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Postoperative Acute Kidney Injury is Associated with Hemoglobinemia and an Enhanced Oxidative Stress Response

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Abstract

Acute kidney injury (AKI) frequently afflicts patients undergoing cardiopulmonary bypass (CPB) and independently predicts death. Both hemoglobinemia and myoglobinemia are independent predictors of postoperative AKI. Release of free hemoproteins into the circulation is known to cause oxidative injury to the kidneys. This study tested the hypothesis that postoperative AKI is associated with both enhanced intraoperative hemoprotein release and increased lipid peroxidation assessed by measuring F₂-isoprostanes and isofurans. In a case-control study, nested within an ongoing randomized trial of perioperative statin treatment and AKI, we compared levels of F₂-isoprostanes and isofurans with plasma levels of free hemoglobin and myoglobin in 10 cardiac surgery AKI patients to 10 risk-matched controls. Peak plasma free hemoglobin concentrations were significantly higher in AKI subjects (289.0±37.8 versus 104.4±36.5 mg/dl, P=0.01), whereas plasma myoglobin concentrations were similar between groups. The change in plasma F₂-isoprostane and isofuran levels (repeated measures ANOVA P=0.02 and P=0.001, respectively) as well as the change in urine isofuran levels (P=0.04) was significantly greater in AKI subjects. In addition, change in peak plasma isofurans levels correlated not only with peak free plasma hemoglobin concentrations (r²=0.39, P=0.001) but also with peak change in serum creatinine (r²=0.20, P=0.01). Postoperative AKI is associated with both enhanced intraoperative hemoprotein release and enhanced lipid peroxidation. The correlations among hemoglobinemia, lipid peroxidation, and AKI indicate a potential role of hemoprotein-induced oxidative damage in the pathogenesis of postoperative AKI.

Keywords

Acute kidney injury; oxidative stress; isoprostanes; isofurans; hemoprotein; cardiac surgery; cardiopulmonary bypass; NGAL

Acute kidney injury (AKI) complicates the postoperative course in up to 30% of all cardiac surgery patients and independently predicts in-hospital mortality, morbidity, and mid-term and long-term survival.[1–3] Renal hypoperfusion, use of cardiopulmonary bypass (CPB),

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the systemic inflammatory response syndrome, and microembolization contribute to AKI following cardiac surgery.[4–6] Other implicated risk factors include hypothermia, non-pulsatile blood flow, hemoglobinemia and myoglobinemia.[7–11]

Hemolysis, a common consequence of CPB, increases plasma concentrations of free hemoglobin and decreases circulating haptoglobin.[12] Plasma concentrations of myoglobin also increase rapidly after CPB, indicative of striated muscle injury.[9,13] In fact, plasma concentrations of free hemoglobin and myoglobin have been shown to be independent predictors of AKI following CPB.[9,11] Both free hemoglobin and myoglobin can undergo redox cycling which leads to oxidative damage.[14–16] Increased intraoperative oxidative stress may also contribute to the pathophysiology of postoperative organ dysfunction. For example, markers of oxidative stress precede inflammatory markers during surgery and are associated with postoperative pulmonary dysfunction in children undergoing CPB.[17]

Measurement of F₂-isoprostanes, products of non-cyclooxygenase free radical-induced peroxidation of arachidonic acid, has been shown to be one of the most reliable approaches to assess oxidative stress status *in vivo*. [18] One limitation with measuring F₂-isoprostanes as an indicator of oxidative stress, however, is that the formation of F₂-isoprostanes is suppressed by elevated concentrations of oxygen.[19] In contrast, the formation of products of lipid peroxidation termed isofurans is favored at elevated concentrations of oxygen.[19] Accordingly, simultaneous measurement of both F₂-isoprostanes and isofurans provides the most reliable approach to assess oxidative stress status under conditions of varying concentrations of oxygen, such as during CPB surgery. In addition, F₂-isoprostanes are potent vasoconstrictors and can contribute to organ dysfunction associated with rhabdomyolysis, subarachnoid hemorrhage and hemolytic disorders.[16,20–22] Therefore, we quantified F₂-isoprostanes, isofurans, and circulating free hemoglobin and myoglobin in patients undergoing CPB surgery to test the hypothesis that postoperative AKI following CPB is correlated with extent of heme protein release and oxidative damage.

Methods

Study Population

Data were obtained from participants in the ongoing “The effect of atorvastatin on Acute Kidney Injury following cardiac surgery” (Statin AKI) study (NCT00791648). The Statin AKI study has been approved by the Vanderbilt University Institutional Review Board for Research on Human Subjects and is conducted according to the Declaration of Helsinki. All subjects provided written informed consent. The Statin AKI study tests the hypothesis that perioperative atorvastatin reduces the incidence of AKI following elective cardiac surgery. Statin naïve subjects are randomized to treatment with placebo or atorvastatin (80mg the day prior to surgery and then 40mg daily until hospital discharge). Subjects using statin preoperatively are randomized to treatment with placebo or atorvastatin (80mg the morning of surgery and 40mg on postoperative day 1). Home statin treatment is resumed on postoperative day 2 in subjects using statins preoperatively. Exclusion criteria for the Statin AKI study include age < 18 years, current acute coronary syndrome (defined as myocardial injury with troponin-I > 0.05 ng/ml), liver dysfunction (defined as transaminases > 120 U/L, bilirubin > 3 mg/dl, or a diagnosis of cirrhosis), history of liver dysfunction (defined as above) with prior statin use, history of myopathy (defined as myalgia with concomitant creatinine kinase > 2 times upper limit of normal) with prior statin use, use of potent CYP3A4 inhibitors (azole antifungals, macrolide antibiotics, protease inhibitors, or nefazodone), current hemo- or peritoneal dialysis, history of kidney transplant, pregnancy, or use of cyclosporine. Randomization is stratified by diabetes and stage of chronic kidney disease.

The primary end point of the Statin AKI study is the occurrence of AKI. AKI is defined using Acute Kidney Injury Network (AKIN) consensus guidelines for the staging of AKI: stage 1 AKI (50% or 0.3 mg/dL increase in serum creatinine concentration within 48 hours of surgery), stage 2 AKI (100% increase in serum creatinine within 48 hours of surgery), and stage 3 AKI (200% increase in serum creatinine within 48 hours of surgery or serum creatinine > 4.0 mg/dL with an acute increase of at least 0.5 mg/dL).[23] Baseline creatinine was determined during the preoperative anesthesiology assessment clinic visit for outpatients and on the morning of surgery for inpatients. The AKIN urine output criteria for AKI diagnosis are not used due to confounding by intravascular hypovolemia and diuretic use,[24] both of which are common among cardiac surgery patients. Secondary end points include perioperative oxidant stress, use of dialysis, myocardial infarction, stroke, ICU delirium, length of ICU and hospital stay, and death.

Case and Control Subject Selection

Study subjects were selected from the first 80 subjects of the Statin AKI study. Using F₂-isoprostane data from an unrelated cardiac surgery cohort, 10 pairs of subjects are needed to detect a difference of 31 pg/ml F₂-isoprostanes or isofurans between AKI and non-AKI groups, with a within group standard deviation of 24 pg/ml, a type I error rate of 0.05, and 80% power.

Seventy-six of the first 80 Statin AKI study subjects completed the parent study, 46 of which underwent CPB, and 11 of those (23.9%) developed AKI (Figure 1). Using the Acute Kidney Injury in Cardiac Surgery (AKICS) score,[25] AKI risk was calculated for all subjects. The AKICS score predicts AKI following cardiac surgery based on baseline creatinine, age, congestive heart failure, preoperative blood glucose, combined surgery, duration of CPB, postoperative central venous pressure, and postoperative vasoactive infusion and mechanical (intra-aortic balloon counterpulsation) hemodynamic support. Ten AKI and ten non-AKI risk-matched control subjects were selected from these 46 patients. Each control subject was matched individually to an AKI case subject using the AKICS score. When the AKI risk score of more than one control candidate matched the AKI risk score of an AKI subject, the control candidate with the lowest estimated glomerular filtration rate (eGFR; calculated using the modification of diet in renal disease formula[26]) was selected. No data from case or control subjects were reviewed prior to subject selection other than AKICS score variable and AKI diagnosis data.

Patient Treatment

Anesthetic management, CPB, surgical management, and transfusion and resuscitation management were conducted according to institutional protocols. Subjects received general endotracheal anesthesia, consisting of induction with a combination of propofol, midazolam, fentanyl, or etomidate and maintenance with isoflurane, vecuronium, and fentanyl. Monitoring included standard modalities (EKG, temperature, invasive blood pressure, pulse oximetry, and gas monitoring), central venous pressure or pulmonary artery catheter monitoring, and transesophageal echocardiography (TEE). Anticoagulation for CPB was achieved by administering 400 U/kg unfractionated porcine heparin, and an activated coagulation time > 400 seconds was maintained during CPB. Temperature management involves cooling to 28°C to 30°C, temperature-uncorrected blood gas management (α stat), and cold antegrade and retrograde cardioplegia techniques. At the conclusion of CPB, anticoagulation was reversed with 250 mg protamine, and an additional 50 mg was administered during the next 10 minutes in the presence of ongoing microvascular bleeding and an activated coagulation time 10% greater than baseline.

Vasopressors and inotropes were used for separation from CPB for the following criteria: left ventricular ejection fraction <40%, CPB time >120 minutes, a cardiac index <2 L / min / m², or evidence of new-onset ventricular dysfunction by TEE. Subjects were transfused with packed red blood cells to a hemoglobin concentration target of 10 g/dL in the setting of persistent hemorrhage. Platelets were administered if hemorrhage persisted following protamine reversal of heparin, poor clot quality existed, and the absolute platelet count was <80,000, or if CPB time exceeded 120 minutes. Likewise, fresh frozen plasma was administered if hemorrhage persisted following protamine reversal of heparin, poor clot quality exists, and the INR exceeded 1.5. Six % hetastarch and isotonic saline were administered if the subject was hypotensive, cardiac filling pressures were low, and the left ventricle reflected hypovolemia by TEE.

Subjects were transported to the intensive care unit (ICU) intubated, mechanically ventilated, and sedated on propofol infusion. Postoperative care was at the discretion of the intensivist with consultation from the subject's surgeon. If subjects were normothermic and hemodynamically stable, and chest tube drainage was <100 ml/h, propofol was discontinued, and subjects were assessed for extubation. Postoperative medication use and fluid management was at the discretion of the intensive care physicians. Serum creatinine and blood urea nitrogen concentrations were determined daily until hospital discharge.

Blood was collected for measurement of free hemoglobin, myoglobin, F₂-isoprostanes, isofurans and NGAL at anesthetic induction (baseline), 30 minutes into CPB, post CPB, at ICU admission, 6 hours after ICU admission, on postoperative day 1, 2 and 3. Urine samples were collected at the same time points for measurement of F₂-isoprostanes and isofurans. All samples were collected on ice, centrifuged, and stored at -80°C within 30 minutes.

Assays

Free hemoglobin was determined using the 2-wavelength method. As previously described, [27] unhemolyzed plasma was spiked with known concentrations of purified human hemoglobin (Sigma H7379) to generate a calibration curve (0.15 g/L to 10.0 g/L). Samples and spiked EDTA plasma samples were diluted 1:2 with a potassium phosphate buffer pH 7.5, and free hemoglobin measured using the difference of absorbance between 540 and 600nm. Serum myoglobin concentrations were determined using a commercially available ELISA kit (Abnova, Taipei City, Taiwan). Free F₂-isoprostane (non-esterified) and isofuran concentrations were determined by gas chromatography-mass spectrometry as previously described.[19,28] To account for differences in renal function among subjects, urine F₂-isoprostane and isofuran concentrations were normalized to creatinine clearance ([ng/ml] × [plasma creatinine (mg/dl) / urine creatinine (mg/dl)]) and are expressed as ng/ml Cr.Cl as previously described.[15,20] Plasma NGAL was measured as an early biomarker of AKI using a commercially available ELISA assay (Bioporta Diagnostics, Gentofte, Denmark).

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM) unless otherwise indicated. Categorical data were compared between groups using Chi-squared or Fischer's exact tests, as appropriate. Continuous data were compared using Student's *t*-test or Mann-Whitney *U* test, as appropriate. Correlations were determined using Spearman's rho test.

Comparison of the oxidative stress response (F₂-isoprostanes and isofurans) between AKI and non-AKI groups was made using a general linear model-repeated measures analysis of variance (ANOVA) in which the within-subject variable was time (biomarker measured at different time points) and the between-subject variables were AKI status (AKI vs. control) and preoperative statin exposure (statin-using vs. statin-naive). General linear model-

repeated measures repeated measures ANOVA analyzes groups of related dependent variables that represent different measurements of the same attribute by one or more factors and/or variables. Prior statin exposure was included as a between-subject variable because statin use may impact AKI,[2] and in addition, statin treatment has been shown to reduce markers of oxidative stress.[29] Because baseline F₂-isoprostane and isofuran concentrations tended to be higher in the non-AKI control group, we calculated the percentage F₂-isoprostane and isofurans change from baseline in order to compare oxidative stress between AKI and control groups. A 2-tailed P value less than 0.05 was considered statistically significant. Statistical analyses were performed with the statistical package SPSS for Windows (Version 17.0, SPSS, Chicago, IL).

Results

Patient characteristics

Case and control subject selection resulted in 2 groups with similar risk for developing AKI. There were no significant differences in the following risk factors: age, gender, race, creatinine, eGFR, and diabetes (Table 1). Body mass index, history of current smoking, diagnosis of hypertension, atrial fibrillation, and chronic obstructive pulmonary disease, use of β -blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, and statins, were also similar between the two groups. Baseline blood glucose tended to be higher in the AKI group compared to the control group. The type of surgery, duration of CPB, duration of aortic cross-clamp time, mean arterial pressure following CPB, exposure to exogenous blood products, blood loss, surgical re-exploration, use of inotropes, and creatinine at discharge were not significantly different between the two groups (Table 2). Central venous pressure on POD1 tended to be higher in the AKI group.

Hemeprotein Release

Plasma free hemoglobin increased significantly during CPB (from 1.3 ± 1.3 mg/dl at baseline to a peak of 196.7 ± 33.2 mg/dl post CPB; ANOVA, $P < 0.001$) and returned to baseline by POD1. Peak free hemoglobin concentrations were significantly higher in the AKI group as compared to control group (289.0 ± 37.8 versus 104.4 ± 36.5 mg/dl respectively, $P = 0.01$). Serum myoglobin concentrations also increased significantly during surgery (from 59.9 ± 9.1 μ g/L at baseline to a peak of 689.4 ± 116.7 μ g/L upon arrival in the ICU, $P < 0.001$) and remained elevated through the first 3 postoperative days. Myoglobin concentrations were not significantly different between AKI and control groups ($P = 0.21$). The molar ratio between peak hemoglobin and myoglobin was 821, implicating free hemoglobin as the dominant contributor to circulating hemeproteins during CPB surgery.

Oxidative Stress Response

Plasma concentrations of both F₂-isoprostanes and isofurans increased significantly during CPB and returned to baseline on POD1 (ANOVA $P = 0.01$ F₂-isoprostanes; $P = 0.003$ isofurans; Figure 2 **Panel A**). The magnitude of increase, measured by the percentage change from baseline, was significantly greater for plasma isofurans compared to plasma F₂-isoprostanes (ANOVA $P = 0.02$, Figure 2 **Panel B**). This is consistent with cardiac surgery patient care, in which blood is highly oxygenated during CPB, and patients receive supplemental oxygen after surgery. Urine F₂-isoprostane and isofuran concentrations increased significantly after surgery (ANOVA $P = 0.001$ F₂-isoprostanes; $P = 0.008$ isofurans; Figure 3 **Panel A**). Similar to the plasma biomarkers, the magnitude of increase was significantly greater for urine isofurans compared to urine F₂-isoprostanes (ANOVA $P = 0.01$, Figure 3 **Panel B**). Furthermore, the magnitude of increase of urine isofurans was significantly greater compared to plasma isofurans (ANOVA $P = 0.02$).

Baseline plasma F₂-isoprostane (63.3±2.0 vs. 43.1±4.6 pg/ml; P=0.001), urine F₂-isoprostane (3.6±0.8 vs. 2.0±0.3 ng/ml Cr.Cl; P=0.04), and urine isofuran (4.2±0.5 vs. 2.5±0.3 ng/ml Cr.Cl; P=0.048) concentrations were significantly higher in active smokers, all of whom were in the control group, as compared to non-smokers. These data are consistent with previous reports of increased concentrations of F₂-isoprostanes in smokers[30] and may account for the increased baseline urine and plasma markers of oxidative stress in the non-AKI control group (Table 3). Consequently, to compare oxidative stress between the AKI and control group and account for differences in baseline biomarkers, we calculated the percentage change from baseline for F₂-isoprostanes and isofurans.

Plasma F₂-isoprostane (Figure 4 **Panel A**) and isofuran (Figure 4 **Panel B**) concentrations increased significantly more during surgery among subjects subsequently diagnosed with AKI than among non-AKI risk matched control subjects (ANOVA P=0.02 F₂-isoprostanes and P=0.001 isofurans). The change in urine F₂-isoprostane concentrations tended to be higher in the AKI group (P=0.08; Figure 5 **Panel A**). Although the early increase in urine isofuran concentrations was similar between study groups (P=0.97) there was a sharp divergence in response following surgery such that urine isofuran concentrations continued to rise, peaked on postoperative day 1, and remained elevated on postoperative day 2 in the AKI group (P=0.04; Figure 5 **Panel B**). In addition, the change in peak plasma isofuran concentrations correlated not only with peak free hemoglobin concentrations (r²=0.39, P=0.001; Figure 6 **Panel A**) but also with the peak change in creatinine (r²=0.20, P=0.01; Figure 6 **Panel B**).

Since preoperative inhibition of the renin-angiotensin system may affect the risk of postoperative AKI, we also evaluated the impact of ACE inhibition or angiotensin receptor blockade on the oxidative stress response.[31] Neither preoperative ACE inhibitor nor angiotensin receptor blocker treatment affected the plasma isofuran response (P=0.33 and P=0.17, respectively), and the plasma isofuran response remained significantly greater in AKI subjects (P<0.04) even after controlling for potential confounding by ACE inhibitor or angiotensin receptor blocker use. Mock-unblinding of the study drug assignment from the parent trial (drug A = placebo or atorvastatin and drug B = placebo or atorvastatin) and comparison of free hemoglobin, myoglobin, F₂-isoprostane, and isofuran concentrations between subjects randomized to drug A versus drug B did not reveal any significant effect of study drug on these measurements.

Markers of Acute Kidney Injury

Plasma NGAL concentrations increased 2-fold (from 92.2±6.5 ng/ml at baseline to a peak of 184.7±33.7 post CPB, P=0.01) and remained elevated throughout the first 3 postoperative days. Baseline NGAL concentrations were similar between AKI and control groups (93.1±6.7 vs. 91.2±11.4 ng/ml, P=0.65). There was a trend towards increased NGAL concentrations at 6 hours after ICU arrival among AKI subjects (162.9±15.9 vs. 121.1±28.5 ng/ml, P=0.08), and NGAL concentrations 6 hours after ICU admission correlated with peak free hemoglobin (r²=0.28, P=0.02), peak myoglobin (r²=0.17, P=0.045), peak postoperative creatinine (r²=0.34, P=0.001) but not with concentrations of F₂-isoprostanes or isofurans.

Discussion

This study examined the hemeprotein and oxidative stress response of adult patients undergoing CPB surgery that did and did not develop postoperative AKI. CPB was associated with hemoglobinemia, myoglobinemia, and oxidative stress. Subjects who developed AKI had significantly greater hemoglobinemia and oxidative stress compared to controls. In addition, the correlations among free hemoglobin, markers of oxidative stress,

and AKI suggest that free hemoglobin may contribute to the oxidative stress during CPB surgery and subsequent kidney injury.

Our observation that CPB generates an oxidant stress is consistent with prior studies.[17,32] This is the first study, however, to measure both F₂-isoprostanes and isofurans during surgery. The increase in isofuran concentrations from baseline was considerably greater than the increase in F₂-isoprostane concentrations. This can be explained by the fact that the formation of isofurans is favored by elevated oxygen tensions. Free radical-induced lipid peroxidation generates F₂-isoprostanes and isofurans from the same lipid intermediate, and formation of one is mutually exclusive of formation of the other. As oxygen tension increases, the lipid radical intermediate preferentially reacts with molecular oxygen to generate isofurans. In conditions of lower oxygen tension, the radical intermediate undergoes exocyclization, and F₂-isoprostanes are formed.[33] The high oxygen tensions that typically accompany cardiac surgery and CPB would favor the formation of isofurans. Oxygen delivery and organ perfusion can vary considerably during surgery and in the early postoperative period. Accordingly, both F₂-isoprostane and isofuran concentrations should be measured simultaneously in cardiac surgery patients to obtain an accurate assessment of the extent of lipid peroxidation.

The mechanisms that generate intraoperative oxidative stress and contribute to the pathophysiology of postoperative AKI are incompletely understood and are likely multifactorial. Isoprostanes and isofurans are generated wherever free radicals are generated. An important concept is that both are initially formed predominantly esterified in phospholipids because the vast majority of the arachidonic acid they are formed from is esterified in phospholipids. Subsequently, they are hydrolyzed from phospholipids, appear in their free form, and may be measured in plasma.[33] Following oxidative damage in peripheral tissues isoprostanes and isofurans are hydrolyzed from phospholipids in peripheral tissues, and appear in the free form in the circulation and subsequently, in the urine following glomerular filtration. With this scenario, the relative increase in levels of both isoprostanes and isofurans in the circulation and urine is similar. However, when there is also oxidative damage in the kidney itself, the additional amount of isoprostanes and isofurans formed in the kidney are excreted directly into the urine.[34]. Thus, when there is both systemic and renal oxidative injury, the relative increase in levels of isoprostanes and isofurans measured in the urine are higher than the relative increase in levels measured in the circulation. Our finding of increased lipid peroxidation in the urine compared to plasma therefore supports renal oxidative injury. The elevated and persistent levels of isofurans in the urine of AKI subjects may be explained by increased oxidative stress in the presence of dysfunctional renal mitochondria, a feature of ischemia-reperfusion and hemeprotein-induced renal injury.[35,36] Theoretically, when mitochondria are dysfunctional, cellular oxygen tension is increased due to diminished mitochondrial oxygen consumption. This elevated oxygen environment favors the formation of isofurans during conditions of oxidative stress.

Hemeproteins can injure renal cells through various mechanisms including lipid peroxidation, vasoconstriction, and cast formation.[35] Despite the implication of oxidative stress in the pathogenesis of AKI in animal models,[20,37,38] evidence for oxidative stress-mediated AKI in patients following CPB remains limited. No antioxidant therapies (N-acetylcysteine, Vitamin E, or mannitol) have reduced the risk of AKI in patients undergoing cardiac or major aortic surgery.[39] Questions remain, however, about proper antioxidant dosing and duration of treatment. For example, with vitamin E treatment, high doses (~1600 I.U./day) are required for several months to significantly reduce markers of lipid peroxidation in humans.[40] Haptoglobin, a scavenger of plasma free hemoglobin, could potentially reduce lipid peroxidation by removing free hemoglobin from the circulation.[41]

However, plasma haptoglobin levels fall below detectable concentrations rapidly after onset of CPB.[42,43] The administration of haptoglobin during CPB is a potential strategy to prevent hemeprotein-induced lipid peroxidation and renal injury but remains largely experimental.[43]

We found increased levels of circulating free hemeproteins and increased lipid peroxidation in patients who developed AKI and correlations between levels of free hemoglobin, the extent of lipid peroxidation, and postoperative increases in levels of serum creatinine. These findings suggest that hemeprotein-induced oxidative injury may contribute to postoperative AKI. Further evidence supporting a role of hemeproteins in the pathogenesis of postoperative AKI is suggested by the finding that increased free hemoglobin and myoglobin both independently predict postoperative AKI,[9,11] and free hemoglobin and myoglobin contribute to hemeprotein redox cycling induced oxidative damage and renal failure in animal models.[14–16,20] We recently showed that acetaminophen is a potent hemoprotein reductant *in vitro* and that treatment of rats with rhabdomyolysis with acetaminophen markedly decreased lipid peroxidation in the kidney and protected against renal failure at plasma concentrations that were within the normal human therapeutic range. [20] This finding provides the rationale for further studies to assess whether treatment of humans undergoing CPB surgery with acetaminophen might be effective in diminishing the incidence and/or severity of AKI.

In addition to measuring changes in postoperative creatinine, we also measured plasma NGAL concentrations as an early marker of AKI. NGAL is rapidly induced in renal tubular epithelium following injury, and NGAL:siderophore:iron complexes may constitute a physiological renoprotective mechanism.[44] Urinary NGAL has been found to be a highly sensitive and specific marker of AKI as early as 2 hours after pediatric cardiac surgery.[45] In adults, however, plasma NGAL measured immediately after CPB has poor sensitivity and specificity for AKI diagnosis and a positive predictive value of only 16.3%.[46] This may explain in part our finding of only a modest increase in plasma NGAL concentrations measured early after CPB in AKI subjects but could also reflect the fact that this study was not powered to detect a difference in plasma NGAL concentrations.

Study Limitations

Despite careful matching of subjects based on the AKICS score for the prediction of AKI following cardiac surgery, we observed higher baseline markers of oxidative stress in control subjects compared to AKI subjects. The exclusive prevalence of active smokers in the control group most likely accounted for the higher baseline markers of oxidative stress in control subjects and is consistent with the finding that smoking increases oxidative stress. [30] Consequently, to adjust for baseline differences in oxidative stress markers and compare oxidative stress between AKI and control groups, we analyzed differences in change from baseline of oxidative stress markers. In addition, the relatively small sample size may limit the generalizability of our study findings. Also, while not statistically significant, more subjects in the AKI group were taking an ACE inhibitor or angiotensin receptor blocker. Preoperative use of ACE inhibitors and angiotensin receptor blockers has been associated with an increased risk of postoperative AKI in some but not in other studies. [1,31,47] Prior studies have indicated that ACE inhibition enhances the bradykinin response during CPB.[48] Moreover, bradykinin increases oxidative stress and worsens ischemia/reperfusion renal injury in an animal model.[49] Thus, ACE inhibition, through an increase in bradykinin concentrations, could increase oxidative stress and contribute to AKI. Although preoperative ACE inhibitor use did not affect the oxidative stress response we cannot exclude a potential contribution of enhanced bradykinin concentrations to the oxidative stress response during CPB. Whether and how increased lipid peroxidation is involved in postoperative AKI remains to be elucidated and it is possible that our findings

represent a surrogate marker for the actual pathophysiological events that lead to AKI. Reducing CPB-induced hemolysis and hemoglobinemia (by improving circuit design, the use of centrifugal pumps[42] or the administration of haptoglobin[43]) may be a more important therapeutic goal than preventing lipid peroxidation. However, despite improvements in the CPB machine, hemolysis still occurs and therefore alternative strategies are needed to attenuate hemeprotein-induced lipid peroxidation and potential renal injury.

In summary, our study findings indicate that 1) plasma and urine concentrations of isofurans and to a lesser extent F₂-isoprostanes increase significantly during and after CPB, 2) CPB surgery is associated with both hemolysis and rhabdomyolysis but greater amounts of hemoglobin are released compared to myoglobin, 3) hemoglobinemia is associated with increased plasma isofurans 4) patients who develop AKI have greater hemoglobinemia and oxidative stress compared to controls and 5) the correlations among hemoglobinemia, oxidative stress, and kidney injury indicate a potential role of hemeprotein-induced oxidative injury in the pathogenesis of postoperative AKI. Future studies are needed to determine the predictive value of elevated isofurans in AKI and whether pharmacological inhibition of hemeprotein-induced oxidative stress will attenuate oxidative damage and preserve renal function in humans.

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List of Abbreviations

ACE	angiotensin-converting enzyme
AKI	acute kidney injury
CPB	cardiopulmonary bypass
GFR	glomerular filtration rate
ICU	intensive care unit
NGAL	neutrophil gelatinase-associated lipocalin
POD	postoperative day

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Figure 1.
Case and Control Subject Selection

Figure 2.

Oxidative stress response in **A**) all subjects as measured by plasma F₂-isoprostane (F₂-isoP) and isofuran (IsoF) concentrations and **B**) as a percentage change from baseline. Base, baseline; 30min, 30min of cardiopulmonary bypass (CPB); post, post CPB; ICU, intensive care unit; 6hrs, 6 hours after arrival in ICU; POD, postoperative day. Plasma concentrations of both F₂-isoprostanes and isofurans increased significantly during surgery (ANOVA P=0.01 isoprostanes; P=0.003 isofurans). The percentage change from baseline was significantly greater for isofurans compared to F₂-isoprostanes (ANOVA P=0.02 for effect of biomarker). *P<0.05, †P<0.01 versus baseline. §P<0.01 versus F₂-isoprostanes.

Figure 3.

Oxidative stress response in **A**) all subjects as measured by urine F₂-isoprostane (F₂-isoP) and isofuran (IsoF) concentrations and **B**) as a percentage change from baseline. Base, baseline; 30min, 30min of cardiopulmonary bypass (CPB); post, post CPB; ICU, intensive care unit; 6hrs, 6 hours after arrival in ICU; POD, postoperative day. Urine concentrations of both F₂-isoprostanes and isofurans increased significantly after surgery (ANOVA $P=0.001$ isoprostanes; $P=0.008$ isofurans). The percentage change from baseline was significantly greater for isofurans compared to F₂-isoprostanes (ANOVA $P=0.01$ for effect of biomarker). * $P<0.05$, † $P<0.01$ versus baseline. ‡ $P<0.05$ versus isofurans, § $P<0.01$ versus F₂-isoprostanes.

Figure 4.

Plasma oxidative stress response as measured by percentage change from baseline for **A)** plasma F₂-isoprostanes (F₂isoP) and **B)** plasma isofurans (isoF) in subjects who developed postoperative acute kidney injury (AKI) compared to controls. Base, baseline; 30min, 30min of cardiopulmonary bypass (CPB); post, post CPB; ICU, intensive care unit; 6hrs, 6 hours after arrival in ICU; POD, postoperative day. Plasma F₂-isoprostane and isofuran concentrations increased significantly more during and following surgery in subjects who developed AKI (ANOVA P=0.02 and P=0.001, respectively). *P<0.05 versus controls.

Figure 5. Urine oxidative stress response as measured by percentage change from baseline for **A**) urine F₂-isoprostanes (F₂isoP) and **B**) urine isofurans (isoF) in subjects who developed postoperative acute kidney injury (AKI) compared to controls. Base, baseline; 30min, 30min of cardiopulmonary bypass (CPB); post, post CPB; ICU, intensive care unit; 6hrs, 6 hours after arrival in ICU; POD, postoperative day. Urine isofuran concentrations continued to rise and remain elevated in AKI subjects as compared to non-AKI control subjects (ANOVA P=0.04). *P<0.05 versus controls.

Figure 6.

Correlations between **A**) percentage change from baseline in peak plasma isofurans vs. peak plasma free hemoglobin and **B**) percentage change from baseline in peak plasma isofurans vs. percentage change from baseline in peak creatinine. ● acute kidney injury (AKI), ○ control.

Table 1**Baseline Subject Characteristics**

Characteristic	Control (N=10)	AKI (N=10)	P-value
Age (years)	65.7±3.9	69.9±4.2	0.33
Gender, women, N (%)	3 (30)	4 (40)	1.0
Race, Caucasian, N (%)	10 (100)	10 (100)	
Body Mass Index (kg/m ²)	26.5±1.4	29.2±1.5	0.15
Medical History, N (%)			
Current Smoker	3 (30)	0 (0)	0.21
Hypertension	9 (90)	9 (90)	1.0
Diabetes	1 (10)	3 (30)	0.58
Atrial fibrillation	6 (60)	5 (50)	1.0
COPD	2 (20)	1 (10)	1.0
Preoperative medications, N (%)			
β-Blocker	7 (70)	5 (50)	0.65
ACEI	1 (10)	4 (40)	0.30
Angiotensin receptor blocker	2 (20)	3 (30)	1.0
Statin (pre-randomization)	4 (40)	4 (40)	1.0
Preoperative laboratory data			
Creatinine (mg/dl)	1.20±0.12	1.23±0.13	0.76
eGFR (ml/min/m ²)	59.0±6.2	61.3±9.6	0.76
Glucose (mg/dl)	93.5±2.8	114.6±9.4	0.09
LV ejection fraction (%)	51.0±3.6	53.0±3.2	0.81

COPD, chronic obstructive pulmonary disease; ACEI, angiotensin-converting enzyme inhibitor; eGFR, estimated glomerular filtration rate; LV, left ventricle.

Table 2**Intra- and Postoperative Subject Characteristics**

Characteristic	Control (N=10)	AKI (N=10)	P-value
Type of Surgery			
CABG surgery only, N (%)	1 (10)	1 (10)	1.0
Valve surgery only, N (%)	6 (60)	7 (70)	1.0
CABG and Valve surgery, N (%)	3 (30)	2 (20)	1.0
Cardiopulmonary bypass time, min	154±17	150±20	0.89
Aorta cross-clamp time, min	113±18	118±12	0.84
Mean blood pressure, intraop, mmHg [*]	72±3	68±2	0.31
Intraoperative blood product transfusion			
Packed red blood cells, N (%)	5 (50)	7 (70)	0.65
Fresh frozen plasma, N (%)	3 (30)	6 (60)	0.37
Platelets, N (%)	6 (60)	4 (40)	0.66
Cryoprecipitate, N (%)	0 (0)	1 (10)	1.0
Chest tube output first 4hrs ICU (mL)	414±146	254±46	0.21
Chest tube output first 24hrs ICU (mL)	1047±233	688±83	0.23
Surgical Re-exploration, N (%)	2 (20)	1 (10)	1.0
Two or more inotropes on POD1, N (%) [†]	1 (10)	4 (40)	0.30
Central venous pressure on POD1, mmHg	11.8±1.4	14.9±1.1	0.09
Creatinine at discharge (mg/dl)	1.17±0.11	1.51±0.23	0.20

^{*} Following cardiopulmonary bypass.

[†] Inotropes include epinephrine, norepinephrine, milrinone, dobutamine, and dopamine. CABG, coronary artery bypass grafting; POD1, postoperative day 1; ICU, intensive care unit.

Table 3Baseline plasma and urine F₂-isoprostane and isofuran concentrations

Biomarker	Control (N=10)	AKI (N=10)	P-value
Plasma (pg/ml)			
F ₂ -isoprostanes	52.5±6.5	39.9±4.9	0.14
Isofurans	37.7±5.7	27.4±5.4	0.07
Urine (ng/ml Cr.Cl)			
F ₂ -isoprostanes	2.9±0.4	1.6±0.3	0.01
Isofurans	3.3±0.5	2.2±0.3	0.08