Vascular endothelial cell changes in postcardiac surgery acute kidney injury

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Qureshi SH, Patel NN, Murphy GJ. Vascular endothelial cell changes in postcardiac surgery acute kidney injury. Am J Physiol Renal Physiol 314: F726–F735, 2018. First published December 20, 2017; doi:10.1152/ajprenal.00319.2017.—Acute kidney injury (AKI) is common complication of cardiac surgery; however, the phenotype of this condition is poorly defined. The aim of this study was to characterize changes in endothelial structure and function that underlie postcardiopulmonary bypass (post-CPB) AKI. Adult pigs (n = 16) were randomized to undergo the following procedures (n = 8 per group): group 1: sham operation, neck dissection with 2.5 h of general anesthesia; and group 2: CPB, 2.5 h of cardiopulmonary bypass. CPB resulted in the depletion of specific epitopes of glycosaminoglycans side chains of the endothelial glyocalyx: Dolichos biflorus agglutinin: mean difference (MD) [95% confidence interval (CI)], P value: −0.26 (−0.42, −0.09), P = 0.0024, Triticum vulgaris (wheat germ) agglutinin: −0.83 (−1.2, −0.38), P = 0.0005, and Ulex europaeus agglutinin 1: −0.25 (−0.49, −0.009), P = 0.041; endothelial membrane protein: thrombomodulin: −3.13 (−5.6, −0.65), P = 0.02; and adherens junction: VE-cadherin: −1.06 (−1.98, −0.145), P = 0.02. CPB also resulted in reductions in microvascular cortical perfusion: −0.62 (−1.02, −0.22), P = 0.006, and increased renal cortex adenosine levels: 2.32 (0.83, 3.8), P = 0.0059. These changes were accompanied by significant reduction in creatinine clearance at 1.5 h postintervention, MD 95% CI: −51.7 (−99.7, −3.7), P = 0.037, and at 24 h, MD (95% CI): −47.3 (−87.7, −7.6), P = 0.023, and proteinuria immediately postintervention MD (95% CI): 18.79 (2.17, 35.4), P = 0.03 vs. sham. In our experimental CPB model, endothelial injury was associated with loss of autoregulation, increase in microvascular permeability, and reduced glomerular filtration. Interventions that promote endothelial homeostasis may have clinical utility in the prevention of postcardiac surgery AKI.

INTRODUCTION

The Clinical Problem

Acute kidney injury (AKI) postcardiopulmonary bypass (CPB) is a common complication with key determinants being CPB perfusion pressures and nadir hematocrits (21, 35). Reported AKI rates vary between 5 and 38% depending on the definition used (12, 22, 35, 36) and are consistently associated with increases in mortality. Severe AKI, defined as the need for new renal replacement therapy, is associated with a 22-fold rise in mortality (60–70%) (5, 50). Improved understanding of the underlying disease mechanisms is required if effective treatments are to be developed. Experimental studies have consistently shown that AKI whether as the result of experimental ischemia-reperfusion (46) or red cell transfusion (29) is characterized by renal endothelial dysfunction. Moreover, interventions that prevent kidney injury, such as endothelin-1A receptor blockade, the administration of sildenafil citrate (30), or the use of fresh, as opposed to stored, red cells (29), also prevent loss of endothelial homeostasis. Despite strong experimental and clinical (16) evidence linking endothelial injury to AKI, the molecular processes underlying the observed changes in endothelial function remain poorly characterized. We suggest that improved understanding of these processes will lead to more effective treatments. The central hypothesis was that structural alterations in constituents of the glomerular endothelial membrane result in renal vascular endothelial dysfunction in the context of AKI. We focused our investigation on the glycosaminoglycan (GAG) and core protein elements that sustain the barrier (38), anti-inflammatory (20), and flow characteristics of the endothelium (33, 49). Since the endothelial glyocalyx is a regulator of nitric oxide-mediated blood flow (49) and a major determinant in regulation of coagulation, fibrinolytic and hemostatic pathways (6, 34), we speculated that post-CPB microstructural disruption in GAG or glyocalyx core proteins underlie endothelial vasomotor dysregulation leading to hypoxia, cortico-medullary metabolic stress, and altered permeability that characterize AKI in humans.

MATERIALS AND METHODS

Animals

The study received Institutional Review Board approval and adhered to ARRIVA guidelines (https://www.nc3rs.org.uk/arrive-guidelines). Animals received care in accordance with, and under the license of, the Animals (Scientific Procedures) Act 1986 and conformed 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).

These experimental methods have been previously described in detail (30).

Sixteen female large, white, Landrace crossbred pigs &4 mo old and weighing 50–70 kg were used. Animals were paired housed, with no restriction to water pre- and postsurgery; however, they were fasted 12 h pre surgery. There were no adverse events recorded in either group.

Intervention Allocation and Blinding

Sixteen animals (n = 8 per group) were quasirandomized preintubation. Research staff undertaking the experiments and collecting tissue and urine samples were not blinded. However, all laboratory measurements and analyses were carried out in a blinded manner. A unique ID identified each subject, and no attribution to the interven-
tion allocation could be assimilated from the identifiers. The allocation groups were as follows; group 1: sham operation with 2.5 h of general anesthesia; group 2: CPB consisting of 2.5 h of cardiopulmonary bypass with general anesthesia.

Anesthesia and CPB

Anesthesia and CPB were performed by a modification of our protocol described previously (24). 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 20-G Vygon catheter placed in the left common carotid artery. Urine output was measured via a urethral silastic 14-Fr catheter.

After an initial stabilization period (1 h), CPB was established between minimally invasive Smart Cannulae (Smartcanu, Lausanne, Switzerland) placed in the aorta and right atrium through the right internal carotid artery and external jugular vein, respectively. All animals received heparin (300 IU/kg), and activating clotting time was maintained for >480 s. The CPB circuit, composed of a Jostra Quadrox oxygenator, a VH4201 hard shell reservoir with standard PVC/silicon tubing (all Maquet Cardiopulmonary, Hirrlingen, Germany) was primed with Hartman’s solution (1,500 ml) and heparin (5,000 IU). Normothermic (38–39.8°C in pigs), nonpulsatile CPB was maintained for 90 min, and at 24 h (a period of 90 min) using the following protocol described previously (24). 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 20-G Vygon catheter placed in the left common carotid artery. Urine output was measured via a urethral silastic 14-Fr catheter.

Assessment of Tissue Perfusion and Gas Exchange

Prespecified analyses including serum lactate (mmol/l), mixed venous saturations (%), and gas exchange of PO2 (mmHg) and CO2 (mmHg) were measured at baseline, during intervention, at 1.5 h postintervention, and at 24 h in both sham and CPB animals (n = 8) each. Work of breathing (Joules) was assessed using SERVO-I Universal Ventilator (Maquet, Rastatt, Germany), which used volume-controlled ventilation with a tidal volume of 10 ml/kg, FiO2 of 0.5, respiratory rate of 12 breaths/min, and peak end expiratory pressure of 5 cm H2O.

Renal Function and Injury

Clinical definitions of AKI are based on reduction in glomerular filtration rate (GFR) (14). In previous studies, we have documented a strong correlation between calculated creatinine clearance (CrCl) and GFR measured by Cr51 EDTA clearance: regression coefficient for CrCl vs. 51Cr-EDTA clearance: 0.74 at postintervention and 0.78 at 24 h, both P < 0.001 (10). In the swine model, CPB results in a reduction in CrCl of 25–33% (24), which is consistent with previous clinical definition of AKI: the Risk, Injury, Failure, loss of kidney function, and End (RIFLE) definition assumed that AKI equated to a 25% reduction in GFR (2). In the porcine model, serum and urine samples were obtained for serum and urinary creatinine, respectively, and CrCl was calculated for baseline period (over 60 min), postintervention period (90 min), and at 24 h (a period of 90 min) using the following equation: (creatinine clearance (ml/min) = [urine creatinine concentration (µmol/ml) x urine volume (ml/min)/plasma creatinine concentration (µmol/ml)]). These measurements were completed in n = 8, sham and CPB. Interleukin-18 (IL-18), a specific marker of acute kidney injury, was detected in the urine more than 12 h post-CPB. As previously described (30), IL-18 was measured in urine samples using ELISA (Bender Med-Systems, Vienna, Austria).

Microvascular Perfusion In Renal Cortex

To assess renal endothelial function at 24 h, we measured in vivo renal cortical perfusion (postmidline laparotomy) and the response to a supraparenal intra-aortic acetylcholine (Ach) (endothelial-dependent response). Cortical perfusion was measured using a microvascualr flow probe (miniature surface probe; MSP 300XP; Oxford Optromx, Oxford, UK) placed on the left renal capsular and connected to an OxyLab LDF, single-channel laser-Doppler tissue blood perfusion monitor (Oxford Optromx). This system continuously monitors tissue blood flow using laser-Doppler flowmetry (LDF). LDF works by illuminating a small region of tissue under observation with low-power laser light from a sensor containing optical fiber light guides. Laser light from one fiber is directed toward the tissue and is scattered within the tissue. Some of the scattered laser light is incident upon moving red blood cells within the tissue, and this light becomes Doppler-frequency shifted as a result. A second optical fiber collects this backscattered, Doppler-shifted light from the tissue and returns it to the detector within the monitor. The photodetected signal comprises a broad spectrum of Doppler-frequency-shifted signals generated as a result of the movement of red blood cells within the tissue. Microvascular blood flow is electronically calculated as the product of mean red blood cell velocity and mean red blood cell concentration in the volume of tissue under illumination from the sensor. The OxyLab contains in-built calibrations for the surface probe. The OxyLab monitor was connected to a laptop via a Powerlab 8/35 (PL3508; AD Instruments, Oxford, UK), which gave an output of cortical microvascular flow in blood per unit, which is an arbitrary nonabsolute unit for blood perfusion.

To avoid vasoconstriction (as a result of manipulation) confounding our results, a 10-min rest or nonmanipulation period was provided before baseline measurements. This was followed by a 5-min period of baseline flow measurements and then infusion of Ach for 5 min during which flow was continuously monitored. The mean flow during the time period was calculated, and flow measurements were performed in triplicate both with and without acetylcholine. Endothelial dysfunction was determined by the change in renal blood flow with or without an Ach infusion (0.1–10 µg·kg·min−1) administered via a 14-G peripheral venous cannula (BD Venflon, Becton Dickinson, Oxford) inserted into the supraparen abdominal aorta. Data are displayed as baseline measurements and change from baseline in n = 4 sham and CPB animals each. Between-group differences were assessed using t-tests.

Microvascular Permeability Assessment

We used the protein/creatinine ratio as an index of microvascular permeability. Urine protein and albumin concentrations adjusted to urinary creatinine concentrations were calculated at baseline (i.e., before institution of CPB) and 24 h postintervention (n = 8 sham and CPB groups each) from swine. These were determined by immuno-turbidimetry using Cobas Mira analyzer (Roche Diagnostics, West Sussex, UK).

Tissue Harvest-Snap Freezing, Cryosectioning, and Protein Extraction

At 24 h postintervention, 5–10 g of right renal cortex and medulla were harvested via midline laparotomy and snap frozen at −80°C. Blocks of cortex were then sectioned into 5-µm thick slices and

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frozen until staining. Stored blocks of renal cortex were made to thaw and immediately homogenized and solubilized with RIPA buffer for protein extraction for Western blotting. Tissue blocks of renal cortex and medulla were homogenized in liquid nitrogen with a mortar and pestle and treated with extraction solutions for purine high-performance liquid chromatography as previously described (19).

**Immunofluorescence Staining of Renal Tissue**

Dual-stained immunofluorescence was undertaken on 5-μm cortical frozen sections fixed with methanol, blocked with 3% BSA/5% FCS, and stained using Dolichos biflorus agglutinin (DBA) [ligand motif: N-acetylgalactosamine (GalNAc); Triticum vulgaris (wheat germ) agglutinin (TBA) [ligand motif: N-acetylgalactosamine (GalNAcβ1-4GlcNAcβ1-4GlcNAc, Neu5Ac, sialic acid)] (WGA); glycinine max (soybean) agglutinin (SBA) [ligand motif: N-acetylgalactosamine (GalNAc)]; and Ulex europaeus agglutinin (UEA) [ligand motif: fucose-1,2-galactose (Fucα1-2Gal-R)], as markers of endothelial glycocalyx [concentration 2 mg/ml; BK-1000; Vector Laboratories] at 1:50 dilution and costained with VE-cadherin (Santa Cruz Biotechnology) and glypican-1, 1:500 (all proteins: dyndecan-1, 1:500; dyndecan-4, 1:500; perlecan, 1:500 (all Zymed Laboratories, San Francisco, CA); and glypicanc, 1:500 (Santa Cruz Biotechnology); and endothelial surface markers thrombospondin, 1:1,000 (BionRBT); and von Willebrand factor (vWF), 1:1,000 (DakoCyto) in a single staining experiment. This was an exploratory analysis, and no formal power calculation was performed. However, this study was based on previous work (24), where for one baseline and two postintervention measurements of primary end point; creatinine clearance, it was estimated that a study with eight recipients per group will have 90% power to detect a large effect size of 0.7 SD (equivalent to a difference of 16 ml/h in creatinine clearance between groups assuming a within-group SD of 23.5). We hence demonstrated (24) significant differences in renal function between control and intervention groups and subsequently detected changes in cell markers of injury in groups of n = 4. We therefore performed our analyses in a total of 16 pigs, with 4 pigs per group for endothelial function tests, and 4 pigs per group for tissue analyses.

**RESULTS**

All animals proceeded to recovery on day 0 and underwent post-CPB reevaluation of renal function, and death on day 1.

**Renal Injury**

There was significant post-CPB reduction in creatinine clearance: MD (95% CI): −51.7 (−99.7, −3.7), P = 0.03; and at 24 h, MD (95% CI): −47.3 (−87.7, −7.6), P = 0.023, (Fig. 1A). The test for interaction of treatment vs. time was significant, P = 0.011. The effect estimates represented a percentage reduction in CrCl of −14% (−60–32%) at 1.5 h and −30% (−72.8–14.6) at 24 h. There was also significant release of the urinary biomarker IL-18 at 24 h in CPB pigs: MD (95% CI): 209 (124.9, 294), P = 0.0001, compared with controls (Fig. 1B).

**Endothelial Dysfunction And Renal Cortical Purine Metabolism At 24 Hours Post-CPB**

To test the effect of CPB on endothelial responsiveness when stimulated with Ach, the relative change in cortical blood flow was measured at 24 h postintervention in control and CPB animals. The fold change response (i.e., adjusted to baseline with no Ach stimulation) in cortical blood flow in sham kidneys was significantly higher compared with the CPB group 0.62 (1.02, 0.22), P = 0.006, (Fig. 1C), suggesting that CPB had blunted the Ach-mediated, endothelial cell-driven vasodilation and hence cortical blood flow. CPB-induced renal stress was associated with significant reductions in cortical
ATP, implying significant impairment of mitochondrial function (4) along with increases in the cortical vasoconstrictor adenosine (Fig. 1D). This was associated with significant increases in cortical GTP 0.92 (0.04, 1.82), and an important source of energy for gene transcription, translation, and cytoskeletal homeostasis (44).

Transendothelial Protein and Albumin Leak

CPB led to increased proteinuria postintervention MD (95% CI): 17 (1.29, 33.4), P = 0.03 (Fig. 2A). The test for interaction treatment vs. time was not significant, P = 0.181. Changes in urine albumin levels showed wide variability and were not statistically significant (Fig. 2B).

Tissue Analysis

Glycocalyceal GAG modifications in response to renal cortical stress. To test the effect of CPB on modification of glycocalyx GAG motifs, dual stain immunofluorescence was carried out using the GAG markers DBA, WGA, SBA, and UEA lectins (Fig. 3A, green stain) and costained with the endothelial marker VE-cadherin (Fig. 3A, red stain). There was significant loss of the DBA-specific motif GalNAc: MD (95% CI), P value: −0.26 (−0.42, −0.09), P = 0.0024; the WGA-specific motif GlcNAcβ1-4GlcNAcβ1-4GlcNAc, Neu5Ac: 0.83 (−1.2, −0.38), P = 0.0005; and the UEA-specific motif Fuca1–2Gal-R: −0.25 (−0.49, −0.009), P = 0.041, of GAG.
Our findings underscore that CPB leads to significant modification of the GAG epitopes DBA-specific GalNAc (galactose-binding lectin), WGA-specific GlcNAcBl-4GlcNAcBl-4GlcNAc, Neu5Ac (glucose-binding lectin), and UEA-specific Fucα1-2Gal-R (fucose-binding lectin). These glucose- and galactose-binding lectins that we have measured in renal cortex are extremely sensitive to reactive oxygen species and other modulators of inflammation (40). Using whole blood transcriptomics from cardiac surgery patients (18) and from our translational models of CPB-mediated AKI (41), we have shown the critical role of hypoxia inducing factor-α and NF-κB activation in CPB-mediated AKI, both of which have now been implicated in glycocalyx damage (26). Interestingly, these GAG epitopes are known to provide binding sites for proinflammatory molecules such as IL-18 (9), released by macrophages and now an established post-CBP AKI biomarker (27, 48) utilized and demonstrated here to be elevated in CPB pigs. This post-CBP cellular inflammation essentially driven by MAC-387-positive macrophages has been previously characterized by our group (31, 32).

We did not observe any changes in the expression of core proteins at 24 h. Syndecan-1 core protein is an integral proteoglycan that has multiple GAGs bound to it and is a key negative regulator of endothelial cell-leucocyte interaction (11). The release of syndecan-1 is time course dependent, and as shown by us and corroborated by others (23) is resistant to shedding.

The initiation phase of AKI is characterized by impaired oxygenation and disequilibrium of ATP and GTP nucleotides (1). Our data suggest significant depletion of cortical ATP and accumulation of adenosine at 24 h post-CPB parallel to a reduction in cortical blood flow as well as creatinine clearance. Other investigators have suggested a vasoconstrictor mechanism for adenosine-led reduction in GFR (8) which has been corroborated in human studies (16).

We have observed significant proteinuria associated with loss of VE-cadherin on the background of cortical ATP depletion, a plausible observation as ATP is a strong analog to VE-cadherin in cell-cell junctions (15). Our results suggest VE-cadherin loss to be a potential determinant of proteinuria but not albuminuria.

The reduction in the expression of thrombomodulin, a key endothelial marker with significant anti-inflammatory role (45), was observed in CPB animals. Thrombomodulin expression is downregulated in the setting of sepsis and inflammation via activation of NF-κB (42). We have previously shown CPB led activation of NF-κB (41), and our current findings support downregulation of thrombomodulin as a potential mechanism attributed to CPB.

We also observed loss of endothelial vWF in CPB animals. The expression of vWF is known to be endothelial nitric oxide synthase dependent (25). A reduction in endothelial nitric oxide synthase expression in CPB-mediated AKI has been previously observed and reported by our group (30).

**Limitations**

We only performed low-grade ischemia via preferentially oxygenating swine via CPB and neither cross clamped the heart (i.e., elicited cardiac ischemia) nor performed deep hypothermic circulatory arrest (cardiopulmonary and systemic

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**Fig. 2.** Transendothelial protein and albumin leak (sham: n = 8; CPB: n = 8). A: protein/creatinine ratio (mg/mmol) measured at baseline, 1.5 h, and 24 h and assessed using repeated-measures ANOVA. *P < 0.05, CPB baseline vs. 1.5 h. B: albumin/creatinine ratio (mg/mmol) measured at baseline, 1.5 h, and 24 h and assessed using repeated-measures ANOVA; not significant.

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**DISCUSSION**

**Principal Findings**

This study reports the effects of CPB on the renal vascular endothelium, glycocalyx GAG glycosylation patterns, core proteins, biologically active surface markers, and the adherens junctional marker VE-cadherin.

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**Limitations**

We only performed low-grade ischemia via preferentially oxygenating swine via CPB and neither cross clamped the heart (i.e., elicited cardiac ischemia) nor performed deep hypothermic circulatory arrest (cardiopulmonary and systemic
ischemia). We cannot exclude that a more severe injury may produce different results. Nonetheless, CPB causes significant alterations in circulatory flow dynamics by increasing shear stress, hemodilution, and nonpulsatile flow, and it could be possible that GAG components are easily damaged with such altered flow conditions whereas core proteins (such as syndecan-1) are protected but sensitive to ischemia-reperfusion associated ROS. Similarly, the fact that core proteins have varied distributions (syndecan-1, syndecan-4, and glypican are membrane bound where as perlecan is associated with extracellular matrix, and all core proteins are associated with different GAG chains) means that they will be exposed to varied levels of a given insult. The other conundrum is that GAG and core proteins are in dynamic equilibrium (37) with each other and is

Fig. 3. A: glyocalceal glycosaminoglycan (GAG) modifications in response to renal cortical stress. Immunofluorescence: effect of CPB on coexpression of GAG component of glyocalyx: a: effect of CPB on coexpression of GAG component of glyocalyx using Dolichos biflorus agglutinin (DBA) lectin and the endothelial cell (EC) cell-cell junctional marker VE-cadherin. Green: DBA lectin; red: VE-cadherin; blue: DAPI. b: Triticum vulgaris (wheat germ) agglutinin (WGA) lectin and the EC cell-cell junctional marker VE-cadherin. Green: WGA lectin; red: VE-cadherin; blue: DAPI. c: effect of CPB on coexpression of GAG component of glyocalyx using glycine max (soybean) agglutinin (SBA) lectin and the EC cell-cell junctional marker VE-cadherin. Green: SBA lectin; red: VE-cadherin; blue: DAPI. d: effect of CPB on coexpression of GAG component of glyocalyx using Ulex europaeus agglutinin 1 (UEA) lectin and the EC cell-cell junctional marker VE-cadherin. Green: UEA lectin; red: VE-cadherin; blue: DAPI. Immunofluorescence was carried out on 5-µm frozen sections of renal cortex. Imaged at ×1100 primary objective. Twenty-four glomeruli where analyzed in each group (sham and CPB; 4 animals per group). Scale bar = 50 µm. B: densitometry for A is shown using unpaired t-test, and significance cut off was *P < 0.05 (n = 4 per group). CPB led to significant reductions in staining densities of DBA-, WGA-, and UEA-specific motifs of GAG side chains. C: assessment of colocalization of lectins (DBA, WGA, SBA, and UEA) on endothelial membrane using 5-mm frozen sections of renal cotex obtained at 24 h from sham and CPB animals (sham: n = 1; CPB: n = 1). This was assessed using TCS-NT Confocal laser scanning microscope (Leica Microsystems) to look for superimposition of lectins (green stain) and the endothelial marker VE-cadherin (red stain) using ×100 magnification. The images represents good visual colocalization (blue arrows) of all 4 lectins in the following sequence DBA > WGA > SBA > UEA.
Fig. 4. Expression of glycocalyx core proteins in response to CPB (sham: n = 4; CPB: n = 4). A-1 and A-2: Western blot analyses with densitometry. Representative Western blots for syndecan-1 (90 kDa), syndecan-4 (50 kDa), glypican (60 kDa), and perlecan (420 kDa) are shown. CPB did not effect expressions of these core proteins at 24 h. β-Tubulin (55 kDa) was used as internal control. B-1 and B-2: Expressions of von Willebrand factor (vWF) and thrombomodulin in response to CPB (sham: n = 4; CPB: n = 4); Western blot analyses with densitometry vWF (250 kDa) and thrombomodulin (105 kDa). CPB led to borderline reduction (P = 0.08) in vWF and significant reduction in the expression of thrombomodulin (P = 0.02). β-Tubulin (55 kDa) was used as internal control. Analyses were undertaken using unpaired t-test. *P < 0.05.
it unclear whether they demonstrate true loss or regeneration whether at 24 h time point.

Plasma thrombomodulin levels are known to rise after CPB (3), and our observations of loss of endothelial-bound thrombomodulin in CPB kidneys could have led to demonstrable higher plasma levels; however, we did not measure them in serum.

The results of core-protein expression cannot be interpreted as glomerular specific as they were analyzed from cortical lysates also containing epithelial cells. In addition, although we stained for core proteins using immunofluorescence but found the expression unreliable and difficult to reproduce. We purposely refrained from enzymatic degradation of GAGs to expose coreproteins, as it would have affected the stability of core proteins (47) with resultant unreliable quantification. In addition we did not measure the expression of GAGs or core proteins in the peritubular capillaries.

We did not include male swine as experimental subjects due to one-corkscrew anatomy of the male urethra with the potential of traumatic injury during transurethral catheterization (17) and used female pigs due to their lesser stress and aggression in confinement, which we believe translates into less experimental heterogeneity.

Last, this should be considered an “acute” study and no extrapolations regarding reversibility or progression of post-CPB pathogenesis can be drawn. The present model affords recovery and evaluation up to 24 h to assess the processes akin to cardiac surgical patients (i.e., AKI at 24 h post-CPB). However, using same model, we evaluated interventions that prevent kidney injury, such as endothelin-1A receptor block-

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**Fig. 5.** Effect of CPB on endothelial integrity. A-1: immunofluorescence on 5-μm frozen sections from renal cortex obtained at 24 h post-CPB and stained for the EC cell-cell junctional protein VE-cadherin (red) (sham: n = 4; CPB: n = 4). A-2: staining densitometry using unpaired t-test, CPB led to significant loss of VE-cadherin staining from glomeruli. Imaged at ×40 primary objective. *P < 0.05. Twenty-four glomeruli, 4 animals per condition where analyzed in each condition. Scale bar = 50 μm. B-1: Western blot quantification using antibodies against VE-cadherin (130 kDa) in renal cortex lysates (sham: n = 4, CPB: n = 4), adjusted to β-tubulin (55 kDa) as internal control. B-2: densitometry using unpaired t-test. CPB led to significant depletion of this cell-cell adhesion marker at 24 h (*P = 0.029).
ade (32), the administration of sildenafil citrate (30), or the use of fresh, as opposed to stored, red blood cells (29), to prevent acute lung injury.

Translation Relevance

Renoprotective interventions developed in experimental models have not been shown to translate into clinical benefits (28). This study makes two conclusions: first, these findings may point toward new biomarkers for risk stratification and diagnosis (an unmet clinical need), and second, prevention and treatment strategies should address the possible mechanisms identified in this study; all of the changes that we have observed are common to Toll-like factor/NF-κB activation. We have reported changes in NF-κB signaling in post-CPB AKI previously (41). In addition, the current findings have clinical relevance; for example, they point to the futility of forced diuresis or natriuresis as renoprotective strategies in post-surgery patients, i.e., forced diuresis could play a role in changing the fluid status of a patient but is unlikely to alter structural alterations resulting from CPB and hence neither prevents nor modifies the course of AKI (7). We propose the use of glyocalyx as a potential biomarker (13) or even microparticle-glyocalyx complexes (39) to detect early organ injury. Novel methods of glyocalyx preservation against cellular proinflammatory mediators (43) should also undergo experimental and clinical evaluation.

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DISCLOSURES

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