig. 3, C and D). Transfusion in Sham pigs also reduced eNOS expression and DBA lectin staining. Transfusion in CPB pigs did not reverse the reductions in eNOS but increased levels of DBA lectin staining relative to CPB alone (Fig. 3, C and D).

**Inflammation and platelet activation.** CPB resulted in the appearance of activated PAC-positive platelets and inflammatory cells (MAC387) localized primarily to the glomeruli (Fig. 3, E and F). Transfusion in Sham pigs resulted in a significant increase in activated platelet counts and a small increase in inflammatory cells in association with increased vWF staining (data not shown). In CPB pigs, transfusion had the opposite effect, reducing the numbers of inflammatory cells and activated platelets per square millimeter. (Fig. 3, E and F).

**DISCUSSION**

**Main Findings**

In this study, CPB and associated dilutional anemia caused acute kidney injury in swine, characterized by renal endothelial injury and dysfunction, loss of NO bioavailability, intrarenal vasoconstriction, medullary hypoxia, cortical ATP depletion, glomerular sequestration of activated platelets and inflammatory cells, and evidence of proximal tubule epithelial cell stress. RBC transfusion in the absence of CPB also resulted in renal injury, characterized by endothelial injury, microvascular endothelial dysfunction, platelet activation, and equivalent cortical tubular epithelial phenotypic changes to those observed in CPB pigs, although significant intrarenal vasoconstriction and reductions in creatinine clearance were not evident. In contrast, reversal of anemia during CPB with RBC transfusion prevented the reductions in creatinine clearance, loss of NO bioavailability, platelet activation, inflammation, and epithelial cell injury attributable to CPB but did not prevent the development of significant intrarenal vasoconstriction and endothelial dysfunction. These findings are at odds with clinical evidence suggesting that RBC transfusion during CPB increases renal injury. They also highlight the complexity of the renal response to injury and underline the value of developing large-animal recovery models with assessment at multiple time points as a means to more fully understand the pathophysiological processes underlying our clinical observations.

**Study Strengths and Limitations**

In this study, we evaluated the interaction of two risk factors for post-cardiac surgery acute kidney injury identified from clinical studies in a large-animal recovery model of extracorporeal circulation with considerable homology to that which occurs in humans (1, 16). We established pump flows, MAP, and $D_{O_2}$ during CPB that were at or above perfusion targets in humans (7). During CPB, the mean Hct in CPB-only pigs was 0.24. This is higher than the Hct threshold (>0.18) for tissue oxygen supply dependency in swine (35) and represents the upper limit for Hct transfusion triggers in clinical studies (30, 36). CPB resulted in modest elevations in arterial lactate, indicative of tissue hypoxia, but levels were typical of values reported post-CPB in cardiac surgery patients (24). These apparently normal perfusion indices resulted in significant reductions in creatinine clearance at 24 h, however, showing considerable homology in terms of renal dysfunction to a previous randomized trial undertaken by members of this group comparing on- vs. off-pump coronary artery bypass (1). These similarities notwithstanding, there are two important limitations to this model: first, only 4/7 pigs exhibited acute kidney injury as defined clinically (9), and the overall reduction in creatinine clearance from baseline was not statistically significant. This may reflect the absence of other important risk factors for post-cardiac surgery acute kidney injury in the model such as chronic kidney disease or diabetes (5, 8, 33). It may also be attributable in part to the intentional periprocedural volume loading; up to 6,000 ml of crystalloid/24 h, which has well-recognized renoprotective effects. This was intended to avoid confounding from prerenal volume depletion, a recognized differential diagnosis for acute kidney injury in the clinical setting. The equivalent fractional sodium excre-
significant increases in organ injury posttransfusion in observa-
well as the current study; equivalent to
relatively low volumes of RBC transfused in randomized trials as
present study indicates that these results could also be due to the
RBC transfusion. Alternatively, the “subclinical” injury in the
This might be interpreted as evidence of the safety of allogenic
findings are similar to those of recent randomized clinical trials
to rodents (21), suggesting some homology with clinical RBC
storage lesion in pigs have parallels with the tissue hypoxia observed following transfusion of human RBC
to rats (13). This is supported by the higher SvO2 observed in
transfusion pigs relative to CPB alone. In complete
contrast transfusion in Sham pigs reduced SvO2 and caused
organ injury. The differential effects of transfusion on tissue
oxygen extraction and organ injury were dependent on the
degree of tissue hypoxia before transfusion. SvO2 was normal
in Sham+transfusion pigs but was significantly reduced
and lactate elevated in the CPB+transfusion pigs before trans-
fusion. Transfusion in the setting of hypoxia prevented further drops in SvO2, as was seen in CPB pigs. Similar observations
have been reported in clinical studies where RBC transfusion
has been shown to improve tissue oxygenation only in the
setting of critical oxygen extraction (2, 18). Orlov and col-
leagues (18) reported significant improvements in SvO2 only
when pretransfusion SvO2 <75%. Similarly, Casutt and col-
leagues (2) reported that the increase in oxygen utilization
posttransfusion was inversely proportional to the degree of
oxygen uptake before transfusion. Alternatively, these effects
may also have been influenced by the pretransfusion Hct; CPB

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Sham</th>
<th>CPB</th>
<th>Sham+Transfusion</th>
<th>CPB+Transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free water clearance*, ml/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.29 (0.86)</td>
<td>0.47 (0.55)</td>
<td>1.18 (0.69)</td>
<td>0.28 (0.58)</td>
</tr>
<tr>
<td>1.5 h</td>
<td>3.59 (1.51)</td>
<td>5.22 (2.04)</td>
<td>1.18 (1.01)</td>
<td>0.12 (0.73)</td>
</tr>
<tr>
<td>24 h</td>
<td>0.85 (0.93)</td>
<td>−0.52 (0.17)</td>
<td>1.82 (1.46)</td>
<td>0.35 (0.95)</td>
</tr>
<tr>
<td>Overall</td>
<td>2.42 (0.68)</td>
<td>2.40 (0.77)</td>
<td>1.36 (0.78)</td>
<td>0.44 (0.77)</td>
</tr>
<tr>
<td>Fractional sodium excretion†, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.22</td>
<td>0.55</td>
<td>0.82</td>
<td>0.49</td>
</tr>
<tr>
<td>1.5 h</td>
<td>1.25</td>
<td>1.16</td>
<td>0.63</td>
<td>0.55</td>
</tr>
<tr>
<td>24 h</td>
<td>0.72</td>
<td>1.08</td>
<td>0.74</td>
<td>0.31</td>
</tr>
<tr>
<td>Overall</td>
<td>1.14</td>
<td>1.07</td>
<td>0.59</td>
<td>0.40$</td>
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<tr>
<td>Urinary protein:creatinine ratio‡, mg/mmol</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>21.8</td>
<td>22.7</td>
<td>23.7</td>
<td>19.7</td>
</tr>
<tr>
<td>1.5 h</td>
<td>24.3</td>
<td>40.1</td>
<td>31.8</td>
<td>41.9</td>
</tr>
<tr>
<td>24 h</td>
<td>26.2</td>
<td>40.1</td>
<td>25.8</td>
<td>26.7</td>
</tr>
<tr>
<td>Pooled over time</td>
<td>25.4</td>
<td>40.33</td>
<td>28.38</td>
<td>33.2</td>
</tr>
</tbody>
</table>

*Normally distributed data are expressed as least squares means (SE) and effect sizes reported as mean differences (95% confidence interval). †Nonnormally distributed data are expressed as geometric means and effect sizes reported as ratio of geometric means. The test for an interaction between treatment and time was \( P = 0.551 \) for free water clearance, \( P = 0.632 \) for urinary protein:creatinine ratio, and \( P = 0.622 \) for fractional sodium excretion. Data from postintervention time points were therefore pooled to estimate the overall effect for these outcomes with adjustment for baseline values; free water clearance was estimated as 0.54, fractional sodium excretion was estimated as 0.46, urinary protein:creatinine ratio was estimated as 22.2. ‡\( P < 0.05 \), §\( P < 0.01 \) with Bonferroni adjustment for multiple comparisons.

Findings in Relation to Existing Evidence

Transfusion in CPB pigs increased perfusion pressure and Hct but significantly reduced creatinine clearance at 1.5 h. Reduced glomerular filtration rate at this time point and therefore reduced solute delivery to the distal nephron may be renoprotective by reducing tubular oxygen consumption (13). This is supported by the higher SvO2 observed in CPB+transfusion pigs relative to CPB alone. In complete contrast transfusion in Sham pigs reduced SvO2 and caused organ injury. The differential effects of transfusion on tissue oxygen extraction and organ injury were dependent on the degree of tissue hypoxia before transfusion. SvO2 was normal in Sham+transfusion pigs but was significantly reduced and lactate elevated in the CPB+transfusion pigs before transfusion. Transfusion in the setting of hypoxia prevented further drops in SvO2, as was seen in CPB pigs. Similar observations have been reported in clinical studies where RBC transfusion has been shown to improve tissue oxygenation only in the setting of critical oxygen extraction (2, 18). Orlov and colleagues (18) reported significant improvements in SvO2 only when pretransfusion SvO2 <75%. Similarly, Casutt and colleagues (2) reported that the increase in oxygen utilization posttransfusion was inversely proportional to the degree of oxygen uptake before transfusion. Alternatively, these effects may also have been influenced by the pretransfusion Hct; CPB

Fig. 2. Measures of creatinine clearance, endothelial function, and regional oxygenation. Graphs show measured creatinine clearance at baseline, 1.5 h postintervention, and at 24 h (A), renal artery blood flow at 24 h (B), cortical microvascular flow at 24 h before and in response to acetylcholine (ACh) infusion (C), nitric oxide (NO) bioavailability at 24 h (D), measured PO₂ in the outer medulla at 24 h (E), and renal cortical nucleotide levels at 24 h (F). ATP is expressed as a ratio to ADP (both measured in ng/mg protein), and adenosine is expressed as ng/mg protein. Values are means ± SE. For creatinine clearance, \( P \) values were determined using ANOVA for repeated measures adjusted for baseline estimated at 114.2 ml/min. For ACh studies, \( P \) values were derived from ANOVA with adjustment for baseline values estimated as renal blood flow = 0.24 l/min and cortical microvascular flow = 302 CPU. *\( P < 0.05 \) vs. Sham. †\( P < 0.05 \) vs. CPB. ‡\( P < 0.05 \) vs. Sham+Tx.
Fig. 3. Histological analysis. A: representative hematoxylin and eosin (H&E)-stained photomicrographs of cortical tubules and graphs demonstrating mean proximal tubular diameter. B: differences in protein expression [normalized densitometry ratio of ET-1 (Western blot as shown) to β-actin (not shown)] and localization of ET-1 to dilated injured tubules. Scale bars = 140 μm (A) and 72 μm (B). C: differences in protein expression [normalized densitometry ratio of Western blots as shown to β-actin (not shown)] and immunofluorescence staining for endothelial nitric oxide synthase (eNOS). Scale bars = 47 μm (C) and 70 μm (D). E and F: cell counts/mm² and representative photomicrographs of immunofluorescence for platelet-activating complex (PAC), a specific marker of platelet activation (E), and MAC387, a monocyte marker (F). Scale bars = 31 μm (E) and 50 μm (F). Values are means ± SE (at least n = 4/group). P values were derived from ANOVA with intergroup comparisons using a t-test. *P < 0.05 vs. Sham. †P < 0.05 vs. CPB. ‡P < 0.05 vs. Sham+Tx.
pigs were anemic before transfusion, whereas Sham pigs were not. Studies in rats have shown that tissue hypoxia caused by hemorrhage can be reversed by the transfusion of non-oxygen-carrying glutaraldehyde-fixed erythrocytes, simply due to improved microvascular perfusion at higher viscosities (25). Differentiating between these effects was not possible in the current study because of the strong correlation between pretransfusion Hct and SV\textsubscript{O2}. Controlling for these using additional interventions such as vasodilators, isovolemic hemodilution, or reduced pump flows in different groups would have acted as confounders in our analyses, however. Instead, our anesthetic and perfusion parameters were informed by those used in clinical practice in humans, and, importantly, we have shown that the effects of CPB and transfusion on these parameters were consistent with their effects in a clinical setting.

Transfusion in CPB pigs prevented the reduction in creatinine clearance observed in CPB-only pigs at 24 h but did not reverse the intrarenal vasoconstriction and endothelial dysfunction attributable to CPB, suggesting that this was the result of an increased filtration fraction. This is consistent with the increased NO bioavailability, reduced cortical adenosine, and reduced fractional sodium excretion observed in CPB+transfusion pigs at this time point. Reduced fractional sodium excretion at 24 h in CPB+transfusion pigs compared with CPB only in the presence of equivalent volume loading, and perfusion pressure is also suggestive of activation of the renin-angiotensin-aldosterone pathway, although plasma renin activity was not measured in the current study. The “normal” creatinine clearance in CPB+transfusion pigs at 24 h masks these important pathophysiological responses and highlights the limitations of creatinine clearance as a diagnostic marker. Evaluation of animals at later time points would have yielded useful insights into the longer-term significance of these observations, although importantly, transfusion in CPB pigs also prevented cortical ATP depletion and tubular epithelial cell stress, in association with reduced inflammation and increased NO bioavailability at 24 h.

Observational clinical studies in cardiac surgery indicate that anemia during CPB is associated with acute kidney injury but that reversal of anemia with RBC transfusion increases the likelihood of this outcome still further (5, 25, 32). The apparently contradictory findings in the current study may be explained as follows: first, observational studies have well-recognized limitations that preclude the demonstration of causal relationships, notably bias in the administration of treatments such as transfusion as well as the likelihood that unmeasured confounders may influence study findings. We have shown that incipient tissue hypoxia during CPB is in all likelihood an important and unmeasured confounder in these studies. Second, we have shown that RBC transfusion in the absence of tissue hypoxia may be harmful. It is rare in clinical practice, and presumably also in observational data sets, for patients to receive transfusions because of measurable tissue oxygen debt. Third, clinical definitions of acute kidney injury are based on serum creatinine, an indirect measure of creatinine clearance. We have shown that significant renal endothelial dysfunction, hypoxia, and renal tubular injury can occur despite normal creatinine clearance. Better diagnostic and prognostic biomarkers of acute kidney injury will change current definitions and are likely to alter our interpretation of observational data.

Conclusion

Our study has shown that contrary to the findings of observational studies in cardiac surgery, RBC transfusion during CPB protects against acute kidney injury. The study has also provided novel insights into the pathophysiological processes underlying post-cardiac surgery acute kidney injury, highlighted the limitations of the available clinical evidence relating to the efficacy of transfusion, and underlined the need for translational research into indications for transfusion and prevention strategies for acute kidney injury.

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DISCLOSURES

G. J. Murphy is a consultant for NovoNordisk, Ethicon, Maquet, and Medtronic.

REFERENCES


