Acetaminophen Attenuates Lipid Peroxidation in Children Undergoing Cardiopulmonary Bypass

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Objective: Hemolysis, occurring during cardiopulmonary bypass, is associated with lipid peroxidation and postoperative acute kidney injury. Acetaminophen inhibits lipid peroxidation catalyzed by hemeproteins and in an animal model attenuated rhabdomyolysis-induced acute kidney injury. This pilot study tests the hypothesis that acetaminophen attenuates lipid peroxidation in children undergoing cardiopulmonary bypass.

Design: Single-center prospective randomized double-blinded study.

Setting: University-affiliated pediatric hospital.

Patients: Thirty children undergoing elective surgical correction of a congenital heart defect.

Interventions: Patients were randomized to acetaminophen (OFIRMEV [acetaminophen] injection; Cadence Pharmaceuticals, San Diego, CA) or placebo every 6 hours for four doses starting before the onset of cardiopulmonary bypass.

Measurement and Main Results: Markers of hemolysis, lipid peroxidation (isofurans and F_2-isoprostanes), and acute kidney injury were measured throughout the perioperative period. Cardiopulmonary bypass was associated with a significant increase in free hemoglobin (from a prebypass level of 9.8 ± 6.2 mg/dL to a peak of 201.5 ± 42.6 mg/dL postbypass). Plasma and urine isofuran and F_2-isoprostane concentrations increased significantly during surgery. The magnitude of increase in plasma isofurans was greater than the magnitude in increase in plasma F_2-isoprostanes. Acetaminophen attenuated the increase in plasma isofurans compared with placebo ($p = 0.02$ for effect of study drug). There was no significant effect of acetaminophen on plasma F_2-isoprostanes or urinary makers of lipid peroxidation. Acetaminophen did not affect postoperative creatinine, urinary neutrophil gelatinase-associated lipocalin, or prevalence of acute kidney injury.

Conclusion: Cardiopulmonary bypass in children is associated with hemolysis and lipid peroxidation. Acetaminophen attenuated the increase in plasma isofuran concentrations. Future studies are needed to establish whether other therapies that attenuate or prevent the effects of free hemoglobin result in more effective inhibition of lipid peroxidation in patients undergoing cardiopulmonary bypass. (*Pediatr Crit Care Med* 2014; XX:00–00)

Key Words: acetaminophen; acute kidney injury; cardiac surgery; cardiopulmonary bypass; hemoglobin; lipid peroxidation; pediatrics

Hemolysis frequently occurs during cardiopulmonary bypass (CPB) and leads to an increase in free hemoglobin (1, 2). The adverse effects of free hemoglobin are countered by haptoglobin that binds free hemoglobin to form a complex that is degraded by hemo-oxygenase-1 (3). Evidence that free hemoglobin contributes to postoperative acute kidney injury (AKI) is suggested by the association between hemolysis and postoperative AKI (1, 4, 5), the nephrotoxic effects of hemoglobin-based oxygen carriers (6), and the protective effect of haptoglobin on renal function (7–9). Furthermore, corticosteroids can induce haptoglobin synthesis and prevent complications associated with cell-free hemoglobin (8). Although the mechanism(s) whereby free hemoglobin causes AKI is still debated, we have previously demonstrated that hemoglobinemia is associated with lipid peroxidation and that patients who

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develop postoperative AKI have greater hemoglobinemia and lipid peroxidation compared with controls (1). These findings suggest a potential role of hemeprotein-mediated lipid peroxidation in the pathogenesis of postoperative AKI.

OFIRMEV (acetaminophen, ApAP) injection (Cadence Pharmaceuticals, San Diego, CA) was approved by the U.S. Food and Drug Administration in November 2010 for the treatment of acute pain and fever in children and adults and is used in patients undergoing cardiac surgery as part of a multimodal pain management regimen (10, 11). ApAP acts as a ferryl protein reductant, thus inhibiting hemeprotein-catalyzed lipid peroxidation (12, 13). ApAP inhibits oxidation of arachidonic acid catalyzed by hemoglobin with a half maximal inhibitory concentration of 17.7 ± 2.5 μM (13), which is well within the therapeutic range for humans (5–30 μg/mL; 33–200 μM). In an animal model of rhabdomyolysis-induced kidney injury, ApAP significantly decreased markers of lipid peroxidation and protected kidney function (13). Furthermore, an observational study in critically ill patients suggests that receiving ApAP in the setting of increased free hemoglobin was independently associated with a protective effect against death and lower plasma concentrations of F_2-isoprostanes (14). This pilot study tests the hypothesis that ApAP attenuates lipid peroxidation in pediatric patients undergoing CPB.

METHODS

Thirty children (15 boys and 15 girls with an age range of 2–144 mo) participated in the study (ClinicalTrials.gov Identifier: NCT01228305). This study was approved by the Vanderbilt University Institutional Review Board for Research on Human Patients and conducted according to the Declaration of Helsinki. A legal guardian of all patients provided written informed consent, and patients older than 7 years additionally signed an assent form. Patients were eligible for the study if they were 1) less than 18 years and 2) undergoing elective cardiac surgery requiring CPB for surgical correction of their congenital heart lesion. Patients were excluded for the following reasons: 1) patients with single-ventricle physiology because these patients undergo procedures that frequently require deep hypothermic circulatory arrest (DHCA) that results in global reperfusion oxidant injury, 2) inability of the patient's legal guardian to understand the nature, scope, and possible consequences of the study, 3) severe neurological abnormalities at baseline, 4) weight less than 3 kg, 5) inability of the patient to comply with the protocol, that is, children in whom it was deemed unsafe to have the extra blood draws, 6) patients with major noncardiac congenital malformations, developmental disorders, or serious chronic disorders, 7) previous adverse reaction to ApAP, 8) history of chronic liver disease, and 9) chronic renal insufficiency.

Protocol

Patients were randomly assigned by the investigational pharmacy to treatment (IV ApAP or matching IV placebo). IV ApAP (OFIRMEV injection; Cadence Pharmaceuticals) was given at a standard dose of 15 mg/kg IV for children 2 years old or older and 12.5 mg/kg IV for children 29 days to less than 2 years (OFIRMEV prescriber information). Study drug was given every 6 hours, with the first dose in the operating room and before the onset of CPB, for a total of four doses over a 24-hour study period. Drug administration was double blind. The study drug was prepared and labeled by the investigational pharmacy in an identical fashion. Unblinding of study drug only occurred after completion of the statistical analyses. ApAP plasma concentrations were measured after CPB to assess plasma concentrations achieved during peak hemolysis only after completion of the study and unblinding occurred. Although the patient was receiving study drug (24-hour period), no open-label ApAP was administered.

Anesthesia and CPB

Anesthesia and CPB protocols have been previously described (15). Aminocaproic acid (antifibrinolytic), methylprednisolone, and dexmedetomidine (α-2 agonist) were given intraoperatively at the discretion of the attending anesthesiologist. Briefly, the circuit was primed with a mixture of albumin and plasmalyte-A. Packed RBCs (PRBC) were added to the prime taking into account the patients' age, weight, preoperative hematocrit, and surgical procedure. Plasma was added to the prime for patients less than 10 kg. After prebypass ultrafiltration of this mixture, mannitol, sodium bicarbonate, calcium chloride, and heparin were added to the washed circuit prime in doses adjusted for body surface area. Aortic cross-clamping, with administration of cold high potassium blood cardioplegia solution, was delivered at 20-minute intervals for myocardial protection in all patients. DHCA was not performed. Modified ultrafiltration (MUF), with a pediatric hemoconcentrator (Terumo Cardiovascular Systems Corporation, Ann Arbor, MI), was performed after CPB in all patients.

Blood Sampling and Biochemical Assays

Blood samples were obtained for measurement of free hemoglobin, haptoglobin, F_2-isoprostanes, and isofurans. F_2-isoprostanes and isofurans are sensitive and specific markers of lipid peroxidation in vivo (13, 16, 17) and are increased after CPB (1, 18). Measurement of both isofuran and F_2-isoprostane concentrations provides the most reliable approach to assess oxidative stress status under conditions of varying concentrations of oxygen because the formation of F_2-isoprostanes is suppressed by elevated concentrations of oxygen (17). All blood samples were collected on ice and centrifuged immediately at 0°C for 20 minutes. Plasma was then separated and stored at –80°C until the time of assay. Urine samples were obtained for measurement of F_2-isoprostanes, isofurans, and neutrophil gelatinase-associated lipocalin (NGAL), a marker of AKI. Samples were collected at four time points: 1) after induction of anesthesia, prior to CPB, and administration of study drug (prebypass); 2) following 30 minutes of CPB; 3) after protamine administration (postbypass); and 4) 24 hours after administration of first ApAP dose on postoperative day 1 (POD1). Free hemoglobin was determined using the two-wavelength methods previously described (19). Haptoglobin concentrations were measured using a commercially

available enzyme-linked immunosorbent assay (ELISA) kit (Abcam, Cambridge, MA) according to the manufacturer's instructions. Free F_2-isoprostane (nonesterified) and isofuran concentrations were determined by gas chromatography-mass spectrometry as previously described (17, 20). To account for differences in renal function among subjects, urine F_2-isoprostane and isofuran concentrations were normalized to creatinine clearance ([pg/mL] × [plasma creatinine (mg/dL)/urine creatinine (mg/dL)]) and are expressed as pg/mL Cr.Cl as previously described (13, 21). Urine NGAL was measured using a commercially available ELISA assay (Bioporta Diagnostics, Gentofte, Denmark). ApAP concentrations were quantified with the Roche COBAS INTEGRA 800 System analyzer (Roche Diagnostics Corporation, Indianapolis, IN).

Clinical Data

Clinical outcome data collected included urine output, blood loss (chest tube drainage), blood products transfused, need for surgical re-exploration, time to tracheal extubation, and prevalence of AKI. The definition of AKI was based on the Acute Kidney Injury Network consensus guidelines for the staging of AKI: stage 1 AKI, 0.3 mg/dL increase in serum creatinine concentration or increase to more than or equal to 1.5- to 2-fold from baseline; stage 2 AKI, increase in serum creatinine more than two- to three-fold from baseline; and stage 3 AKI, increase in serum creatinine more than three-fold from baseline. Any patient in whom AKI stage 1 or more developed within 72 hours was defined as having postoperative AKI (22, 23).

TABLE 1. Preoperative Patient Characteristics

Variable	Placebo ($n = 15$)	Acetaminophen ($n = 15$)	p
Age (mo)	34.1 ± 8.9	33.1 ± 9.2	0.97[a]
Gender, male, *n* (%)	7 (46.7)	8 (53.3)	0.72[b]
Race, white, *n* (%)	11 (73.3)	13 (86.7)	0.51[b]
Weight (kg)	15.9 ± 3.8	14.5 ± 2.6	0.84[a]
Weight (percentile for age)	41.4 ± 10.2	35.5 ± 6.7	0.71[a]
Mean arterial pressure (mm Hg)	75.4 ± 3.6	72.7 ± 2.4	0.55[c]
Heart rate (beats/min)	104.7 ± 6.1	110.2 ± 6.1	0.52[c]
Pulse oximetry saturation (%)	98 ± 1.0	98.0 ± 0.7	0.52[a]
Hematocrit (%)	35.7 ± 0.9	38.5 ± 0.7	0.046[a]
Platelet count (k/μL)	345.4 ± 25.7	301.3 ± 13.7	0.23[a]
Creatinine (mg/dL)	0.32 ± 0.02	0.33 ± 0.03	0.69[c]
Potassium (mEq/L)	4.5 ± 0.19	4.9 ± 0.15	0.20[c]
Prior cardiac surgery, *n* (%)	1 (6.7)	1 (6.7)	1.0[d]
Preoperative medications, *n* (%)			
Digoxin	3 (20)	1 (6.7)	0.60[d]
Diuretic	4 (26.7)	1 (6.7)	0.33[d]
Congenital heart defect, *n* (%)			0.67[b]
Atrial septal defect	6 (40)	7 (46.7)	
Ventricular septal defect	4 (26.7)	2 (13.3)	
Tetralogy of Fallot	3 (20)	5 (33.3)	
Other	2 (13.3)	1 (6.7)	
Risk adjusted congenital heart surgery score, *n* (%)			0.52[b]
Category 1	6 (40)	7 (46.7)	
Category 2	9 (60)	7 (46.7)	
Category 3	0	1 (6.7)	

[a]Mann-Whitney *U* test.
[b]Pearson chi-square test.
[c]*t* test.
[d]Fisher exact test.

Statistical Analysis

Data are presented as means ± SEM unless otherwise indicated. Sample size calculations were based on preliminary data of markers of lipid peroxidation in adults and assuming ApAP will reduce these markers by 40%. With these assumptions, a sample size of 14 in each group had 90% power to detect a difference in means of 23 using a two group Satterthwaite *t* test with a 0.05 two-sided significance level. Categorical data were compared between groups using chi-square or Fisher exact test, as appropriate. Continuous baseline data were compared using Student *t* test or Mann-Whitney *U* test, as appropriate. Comparison of free hemoglobin, haptoglobin, and markers of lipid peroxidation between groups (placebo group vs ApAP group) was made using a general linear model-repeated measures analysis of variance in which the within-patient variable was time and the between-patient variable was study drug. Biomarkers that were not normally distributed were log-transformed prior to analysis. A two-tailed *p* value of less than 0.05 was considered statistically significant. Statistical analyses were performed with the statistical package SPSS for Windows (Version 21.0; IBM, New York, NY).

TABLE 2. Intraoperative and Postoperative Patient Characteristics

Variable	Placebo (*n* = 15)	Acetaminophen (*n* = 15)	*p*
PRBC in pump prime, *n* (%)	13 (86.7)	13 (86.7)	1.0[a]
Age of PRBC used in pump prime (d)	7.4 ± 1.2	8.9 ± 1.4	0.48[b]
Fresh-frozen plasma in pump prime, *n* (%)	7 (46.7)	6 (40.0)	0.71[c]
Cardiopulmonary bypass time (min)	77.1 ± 7.9	80.2 ± 13.0	0.53[b]
Cross-clamp time (min)	44.7 ± 7.6	33.8 ± 6.5	0.25[b]
Aminocaproic acid, *n* (%)	2 (13.3)	1 (6.7)	1.0[a]
Prepump steroids, *n* (%)	3 (20)	5 (33.3)	0.68[a]
Dexmedetomidine, *n* (%)	12 (80)	11 (73.3)	1.0[a]
Cell saver volume (mL/kg)	11.1 ± 2.1	10.0 ± 1.9	0.93[b]
Modified ultrafiltration volume (mL/kg)	27.7 ± 5.4	30.5 ± 4.7	0.60[b]
Total transfusions (mL/kg)			
PRBC	48.9 ± 11.1	44.9 ± 11.3	0.95[b]
Platelets	5.6 ± 2.5	4.0 ± 1.6	0.96[b]
Fresh-frozen plasma	11.0 ± 3.3	7.3 ± 2.7	0.39[b]
Urine output first 24 hr (mL/kg)	41.5 ± 4.1	42.8 ± 3.9	0.95[b]
Chest tube output in 24 hr (mL/kg)	23.4 ± 5.0	22.8 ± 4.0	0.98[b]
Surgical re-exploration, *n* (%)	1 (6.7)	0	1.0[a]
Time to extubation (hr)	16.2 ± 7.6	7.4 ± 1.6	0.32[b]
Hospital length of stay (d)	5.6 ± 0.8	4.6 ± 0.3	0.48[b]
Highest postoperative creatinine (mg/dL)	0.48 ± 0.03	0.51 ± 0.03	0.52[d]
AKI within 72 hr, *n* (%)	8 (53.3)	8 (53.3)	1.0[c]
Highest AKI stage within 72 hr, *n* (%)			0.64[c]
No injury	7 (46.7)	7 (46.7)	
Stage 1	7 (46.7)	5 (33.3)	
Stage 2	1 (6.7)	2 (13.3)	
Stage 3	0	1 (6.7)	

PRBC = packed RBCs, AKI = acute kidney injury.

[a]Fisher exact test.

[b]Mann-Whitney *U* test.

[c]Pearson chi-square test.

[d]*t* test.

The definition of AKI was based on the Acute Kidney Injury Network consensus guidelines for the staging of AKI: stage 1 AKI, 0.3 mg/dL increase in serum creatinine concentration or increase to ≥ 1.5- to 2-fold from baseline; stage 2 AKI, increase in serum creatinine > two- to three-fold from baseline; and stage 3 AKI, increase in serum creatinine > three-fold from baseline.

RESULTS

Prerandomization Patient Characteristics

All prerandomization patient characteristics (**Table 1**) were comparable between the two study groups except preoperative hematocrit was lower in the placebo group.

Intra- and Postoperative Patient Characteristics

The use of PRBC and plasma in the pump prime, age of PRBC used in pump prime, CPB time, cross-clamp time, use of aminocaproic acid, steroids, dexmedetomidine, cells saver volume, MUF volume, the amount of blood products given, blood loss as measured by chest tube output in 24 hours, urine output, need for surgical re-exploration, time to extubation, and hospital length of stay were not significantly different between the study groups (**Table 2**). ApAP concentrations measured post bypass were 3.8 ± 0.4 μg/mL (25.0 ± 2.9 μM).

Markers of Hemolysis

CPB was associated with an increase in free hemoglobin ($p < 0.001$ for effect of time) (**Fig. 1*A***) indicating hemolysis. The degree of hemolysis trended to be higher in patients who received ApAP compared with the placebo group ($p = 0.07$ for effect of study drug) (**Fig. 1*B***). Haptoglobin concentrations did not change significantly over the course of the study ($p = 0.94$ for effect of time) (**Fig. 1*C***), and there was no significant difference between the two study groups ($p = 0.29$ for effect of study drug) (**Fig. 1*D***). Because haptoglobin concentrations did not decrease as expected during CPB, we explored potential causes. We considered both the administration of corticosteroids (induce haptoglobin synthesis) (8) and plasma (contains haptoglobin) priming of the pump as potential contributing factors. Patients who received prebypass steroids had higher postbypass haptoglobin concentrations compared with those that did not (51.1 ± 13.9 vs 18.4 ± 4.9 mg/dL, respectively, $p = 0.04$). Haptoglobin concentrations remained elevated in patients who received a plasma pump prime (from a baseline of 36.4 ± 13.0 to 48.6 ± 17.1 mg/dL at 30 min of bypass, $p = 0.75$) compared with a significant decrease in patients who did not receive a plasma pump prime (from a baseline of 25.4 ± 6.3 to 11.9 ± 3.2 mg/dL at 30 min of bypass, $p = 0.02$).

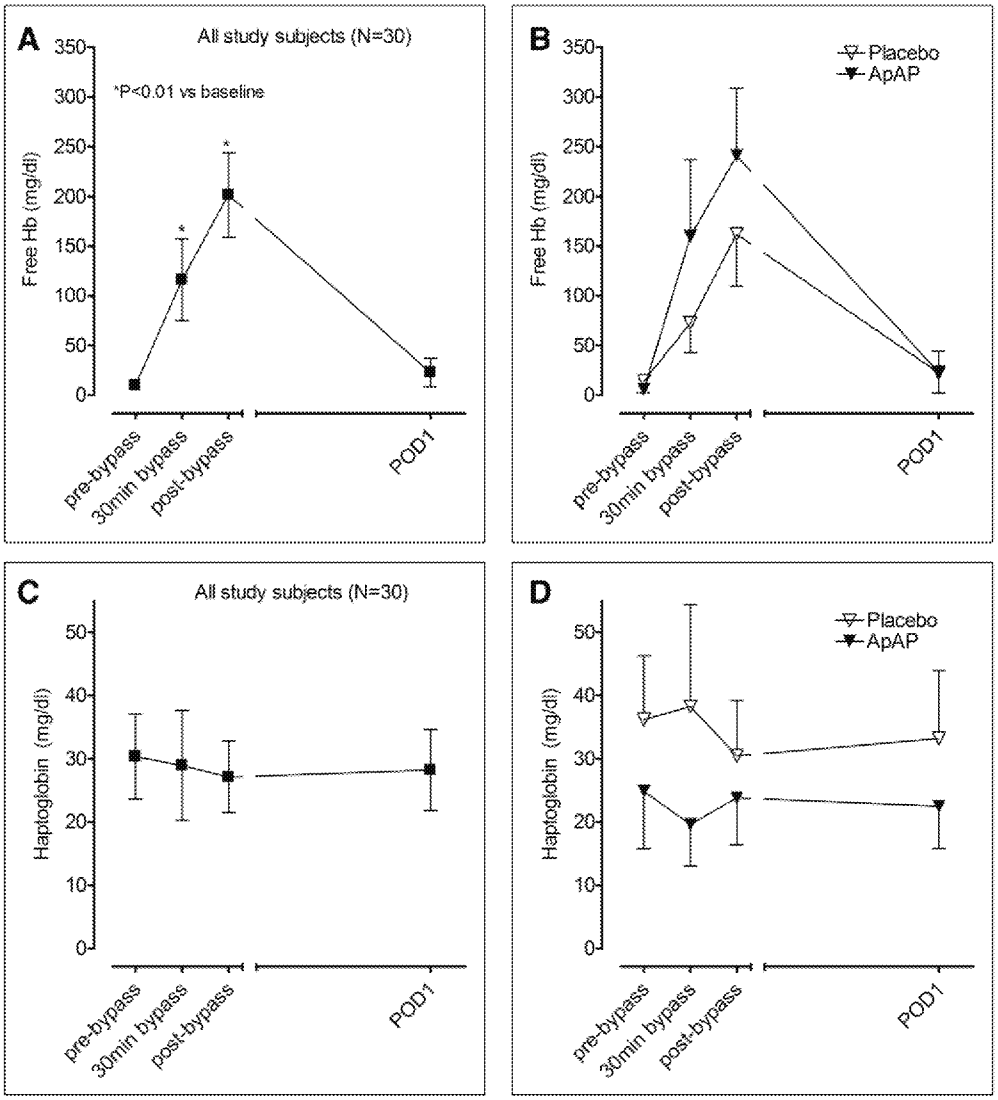

Figure 1. Hemolysis, as indicated by free hemoglobin concentrations in all study patients (**A**) and by study drug group (**B**). Haptoglobin concentrations in all study patients (**C**) and by study drug group (**D**). POD 1 = postoperative day 1, ApAP = acetaminophen.

Markers of Lipid Peroxidation

Both plasma isofuran and F_2-isoprostane concentrations increased significantly during surgery ($p = 0.03$ and $p = 0.05$, respectively, for effect of time) (**Fig. 2*A***). Plasma isofuran concentrations remained elevated on POD1, whereas F_2-isoprostane concentrations returned to baseline levels. The magnitude of increase for POD1 plasma isofurans was significantly greater compared with the magnitude of increase for plasma F_2-isoprostanes (2.6 ± 0.7-fold vs 1.6 ± 0.4-fold increase from baseline, $p = 0.02$). Similar to plasma markers of lipid peroxidation, urine isofurans and F_2-isoprostanes increased significantly during surgery ($p = 0.001$ and $p = 0.003$, respectively, for effect of time) (**Fig. 2*B***) and remained elevated on POD1.

Because baseline concentrations of plasma F_2-isoprostanes (37.7 ± 5.7 vs 46.6 ± 5.0 pg/mL, $p = 0.21$) and isofurans (85.2 ± 21.0 vs 106.4 ± 14.6 pg/mL, $p = 0.12$) tended to be lower in the placebo group compared with the ApAP group, we calculated the fold change from baseline for these biomarkers. The administration of ApAP attenuated the increase in plasma isofurans ($p = 0.02$ for effect of study drug) (**Fig. 3*A***). The effect of ApAP on isofuran concentrations was predominantly seen during the intraoperative period and disappeared by POD1. The increase in plasma F_2-isoprostanes was attenuated by ApAP but was not statistically significant ($p = 0.16$ for effect of study drug) (**Fig. 3*B***). In addition, ApAP did not affect the increase in urine isofuran or F_2-isoprostane concentrations ($p = 0.99$ and $p = 0.47$, respectively, for effect of study drug). There was a significant correlation in postbypass free Hb and postbypass fold change in plasma F_2-isoprostane concentrations ($r^2 = 0.32$, $p = 0.03$) in the placebo group but not in the ApAP group.

Clinical Outcomes

AKI developed in 16 patients (53%) (**Table 2**). There were no significant differences in the highest postoperative creatinine, the prevalence of AKI, or the AKI stage between the placebo and ApAP groups. No patient required postoperative dialysis. The change in urine NGAL concentrations was not significantly different between the placebo and ApAP groups (29.0 ± 14.2 vs 44.6 ± 37.0 ng/mL, respectively, $p = 0.66$). Patients in whom AKI developed tended to have higher postbypass urine NGAL concentrations compared with patients in whom AKI did not developed (77.9 ± 32.1 vs 20.7 ± 6.6 ng/mL, respectively, $p = 0.15$). Peak free hemoglobin concentrations were significantly higher in patients who subsequently developed AKI compared with patients who did not (308.5 ± 67.8 vs 79.1 ± 20.8 mg/dL, $p = 0.02$).

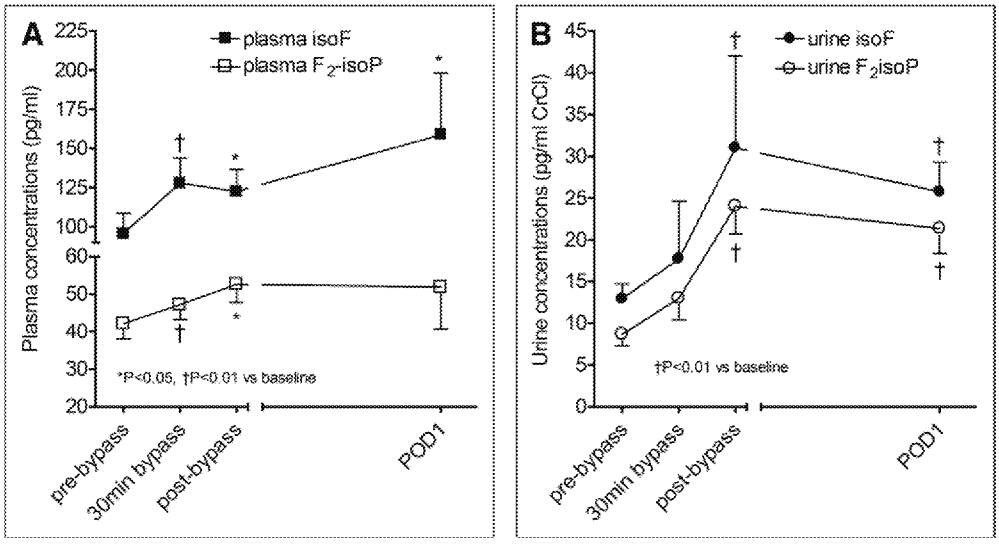

Figure 2. Lipid peroxidation as quantified by isofuran (isoF) and F_2-isoprostane (F_2-isoP) concentrations in plasma (**A**) and urine (**B**) for all study patients. 30 min bypass = 30 min of cardiopulmonary bypass, POD1 = postoperative day 1.

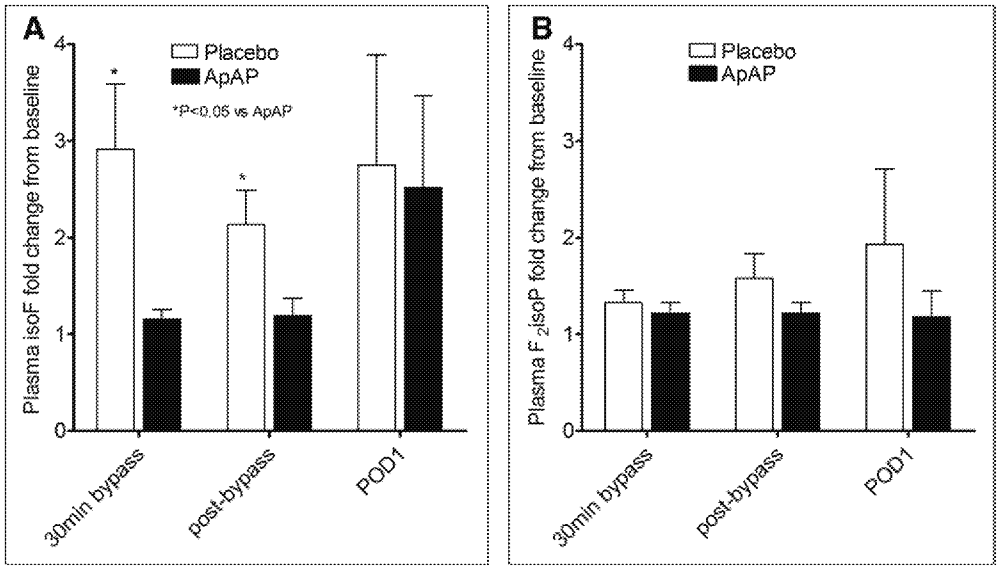

Figure 3. Fold change in plasma isofuran (isoF) (**A**) and plasma F_2-isoprostane (F_2-isoP) concentrations (**B**) from baseline. Acetaminophen (ApAP) attenuated the increase in isoF ($p = 0.02$ for effect of study drug) but not F_2-isoP ($p = 0.16$ for effect of study drug). 30 min bypass = 30 min of cardiopulmonary bypass, POD 1 = postoperative day 1.

DISCUSSION

This study examined the effect of ApAP, a ferryl protein reductant, on markers of lipid peroxidation in children undergoing cardiac surgery requiring CPB. CPB was associated with significant hemolysis and lipid peroxidation. ApAP attenuated the increase in plasma isofuran concentrations but did not affect postoperative creatinine or urine NGAL concentrations.

Our finding of hemolysis, with a subsequent increase in free hemoglobin during CPB, is consistent with prior studies (1, 4, 24). The cause for the greater degree of hemolysis observed in ApAP group is not known. The duration of CPB, transfusion of PRBC, and age of PRBC used for pump prime were similar between the two groups. Although ApAP can cause hemolysis in the presence of glucose-6-phosphate dehydrogenase deficiency, it is in the setting of an overdose; our patients received standard ApAP doses and blood

levels at the end of bypass were below toxic levels. The haptoglobin response during CPB is variable with most studies indicating a decrease in haptoglobin concentrations (7, 25, 26), whereas in another study, haptoglobin concentrations remained within normal limits at the end of CPB (27). The haptoglobin response observed in our study may in part be explained by the administration of prebypass steroids that can induce haptoglobin synthesis as well as plasma priming of the pump that resulted in higher intraoperative haptoglobin concentrations.

Consistent with prior studies, markers of lipid peroxidation increased significantly during CPB, and isofuran concentrations increased to a greater extent compared with F_2-isoprostane concentrations (1, 18, 24). Oxygen administration, which is common after surgery, most likely accounted for the relative increased formation of isofurans compared with isoprostanes because the formation of F_2-isoprostanes is suppressed by elevated concentrations of oxygen (17). ApAP attenuated the intraoperative increase in plasma isofuran concentrations even though patients in the ApAP group tended to have greater hemolysis. The lack of effect of ApAP on plasma F_2-isoprostane concentrations could be the result of the small sample size and being underpowered to detect the small reduction in plasma F_2-isoprostane concentrations observed in the ApAP group. The modest effect of ApAP on plasma markers of lipid peroxidation and the lack of effect on urinary markers of lipid peroxidation could be explained by several reasons. First, despite administering the IV formulation of ApAP at doses usually recommended in the literature, concentrations achieved post bypass were subtherapeutic but still above the IC_{50} for ApAP inhibition of hemoglobin catalyzed oxidation of arachidonic acid. The low concentrations of ApAP postbypass may be due to the increased volume of distribution of bypass which was not accounted for in dosing ApAP. Second, isofurans and F_2-isoprostanes represent global free radical–induced lipid peroxidation and not only lipid peroxidation mediated by hemeprotein redox cycling. Because of this, ApAP would not be expected to completely or near completely diminish lipid peroxidation in these patients. Third, haptoglobin concentrations remained elevated during CPB and could have attenuated hemeprotein-mediated redox cycling by scavenging free hemoglobin.

Although the study was not powered to assess the effect of ApAP on postoperative AKI, we did an exploratory analysis of AKI because ApAP inhibits hemeprotein-mediated lipid peroxidation and protects kidney function in an animal model (13). Although ApAP attenuated intraoperative plasma isofuran concentrations, there were no significant differences in creatinine or NGAL concentrations or the prevalence of AKI between the ApAP and placebo groups. The lack of effect of ApAP on postoperative kidney function is in keeping with the failure of other antioxidants, such as N-acetylcysteine, vitamin C, and vitamin E, to protect the kidney after cardiac surgery (28).

Limitations

Several factors reduce enthusiasm for future studies investigating the effect of ApAP on AKI following cardiac surgery. First, we observed only a modest effect of ApAP on intraoperative lipid peroxidation and no effect on urinary markers of lipid peroxidation. Second, we did not observe an effect of ApAP on markers of AKI although we are underpowered. Third, higher sustained concentrations of ApAP throughout the period of maximum hemolysis may be more effective in inhibiting hemeprotein-mediated lipid peroxidation, but we are limited by the approved ApAP dosing regimen. Although enthusiasm for ApAP as ferryl protein reductant during cardiac surgery may be reduced, we cannot exclude the possibility that more effective inhibition of hemeprotein-mediated lipid peroxidation may reduce markers of AKI and reduce the prevalence of AKI in children undergoing CPB. Deferoxamine, which is not only an iron chelator but also a ferryl protein reductant, may be more effective in reducing lipid peroxidation compared with ApAP and deserves further study (29). The extrapolation of our findings is limited by the small sample size, and larger studies in different populations are necessary to validate or refute our results. Finally, we did not measure ascorbate concentrations (indicator of the endogenous antioxidant status) although prior studies indicate decrease concentrations in children undergoing CPB (24).

In summary, CPB in children is associated with hemolysis and lipid peroxidation. Although ApAP attenuated the increase in plasma isofuran concentrations, it did not affect postoperative creatinine or urine NGAL concentrations. Future studies are needed to establish whether other therapies that attenuate or prevent the effects of free hemoglobin result in more effective inhibition of lipid peroxidation in patients undergoing CPB.

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