ANESTHESIOLOGY

Intraoperative Oxidative Damage and Delirium after Cardiac Surgery

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Postoperative delirium occurs in approximately 25% of cardiac surgery patients.
- Hyperoxic cerebral perfusion during cardiac surgery has been associated with increased postoperative delirium, and oxidative damage may mediate this association.

What This Article Tells Us That Is New

- In a cohort of 400 cardiac surgery patients, intraoperative plasma concentrations of F_2-isoprostanes and isofurans (markers of oxidative damage) were independently associated with both increased postoperative delirium and increased plasma concentrations of ubiquitin carboxyl-terminal hydrolase isozyme L1, a marker of neuronal injury.
- The association between increased systemic markers of oxidative damage and increased neuronal injury was stronger in patients with elevated plasma S100 calcium-binding protein B, a marker of blood–brain barrier disruption. This suggests that blood–brain barrier disruption may increase susceptibility to neuronal injury associated with systemic oxidative stress.

ABSTRACT

Background: Mechanisms of postoperative delirium remain poorly understood, limiting development of effective treatments. We tested the hypothesis that intraoperative oxidative damage is associated with delirium and neuronal injury and that disruption of the blood–brain barrier modifies these associations.

Methods: In a prespecified cohort study of 400 cardiac surgery patients enrolled in a clinical trial of atorvastatin to reduce kidney injury and delirium, we measured plasma concentrations of F_2-isoprostanes and isofurans using gas chromatography-mass spectrometry to quantify oxidative damage, ubiquitin carboxyl-terminal hydrolase isozyme L1 to quantify neuronal injury, and S100 calcium-binding protein B using enzyme-linked immunosorbent assays to quantify blood–brain barrier disruption before, during, and after surgery. We performed the Confusion Assessment Method for the Intensive Care Unit twice daily to diagnose delirium. We measured the independent associations between intraoperative F_2-isoprostanes and isofurans and delirium (primary outcome) and postoperative ubiquitin carboxyl-terminal hydrolase isozyme L1 (secondary outcome), and we assessed if S100 calcium-binding protein B modified these associations.

Results: Delirium occurred in 109 of 400 (27.3%) patients for a median (10th, 90th percentile) of 1.0 (0.5, 3.0) days. In the total cohort, plasma ubiquitin carboxyl-terminal hydrolase isozyme L1 concentration was 6.3 ng/ml (2.7, 14.9) at baseline and 12.4 ng/ml (7.9, 31.2) on postoperative day 1. F_2-isoprostanes and isofurans increased throughout surgery, and the log-transformed sum of intraoperative F_2-isoprostanes and isofurans was independently associated with increased odds of postoperative delirium (odds ratio, 3.70 [95% CI, 1.41 to 9.70]; $P = 0.008$) and with increased postoperative ubiquitin carboxyl-terminal hydrolase isozyme L1 (ratio of geometric means, 1.42 [1.11 to 1.81]; $P = 0.005$). The association between increased intraoperative F_2-isoprostanes and isofurans and increased postoperative ubiquitin carboxyl-terminal hydrolase isozyme L1 was amplified in patients with elevated S100 calcium-binding protein B ($P = 0.049$).

Conclusions: Intraoperative oxidative damage was associated with increased postoperative delirium and neuronal injury, and the association between oxidative damage and neuronal injury was stronger among patients with increased blood–brain barrier disruption.

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Postoperative delirium is a state of acute cerebral dysfunction after anesthesia and surgery.[1] It occurs in 25 to 52% of patients undergoing cardiac surgery and is associated with increased mortality, increased duration of hospitalization, and long-term cognitive decline.[2,3] The underlying pathophysiologic mechanisms of cardiac surgery-induced delirium are unclear but may include acute inflammation, effects of anesthetics, exposure to artificial circulation or altered perfusion, and microemboli.[4] Alterations in systemic and regional perfusion and oxygenation, cardiopulmonary bypass (CPB), and rapid changes in plasma pH and cellular metabolism during cardiac surgery induce an oxidative stress that could contribute to postoperative delirium.[5,6] In

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fact, hydrogen peroxide, a reactive oxygen species generated during tissue ischemia and reperfusion, suppresses neuron function,[7] and delirium-susceptible mice have increased systemic oxidative stress and are protected by treatments that reduce oxidative stress.[8] Oxidative stress leads to oxidative damage, but a functional blood–brain barrier might reduce the injurious effects of circulating oxidants on neurons, unless oxidative damage disrupts the blood–brain barrier.[9]

F_2-isoprostanes and isofurans are oxidative damage end-products of arachidonic acid peroxidation that increase in proportion to oxidative stress, are specific to free radical-initiated oxidation, are stable in plasma, and have been independently associated with other organ injuries after cardiac surgery.[10–13] Data on the relationships among oxidative damage, blood–brain barrier disruption, neuronal injury, and risk of delirium are limited. We conducted this study to test the hypothesis that increased intraoperative oxidative damage is associated with increased postoperative delirium and neuronal injury, and that disruption of the blood–brain barrier modifies these associations.

Materials and Methods

Patients

This observational study was prospectively planned as part of a clinical trial of statin therapy to reduce kidney injury and delirium after cardiac surgery (Clinicaltrials.gov identifier NCT00791648).[14] Adults receiving elective coronary artery bypass grafting (with or without CPB), heart valve surgery, or surgery on the ascending aorta at Vanderbilt University Medical Center (Nashville, Tennessee) were eligible to participate. Patients unable to participate in preoperative cognitive testing or with acute coronary syndrome, hepatitis, chronic liver dysfunction, statin intolerance, cytochrome P450 3A4 inhibitors or cyclosporine use, pregnancy, on dialysis, a history of kidney transplantation or insufficient blood availability were excluded. The Vanderbilt Institutional Review Board approved the study, and all participants provided written informed consent before surgery.

Baseline Assessments

Research nurses measured baseline preoperative cognitive function with the Mini Mental State Exam, estimated severity of illness with the Charlson comorbidity index, and documented past medical history, vital signs, and baseline laboratory data.

Standardized Patient Management

Perioperative anesthetic, surgical, and postoperative patient management was conducted according to institutional protocol (Supplemental Digital Content, Supplemental Methods, http://links.lww.com/ALN/C76).

Postoperative delirium was assessed beginning on the night of surgery, continuing until patients were transferred out of the intensive care unit by research nurses and coordinators who were blinded to treatment and biomarker data. Delirium assessment training is described in the Supplemental Digital Content (http://links.lww.com/ALN/C76). Briefly, trained research personnel assessed patients for level of arousal and delirium twice daily using the Richmond Agitation Scale Score and the Confusion Assessment Method for the Intensive Care Unit tool, respectively. The Confusion Assessment Method for the Intensive Care Unit tool is validated in ventilated and nonventilated patients and is used to assess acute changes or a fluctuating course of mental status, inattention, an altered level of consciousness, and disorganized thinking to diagnose delirium.[15–18] This tool has been frequently utilized to assess for delirium after cardiac surgery.[3,19–21] Participants with a noncomatose state (Richmond Agitation Scale Score −3 or greater) and a positive Confusion Assessment Method for the Intensive Care Unit assessment were considered to have delirium.

Quantification of Oxidative Damage, Blood–Brain Barrier Disruption, and Neuronal Injury

We collected arterial blood at the induction of general anesthesia (baseline), 30 min after initiation of CPB or off-pump coronary artery bypass grafting (CABG), after termination of CPB or completion of off-pump CABG, at intensive care unit admission, and at 9:00 AM on postoperative day 1. Blood was collected in 0.105M sodium citrate- and EDTA-coated tubes, immediately placed on ice, centrifuged for 15 min at 1000G, and plasma separated and frozen at −80°C until thawed for batched analysis. Lab technicians were blinded to the results of delirium assessments and all clinical data. To measure oxidative damage, we quantified the nonesterified free plasma concentrations of F_2-isoprostanes and isofurans using gas chromatography-mass spectrometry. F_2-isoprostanes and isofurans are products of arachidonic acid peroxidation that represent the gold-standard for the assessment of oxidative damage *in vivo* because they increase in direct proportion to oxidative stress and are stable in biologic specimens.[10] F_2-isoprostanes and isofurans are differentially expressed from a common lipid radical intermediate in response to local oxygen tension.[22] When oxygen administration and tissue oxygen tensions are heterogenous both temporally and regionally, such as during cardiac surgery, the combined metric of F_2-isoprostanes and isofurans best reflects systemic lipid peroxidation.

To quantify neuronal injury, we measured plasma concentrations of ubiquitin carboxyl-terminal hydrolase isozyme L1 in duplicate at baseline, intensive care unit admission, and on postoperative day 1, using an enzyme-linked immunosorbent assay (Abnova, Taiwan). Ubiquitin carboxyl-terminal hydrolase isozyme L1 is a neuron-specific enzyme that tags proteins with ubiquitin for proteasome degradation and assists with recycling of ubiquitin among other neuron specific functions.[23] Plasma concentrations of ubiquitin carboxyl-terminal hydrolase isozyme L1

reflect neuronal damage in conjunction with blood–brain barrier disruption, active or passive transport of ubiquitin carboxyl-terminal hydrolase isozyme L1 across the blood–brain barrier, diffusion of ubiquitin carboxyl-terminal hydrolase isozyme L1 across the blood–brain barrier, or glymphatic passage.[24,25] Plasma concentrations of ubiquitin carboxyl-terminal hydrolase isozyme L1 are increased in patients with traumatic brain injury and after controlled hypoxic–ischemic brain injury in preclinical studies.[26,27]

To measure disruption or injury to the blood–brain barrier, we measured S100 calcium-binding protein B concentrations in plasma in duplicate at baseline, intensive care unit admission, and on postoperative day 1 using an enzyme-linked immunosorbent assay (Millipore Sigma, USA). S100 calcium-binding protein B is released from astrocytes after injury or ischemia and has previously been validated as a marker of blood–brain barrier disruption by comparing cerebrospinal fluid-serum albumin quotients and magnetic resonance images after blood–brain barrier injury.[28–30] S100 calcium-binding protein B concentrations may also reflect neuronal injury independent of blood–brain barrier disruption *via* glymphatic passage,[24] and there are some extracranial sources of S100 calcium-binding protein B,[31] although these are considered minor contributors to circulating concentrations.[32,33]

Statistical Analyses

The association between intraoperative oxidative damage and the development of postoperative delirium was estimated using logistic regression, adjusting for potential confounders including age, baseline Mini Mental State Exam score, Charlson comorbidity index, history of diabetes (yes or no), current smoking status (yes or no), history of stroke (yes or no), use of CPB during surgery (yes or no), perioperative atorvastatin treatment (yes or no), and baseline F_2-isoprostanes and isofurans measurements (natural log-transformed). Intraoperative oxidative damage was quantified as the sum of the natural log-transformed intraoperative F_2-isoprostane and isofuran measurements, averaged among the 30 min into CPB or off-pump CABG, the post-CPB or off-pump CABG, and the intensive care unit admission time points. This measure is equivalent to the natural logarithm of the geometric mean of F_2-isoprostanes and isofurans measurements at these three time points. Thus, by exponentiation, the overall measure can be summarized in the original units of the F_2-isoprostane and isofuran measurements. Quantitative factors were modeled using a four-knot natural cubic spline to allow for possible nonlinear associations. These terms were omitted in the absence of significant statistical evidence of nonlinear effects ($P > 0.05$), assessed using a multiple-degree-of-freedom chi-square test. The effect of intraoperative oxidative stress on the incidence of postoperative delirium was summarized using an odds ratio and 95% CI and tested for statistical significance using the associated Wald test. P values less than 0.05 were considered statistically significant.

The effects of intraoperative oxidative damage on neuronal injury, measured as natural log-transformed plasma concentrations of ubiquitin carboxyl-terminal hydrolase isozyme L1 on postoperative day 1, were quantified using linear regression, adjusting for the same set of factors listed previously.

To examine whether the extent of blood–brain barrier disruption, measured by the natural log-transformed plasma concentration of S100 calcium-binding protein B immediately after surgery, modifies the association between intraoperative oxidative damage and the incidence of delirium or the extent of neuronal injury, we added the intensive care unit admission S100 calcium-binding protein B concentration and a cross-product interaction term between intraoperative oxidative damage and intensive care unit admission S100 calcium-binding protein B (F_2-isoprostanes and isofurans × S100 calcium-binding protein B) to the delirium logistic regression and to the ubiquitin carboxyl-terminal hydrolase isozyme L1 linear regression models. We then examined the association between these interaction terms and delirium and ubiquitin carboxyl-terminal hydrolase isozyme L1, respectively.

Finally, as a sensitivity analysis, we measured the association between neuronal injury and the odds of delirium using logistic regression methods, adjusting for perioperative oxidative damage and the other potential confounders listed previously. In addition, we performed a subgroup analysis on patients who received on-pump surgery.

Due to evidence of nonlinear associations among factors, all associations are summarized by comparing the odds or geometric means corresponding to the 90th *versus* 10th percentile of the independent variable, and effect estimates are presented graphically with 95% confidence bands. Multiple-degree-of-freedom chi-square tests were used to evaluate statistical significance.

We selected a sample size for this study based on previous studies of perioperative oxidative damage and established rates of postoperative delirium. To detect a 5 ± 15 pg/ml difference in F_2-isoprostanes and isofurans concentration between patients who did and who did not develop delirium, a concentration of F_2-isoprostanes and isofurans previously demonstrated to be independently associated with kidney injury after surgery,[11] we studied 400 patients and assumed a 25% incidence of delirium in the cohort and a type I error rate of 5%. This number of patients provides 83% power to reject the null hypothesis. In addition, a cohort of 400 patients and 109 events provided the opportunity to reliably fit a model with up to 11 degrees of freedom for logistic regression modeling and 27 degrees of freedom for linear regression modeling.[34] We used R software version 3.4.1 (www.r-project.org, accessed June 2017) for all statistical analyses.

Results

Patient Characteristics and Delirium

Four hundred patients comprised the cohort. The median (10th percentile, 90th percentile) age of patients was 67 (50, 81) yr, 32.8% were female, 30.8% were diabetic, and 70.8% had CPB during surgery (table 1). One hundred-nine patients (27.3%) developed postoperative delirium for a median of 1.0 (0.5, 3.0) days. The median onset of delirium was on the morning of postoperative day 1 (0.5 [0.0, 2.0] days after intensive care unit admission). Patients who developed delirium experienced increased rates of postoperative atrial fibrillation (47.7% *vs.* 30.2%) and acute kidney injury (30.3% *vs.* 20.6%) and remained in the intensive care unit and hospital 2.0 days longer than patients who did not develop delirium. Similar to other trials of short-term statins, study drug administration (atorvastatin *vs.* placebo) did not affect delirium.[14,35,36]

Table 1. Baseline and Intraoperative Patient Characteristics

Patient Characteristics	No Delirium (n = 291)	Delirium (n = 109)	Total (n = 400)
Age, yr	64 (47, 81)	72 (56, 82)	67 (50, 81)
Sex, female	78 (26.8%)	53 (48.6%)	131 (32.8%)
African American ancestry	11 (3.8%)	8 (7.3%)	19 (4.8%)
Height, cm	175 (157, 183)	167 (157, 183)	173 (157, 183)
Weight, kg	85 (63, 101)	77 (61, 102)	82 (62, 109)
Body mass index	27.2 (22.6, 36.1)	27.5 (22.0, 36.9)	27.4 (22.5, 36.5)
Medical history			
ASA Physical Status class	IV (III, IV)	IV (III, IV)	IV (III, IV)
Congestive heart failure	109 (37.5%)	54 (49.5%)	163 (40.8%)
Left ventricular ejection fraction, %	60 (40, 60)	57 (30, 60)	60 (35, 60)
Hypertension	249 (85.6%)	99 (90.8%)	348 (87.0%)
Chronic obstructive pulmonary disease	24 (8.2%)	23 (21.1%)	47 (11.8%)
Current smoking	49 (16.8%)	18 (16.5%)	67 (16.8%)
Obstructive sleep apnea	44 (15.1%)	13 (11.9%)	57 (14.2%)
Charlson comorbidity index	2 (0, 5)	3 (0, 5)	2 (0, 5)
Diabetes	88 (30.2%)	35 (32.1%)	123 (30.8%)
Peripheral vascular disease	79 (27.1%)	34 (31.2%)	113 (28.2%)
Stroke	13 (4.5%)	9 (8.3%)	22 (5.5%)
Education, yr*	12 (10, 16)	12 (9,16)	12 (10, 16)
Mini Mental State Exam score	29 (27, 30)	28 (24, 30)	29 (26, 30)
Trails B score, s	105 (70, 195)	134 (75, 222)	110 (70, 207)
Medication use			
Baseline angiotensin converting enzyme inhibitor use	92 (31.6%)	33 (30.3%)	125 (31.3%)
Baseline statin use	184 (63.2%)	66 (60.6%)	250 (62.5%)
Perioperative statin treatment	151 (51.9%)	53 (48.6%)	204 (51.0%)
Baseline laboratory and hemodynamic data			
Heart rate, beats/minute	63 (49, 83)	66 (49, 85)	64 (49, 83)
Mean arterial Pressure, mmHg	78 (62, 96)	81 (61, 98)	78 (62, 96)
Central venous pressure, mmHg	13 (7, 21)	14 (7, 22)	13 (7, 21)
Cardiac index, liters/min/m^2	2.2 (1.6, 3.0)	2.1 (1.4, 3.2)	2.2 (1.5, 3.0)
Blood glucose, mg/dl	107 (88, 162)	109 (90, 162)	108 (89, 162)
Hematocrit, %	36.0 (29.0, 43.0)	34.0 (28.0, 41.0)	35.0 (29.0, 42.1)
Platelet count, × 10^3/μl	213 (142, 306)	208 (135, 339)	141 (212, 313)
Leukocyte count, × 10^3/l	7.7 (5.0, 10.8)	7.7 (4.7, 11.9)	7.3 (5.0, 10.9)
Arterial pH	7.39 (7.33, 7.47)	7.41 (7.34, 7.47)	7.40 (7.33, 7.47)
$Paco_2$, mmHg	42 (35, 49)	42 (35, 50)	42 (35, 49)
Arterial lactate, mg/dl	0.7 (0.4, 1.4)	0.7 (0.4, 1.4)	0.7 (0.4, 1.4)
Procedure characteristics			
Duration of surgery, min	298 (216, 463)	336 (226, 510)	301 (218, 474)
Coronary artery bypass surgery	144 (49.5%)	50 (45.9%)	194 (48.5%)
Valve surgery	182 (62.5%)	78 (71.6%)	260 (65.0%)
On-pump surgery	196 (67.4%)	87 (79.8%)	283 (70.8%)
Off-pump surgery	95 (32.6%)	22 (20.2%)	117 (29.2%)
Cardiopulmonary bypass time, min†	131 (89, 233)	146 (90, 249)	135 (89, 238)
Aorta cross-clamp use	135 (46.4%)	57 (52.3%)	192 (48.0%)
Circulatory arrest use	9 (3.1%)	2 (1.8%)	11 (2.8%)

Binary characteristics are reported as n (%) and continuous characteristics as median (10th percentile, 90th percentile).

*Education years start at 1st grade. For example, 16 yr of education is 12 grades and 4 yr of post–high school education (college or equivalent). †Among on-pump surgery patients. Medical history diagnoses were obtained from the medical record and from direct patient questioning at the time of enrollment.

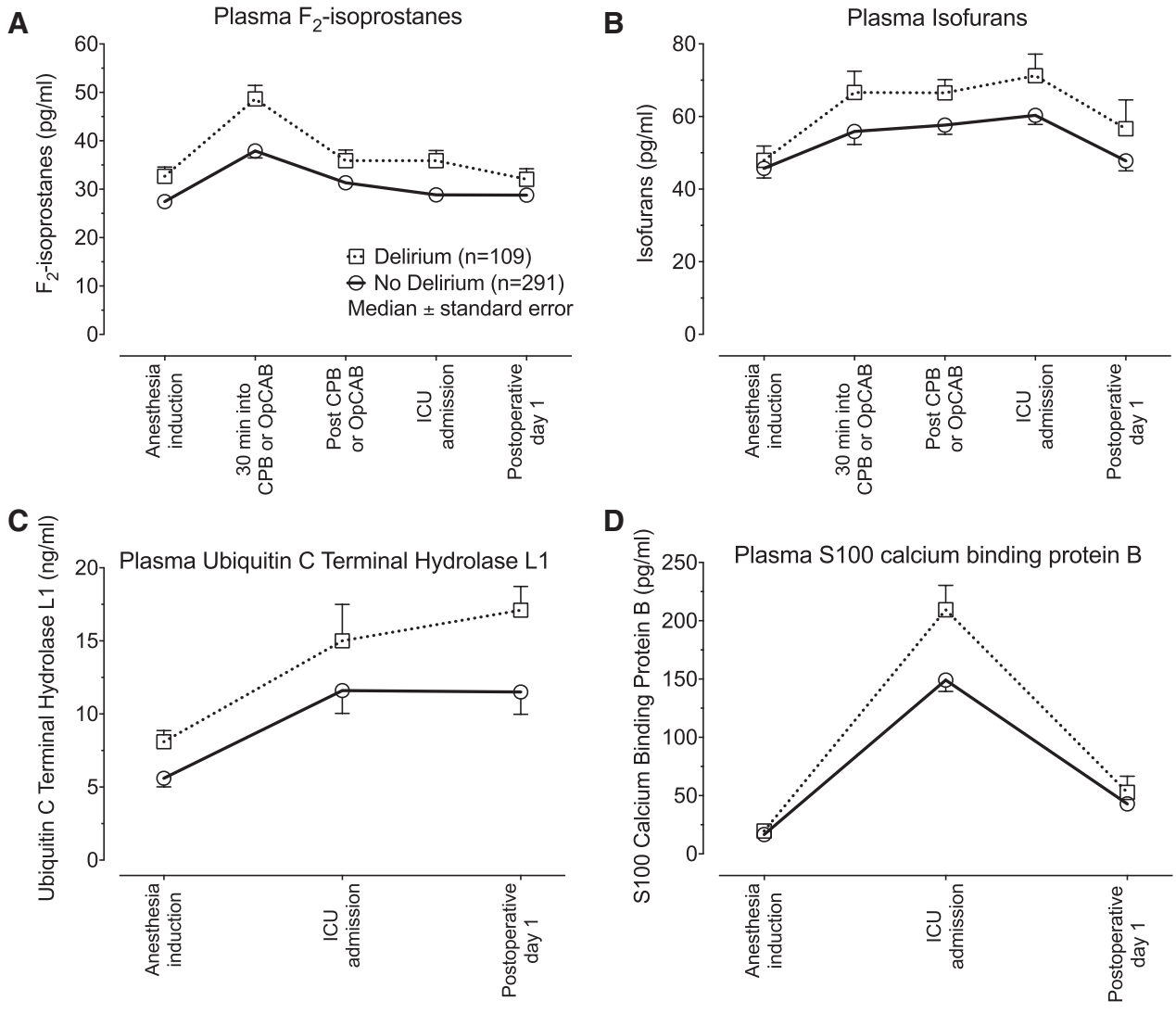

Fig. 1. Plasma concentrations of markers of oxidative damage (F_2-isoprostanes [*A*] and isofurans [*B*]), neuronal injury (ubiquitin carboxyl-terminal hydrolase isozyme L1 [*C*]), and blood–brain barrier disruption (S100 calcium-binding protein B [*D*]) during the perioperative period in patients who developed delirium and those who did not develop delirium.

Oxidative Damage and Postoperative Delirium

The median (10th percentile, 90th percentile) plasma concentration of F_2-isoprostanes was 28.9 pg/ml (14.8, 58.4) at baseline, increased to a peak of 40.8 pg/ml (20.6, 79.6) 30-min into CPB or off-pump CABG, and decreased toward baseline by postoperative day 1 (fig. 1A). The median plasma concentration of isofurans was 46.1 pg/ml (22.3, 110.2) at baseline, increased throughout surgery to a peak of 64.0 pg/ml (32.6, 132.3) at intensive care unit admission, and decreased toward baseline on postoperative day 1 (fig. 1B).

Increased intraoperative oxidative damage was independently associated with increased odds of developing postoperative delirium. Quantitatively, patients at the 90th percentile of intraoperative oxidative damage (combined intraoperative F_2-isoprostane and isofuran concentrations) were, on average, 3.7 times more likely to develop postoperative delirium than patients at the 10th percentile of intraoperative F_2-isoprostanes and isofurans (odds ratio, 3.70 [95% CI, 1.41 to 9.70]; $P = 0.008$; table 2, Model Delirium, independent variable F_2-isoprostanes and isofurans). This analysis was adjusted for the effects of potential confounders between oxidative damage and delirium, risk factors for delirium, and baseline concentrations of F_2-isoprostanes and isofurans. Concentrations of F_2-isoprostane and isofurans at baseline were not associated with delirium ($P = 0.200$). Results were qualitatively similar when individual concentrations of F_2-isoprostanes or isofurans were used in the model, as compared to the combined F_2-isoprostanes and

Table 2. Effects of Oxidative Damage and Blood–Brain Barrier Disruption on Postoperative Delirium and Neuronal Injury

Model	Independent Variable	Delirium Odds Ratio (95% CI)	*P* Value
Delirium	F_2-isoprostanes and isofurans	3.70 (1.41 to 9.7)	0.008
$Delirium_{S100B}$	F_2-isoprostanes and isofurans at S100 calcium-binding protein B 10th percentile	3.03 (0.71 to 13.0)	0.135
	F_2-isoprostanes and isofurans at S100 calcium-binding protein B 90th percentile	4.4 (1.26 to 15.3)	0.020
	S100 calcium-binding protein B	2.50 (1.13 to 5.5)	0.024
	F_2-isoprostanes and isofurans × S100 calcium-binding protein B interaction		0.665
		UCHL1 Ratio of Geometric Means (95% CI)	
UCHL1	F_2-isoprostanes and isofurans	1.42 (1.11 to 1.81)	0.005
$UCHL1_{S100B}$	F_2-isoprostanes and isofurans at S100 calcium-binding protein B 10th percentile	1.02 (0.75 to 1.41)	0.881
	F_2-isoprostanes and isofurans at S100 calcium-binding protein B 90th percentile	1.54 (1.09 to 2.18)	0.014
	S100 calcium-binding protein B	1.30 (1.04 to 1.63)	0.024
	F_2-isoprostanes & isofurans x S100 calcium-binding protein B interaction		0.049

The Delirium model summarizes the independent association between cumulative intraoperative plasma concentrations of F_2-isoprostanes and isofurans and the odds of postoperative delirium. The $Delirium_{S100B}$ model summarizes S100 calcium-binding protein B's modification of the association between F_2-isoprostanes and isofurans and odds of delirium, as well as the independent association between S100 calcium-binding protein B at intensive care unit admission and odds of delirium. The UCHL1 model summarizes the association between F_2-isoprostanes and isofurans and the geometric mean of postoperative ubiquitin carboxyl-terminal hydrolase L1. The $UCHL1_{S100B}$ model summarizes S100 calcium-binding protein B's modification of the association between F_2-isoprostanes and isofurans and the geometric mean of ubiquitin carboxy-terminal hydrolase L1, as well as the association between plasma concentrations of S100 calcium-binding protein B and the geometric mean of ubiquitin carboxyl-terminal hydrolase L1. The effect of S100 calcium-binding protein B is adjusted to the median F_2-isoprostane and isofuran concentration. Due to nonlinearity, all associations are summarized by comparing the odds or geometric means corresponding to the 90th *versus* 10th percentile of the independent variable. All models are adjusted for age, Charlson comorbidity index, baseline Mini Mental State Exam score, current smoking, history of stroke, history of diabetes mellitus, use of cardiopulmonary bypass, study drug, and baseline F_2-isoprostanes and isofurans concentrations.

isofurans metric of oxidative damage. Baseline Mini Mental State Exam and F_2-isoprostanes and isofurans were missing for 12 and 33 patients, respectively. S100 calcium-binding protein B and ubiquitin carboxyl-terminal hydrolase isozyme L1 measurements were missing for 22 and 9 participants, respectively. No other patient data were missing. Records with missing data were excluded from regression analyses.

Oxidative Damage and Postoperative Neuronal Injury

The median (10th percentile, 90th percentile) plasma concentration of ubiquitin carboxyl-terminal hydrolase isozyme L1 at baseline was 6.3 ng/ml (2.7, 14.9), increased during surgery to 12.2 ng/ml (5.0, 42.7) at intensive care unit admission, and peaked at 12.4 ng/ml (7.9, 31.2) on postoperative day 1 (fig. 1C). The mean coefficient of variation for ubiquitin carboxyl-terminal hydrolase isozyme L1 duplicate measurements was 5.23%. Increased intraoperative oxidative damage was independently associated with increased postoperative ubiquitin carboxyl-terminal hydrolase isozyme L1. For example, patients with intraoperative F_2-isoprostane and isofuran concentrations at the 90th percentile had, on average, a postoperative ubiquitin carboxyl-terminal hydrolase isozyme L1 concentration 41.8% greater (ratio of geometric means, 1.42 [95% CI, 1.11 to 1.81]; $P = 0.005$) than patients who had intraoperative F_2-isoprostane and isofuran concentrations at the 10th percentile, adjusted for potential confounders, risk factors for delirium, and baseline F_2-isoprostane and isofuran concentrations (table 2, Model UCHL1, independent variable F_2-isoprostanes and isofurans).

Impact of Blood–Brain Barrier Disruption on the Associations between Intraoperative Oxidative Damage and Delirium and between Intraoperative Oxidative Damage and Neuronal Injury

The median plasma concentration of S100 calcium-binding protein B at baseline was 17.2 pg/ml (10th percentile, 90th percentile: 9.0, 32.3), peaked at 163.4 pg/ml (70.1, 414.0) at intensive care unit admission, and decreased toward baseline by postoperative day 1 (fig. 1D). The mean coefficient of variation for S100 calcium-binding protein B duplicate measurements was 5.99%. Increased S100 calcium-binding protein B plasma concentrations at intensive care unit admission were independently associated with increased development of delirium and with postoperative neuronal injury. Patients with S100 calcium-binding protein B concentrations at the 90th percentile had, on average, 2.5-fold greater odds of developing postoperative delirium (odds ratio, 2.50 [95% CI, 1.13 to 5.51]; $P = 0.024$) than those with concentrations at the 10th percentile, adjusted for potential confounders and delirium risk factors (table 2, Model $Delirium_{S100B}$, independent variable S100 calcium-binding protein B). Patients with S100 calcium-binding protein B concentrations at the 90th percentile at intensive care unit admission also had, on average, 30% greater plasma ubiquitin carboxyl-terminal hydrolase isozyme L1 concentrations on postoperative day 1 (ratio of geometric means, 1.30 [95% CI, 1.04 to 1.63]; $P = 0.024$) than patients with S100 calcium-binding protein B concentration at the 10th percentile (table 2, Model $UCHL1_{S100B}$, independent variable S100 calcium-binding protein B).

The extent of blood–brain barrier disruption did not modify the association between increased oxidative damage

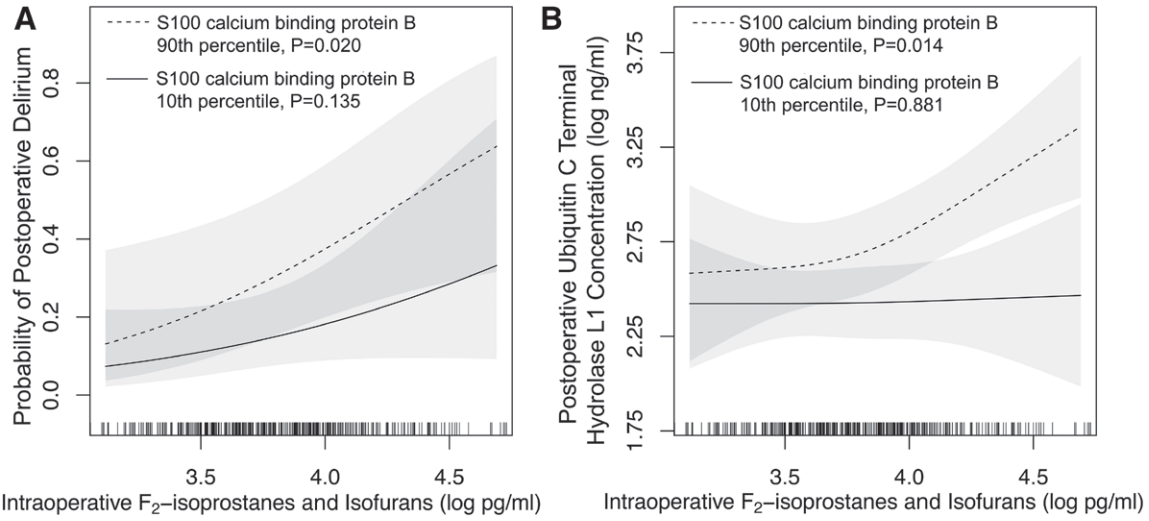

Fig. 2. Intraoperative oxidative damage *versus* postoperative delirium and neuronal injury. Intraoperative oxidative damage, quantified as the plasma concentration of F_2-isoprostanes and isofurans, was independently associated with increased probability of postoperative delirium (*A*) and plasma concentration of ubiquitin carboxyl-terminal hydrolase isozyme L1 on postoperative day 1 (*B*) after adjusting for potential confounders. This association with ubiquitin carboxyl-terminal hydrolase isozyme L1 concentration was differentially modified by the extent of blood–brain barrier disruption (measured as plasma S100 calcium-binding protein B concentration at the time of intensive care unit admission). The association with delirium was not modified by S100 calcium-binding protein B. Shaded areas represent the 95% CI of the probability of delirium (*A*) and ubiquitin carboxyl-terminal hydrolase isozyme L1 concentration (*B*). Tick marks at the bottom of the figure indicate the observed values among the study cohort.

and increased odds of developing delirium (P = 0.665; table 2, Model Delirium$_{S100B}$, F_2-isoprostanes and isofurans x S100 calcium-binding protein B interaction term, fig. 2A) but did modify the association between increased oxidative damage and increased neuronal injury (P = 0.049; table 2, Model UCHL1$_{S100B}$, F_2-isoprostanes and isofurans x S100 calcium-binding protein B interaction term). For example, at the 10th percentile of S100 calcium-binding protein B there was no association between increased oxidative damage and ubiquitin carboxyl-terminal hydrolase isozyme L1 (ratio of geometric means, 1.02 [95% CI, 0.75 to 1.41]; P = 0.881), while at the 90th percentile of S100 calcium-binding protein B, patients with intraoperative F_2-isoprostane and isofuran concentrations at the 90th percentile experienced a 54% increase in postoperative ubiquitin carboxyl-terminal hydrolase isozyme L1 concentrations compared to ubiquitin carboxyl-terminal hydrolase isozyme L1 concentrations among patients at the 10th percentile of F_2-isoprostanes and isofurans (ratio of geometric means, 1.54 [95% CI, 1.09 to 2.18]; P = 0.014). The S100 calcium-binding protein B effect modification on the association between intraoperative oxidative damage and neuronal injury is illustrated in figure 2B.

A *post hoc* subgroup analysis involving on-pump surgery was added during peer review. The results were similar between the subgroup of patients who received on-pump surgery and the total cohort (Supplemental Digital Content, table 1, http://links.lww.com/ALN/C76).

Neuronal Injury and Postoperative Delirium

In the sensitivity analysis, postoperative neuronal injury was also associated with delirium. Specifically, after adjusting for the effects of oxidative damage and potential confounders between ubiquitin carboxyl-terminal hydrolase isozyme L1 and delirium, the odds of developing postoperative delirium were, on average, 2.16-fold greater (odds ratio, 2.16 [95% CI, 1.05 to 4.43]; P = 0.043) in patients with postoperative ubiquitin carboxyl-terminal hydrolase isozyme L1 concentrations in the 90th percentile compared to in patients with postoperative ubiquitin carboxyl-terminal hydrolase isozyme L1 concentrations in the 10th percentile.

Discussion

In this cohort of cardiac surgery patients, increased intraoperative oxidative damage was independently associated with the development of postoperative delirium and with postoperative neuronal injury. Moreover, blood–brain barrier disruption modified the relationship between oxidative damage on postoperative neuronal injury—the association between increased intraoperative oxidative damage and postoperative ubiquitin carboxyl-terminal hydrolase isozyme L1 was stronger in patients with increased S100 calcium-binding protein B than in patients with decreased S100 calcium-binding protein B. Thus, these findings indicate that intraoperative systemic oxidative damage may promote postoperative brain dysfunction and injury and that

blood–brain barrier disruption and neuronal injury may contribute to postoperative brain dysfunction.

Clinicians diagnose delirium in patients using neurocognitive instruments that assess inattention, alterations in behavior, and confusion. These clinical manifestations of delirium are hypothesized to be caused by impaired neuronal function and neuronal networks, persistent γ-aminobutyric acid type A receptor current in the hippocampus, alterations of neurotransmitter availability, residual effects of anesthetics, altered microvascular blood flow, and neuroinflammation.[7,37–40] Evidence that postoperative delirium may be a consequence of oxidative damage and direct neuronal injury, conversely, is limited. For example, many previous studies have focused on the role of anesthetics and sedatives in the development of postoperative delirium.[41] This study demonstrates that *intraoperative* oxidative damage, common during surgery and previously noted to be associated with acute kidney and myocardial injury,[11,42] is independently associated with postoperative brain dysfunction and injury.

Intraoperative oxidative damage is a consequence of excess production or decreased elimination of reactive oxygen species, reactive nitrogen species, and lipid radicals. These compounds are generated during surgery secondary to ischemia and reperfusion, hyperoxygenation, mitochondrial dysfunction, and hemolysis.[43,44] The tissue specific source of intraoperative free radicals and end-products of oxidative stress including F_2-isoprostanes and isofurans is unknown, however, as is the impact of systemic oxidants on the brain and the role of the blood–brain barrier on the movement of F_2-isoprostanes and isofurans between the systemic circulation and the cerebrum. F_2-isoprostanes and isofurans do possess vascular biologic activities that could affect the brain,[45] and a previous study of geriatric patients receiving hip surgery noted increased postoperative F_2-isoprostane concentrations in patients with acute brain dysfunction.[46] In a previous study that also used a subgroup of patients who participated in this clinical trial, we noted that intraoperative hyperoxic cerebral reperfusion, measured by cerebral oximetry, was associated with increased postoperative oxidative damage and delirium, and that postoperative oxidative damage partially mediated this association and was independently associated with delirium.[6] The current study expands upon that research to focus on intraoperative oxidative damage and markers of neuron injury and blood–brain barrier disruption, in order to better characterize the time and nature of the cerebral insult.

Intracellular proteins that are released into the plasma when neurons or the blood–brain barrier are damaged provide a measurement of neurologic injury in humans.[26,27] Increased concentrations of ubiquitin carboxyl-terminal hydrolase isozyme L1 have been associated with increased severity of brain injury and poor neurologic outcomes in patients with traumatic head injuries and with cognitive impairment after critical illness,[47,48] and increased plasma concentrations of S100 calcium-binding protein B have been associated with septic encephalopathy and delirium during critical illness.[9,49] The temporal relationships among these markers and delirium and the effect modification of S100 calcium-binding protein B on the association between F_2-isoprostanes and isofurans and ubiquitin carboxyl-terminal hydrolase isozyme L1 in the current study provide evidence that intraoperative systemic oxidative stress may increase neuronal injury and lead to delirium. Delirium, therefore, may be a behavioral manifestation of oxidative damage-induced neuronal injury, and treatments that decrease oxidative damage may be useful to decrease the development of delirium in patients. This warrants further study. To that end, we are currently conducting a clinical trial to further study this mechanism by randomly assigning cardiac surgery patients to intraoperative normoxia or hyperoxia and assessing for oxidative damage and organ injury.[50]

This study has noteworthy strengths and limitations. Specifically, trained research staff prospectively collected delirium outcomes in the setting of a clinical trial; we measured markers of oxidative damage, neuronal injury, and blood–brain barrier disruption at numerous perioperative time points representing nearly 4,000 samples; and the large cohort included multiple types of cardiac surgery, increasing the generalizability of the results. The observational design of the study, however, prevents determinations of causality and is subject to unknown confounding, although we were able to adjust for several important potential baseline and hospital course confounders. In addition, we did not directly measure cellular events in the brain, but did rely upon well-validated surrogate measurements of the injury pathways. We chose these specific plasma markers because previous studies have shown that these markers reflect the processes—oxidative damage, neuronal injury, and blood–brain barrier disruption—we sought to quantify. These markers, however, are not without limitation. Plasma concentrations of ubiquitin carboxyl-terminal hydrolase isozyme L1, for example, reflect neuronal injury but require translocation of ubiquitin carboxyl-terminal hydrolase isozyme L1 from the brain to the systemic circulation *via* passive and active processes. Plasma concentrations of S100 calcium-binding protein B may also be affected by similar efflux mechanisms,[24] also increase when there is brain injury,[51] and may be affected by extracranial sources.[31,33] Each of these characteristics is a potential limitation to examining S100 calcium-binding protein B as a marker of blood–brain barrier disruption. The increased *magnitude of the association* between intraoperative oxidative damage and postoperative ubiquitin carboxyl-terminal hydrolase isozyme L1 that was observed in patients with elevated S100 calcium-binding protein B at the end of surgery (*i.e.,* effect modification), however, supports the conclusions that blood–brain barrier disruption is associated with an increased correlation between oxidative stress and neuronal injury and that ubiquitin carboxyl-terminal hydrolase

isozyme L1 and S100 calcium-binding protein B are not measurements of the same process. These observations are temporally valid and biologically plausible. Studies using cerebrospinal fluid, although difficult in most surgical populations, or a panel approach to assess neuron-enriched proteins in plasma could provide additional valuable data.

In conclusion, intraoperative oxidative damage was independently associated with the development of postoperative delirium and with postoperative neuronal injury. Increased blood–brain barrier disruption modified the association between increased oxidative damage and increased neuronal injury and also was associated with the development of delirium. These findings support the idea that postoperative delirium may, in part, be a result of neuronal injury and that intraoperative oxidative damage may affect this process. Studies that target intraoperative oxidative damage, blood–brain barrier disruption, and neuronal injury are needed to further evaluate the relationships between these mechanisms of delirium and to determine if reducing intraoperative oxidative damage decreases postoperative brain dysfunction and injury.

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Competing Interests

The authors declare no competing interests.

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