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ORIGINAL ARTICLE

Myocardial Oxidative Stress in Infants Undergoing Cardiac Surgery

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Abstract Cardiac surgery for congenital heart disease often necessitates a period of myocardial ischemia during cardiopulmonary bypass and cardioplegic arrest, followed by reperfusion after aortic cross-clamp removal. In experimental models, myocardial ischemia–reperfusion is associated with significant oxidative stress and ventricular dysfunction. A prospective observational study was conducted in infants (<1 year) who underwent elective surgical repair of a ventricular septal defect (VSD) or tetralogy of Fallot (TOF). Blood samples were drawn following anesthetic induction (baseline) and directly from the coronary sinus at 1, 3, 5, and 10 min following aortic cross-clamp removal. Samples were analyzed for oxidant stress using assays for thiobarbituric acid-reactive substances, protein carbonyl, 8-isoprostane, and total antioxidant capacity. For each subject, raw assay data were normalized to individual baseline samples and expressed as fold-change from baseline. Results were compared using a one-sample t test with Bonferroni correction for multiple comparisons. Sixteen patients (ten with TOF and six with VSD) were enrolled in the study, and there were no major postoperative complications observed. For the entire cohort, there was an immediate, rapid increase in myocardial oxidative stress that was sustained for 10 min following aortic cross-clamp removal in all biomarker assays (all $P < 0.01$), except total antioxidant capacity. Infant cardiac surgery is associated with a rapid, robust, and time-dependent increase in myocardial oxidant stress as measured from the coronary sinus in vivo. Future studies with larger enrollment are necessary to assess any association between myocardial oxidative stress and early postoperative outcomes.

Keywords Congenital heart disease · Cardiac surgery · Myocardial oxidative stress · Ischemia/reperfusion injury

Background

Myocardial dysfunction in infants and children after congenital heart disease surgery is a significant risk factor for morbidity and mortality [10, 13]. Twenty years ago, Wernovsky et al. [22] showed that cardiac output falls by approximately 33 % in the first 6 h after surgery for transposition of the great arteries. The precise mechanisms for postoperative myocardial dysfunction are incompletely understood, but are likely related to a combination of ischemia–reperfusion injury following aortic cross-clamp removal and an inflammatory response to cardiopulmonary bypass [23]. Recently, both in vitro and ex vivo studies suggest that generation of oxidized free radicals plays an important etiologic role in myocardial ischemia–reperfusion injury [14].

Infants represent the largest group of pediatric patients who undergo surgery for congenital heart disease and also have the highest morbidity and mortality [7]. Furthermore, infants and neonates may have immature antioxidant defenses and thus be more susceptible to oxidant stress [12,

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18]. In addition, infants with cyanotic congenital heart disease may be even more susceptible to oxidative stress-induced ischemia–reperfusion injury due to depletion or down-regulation of the intrinsic antioxidant pathways [5, 9, 19]. However, the potential contribution of oxidative stress to myocardial dysfunction has yet to be demonstrated in infants undergoing cardiac surgery. Therefore, the objective of this study was to characterize the myocardial oxidative stress response by measuring biomarkers of oxidative stress directly from the coronary sinus of infants undergoing open-heart surgery.

Methods

This was a prospective observational study in infants (<1 year) who were undergoing elective complete repair of tetralogy of Fallot (TOF) or ventricular septal defect (VSD). Exclusion criteria included additional cardiac lesions requiring intervention, preexisting moderate or severe ventricular dysfunction (as assessed by preoperative echocardiogram), preoperative mechanical ventilation, preoperative cardiogenic shock, and previous surgical palliation. Informed consent was obtained from the parents or legal guardians of all patients. The study was approved by the University of Michigan Human Subject Institutional Review Board.

At the time of operation, each patient was induced with anesthesia, intubated and mechanically ventilated, with placement of a right internal jugular central venous catheter and arterial catheter as per standard institutional practices. A baseline blood sample was obtained from the central venous catheter prior to the initiation of cardiopulmonary bypass (CPB). Cardioplegia (modified Buckberg mixed with four parts blood to one part cardioplegia solution) was administered as an initial arresting dose of 30 ml/kg followed by doses of 15 ml/kg as needed for maintenance. Aortic cross-clamping was then performed according to standard techniques. Coronary sinus blood samples were obtained using a 16-gauge Angiocath catheter with the needle removed (Becton, Dickinson and Company, USA) at 1, 3, 5, and 10 min after aortic cross-clamp removal. Blood samples were placed on ice and immediately centrifuged ($2000g$ for 15 min, 4 °C), and serum was stored at −80 °C for later analysis.

Commercially available assays for oxidative stress markers included thiobarbituric acid-reactive substances (TBARS), protein carbonyls, total antioxidant capacity (TAC), and 8-isoprostanes. All assay kits were purchased from Cayman Chemical (Ann Arbor, MI) and were used according to the manufacturer's instructions. All samples and standards were measured in duplicate.

TBARS assay: Lipid peroxidation in serum was evaluated by the spectrophotometric method based on the reaction between malondialdehyde (MDA) and thiobarbituric acid (TBA). The MDA–TBA adduct formed by the reaction of MDA and TBA under high temperature (95–100 °C) and acidic conditions was measured by absorbance at 530 nm.

Protein carbonyls assay: Serum carbonyl contents were measured using reaction between 2, 4-dinitrophenylhydrazine (DNPH) and protein carbonyls. DNPH reacts with protein carbonyls leading to the formation of stable hydrazone products. The amount of protein-hydrazone product was quantified spectrophotometrically by absorbance at 370 nm. The carbonyl content was then standardized to protein concentration.

TAC assay: TAC measurement was based on the ability of serum antioxidants to inhibit oxidation of 2, 2'-azino-di-[3-ethylbenzthiazolinesulfonate] (ABTS) to the ABTS radical by metmyoglobin. The capacity of serum to prevent oxidation of ABTS was compared with that of Trolox (a water-soluble tocopherol analog) and represented as molar Trolox equivalents.

8-isoprostane assay: Serum 8-isoprostane was measured using an enzyme immunoassay kit based on competition between 8-isoprostane and an 8-isoprostane-acetylcholinesterase conjugate (8-isoprostane tracer) for a limited number of 8-isoprostane-specific rabbit antiserum binding sites. This assay was validated directly by spectrophotometry. The antiserum used in this assay has 100 % cross-reactivity with 8-isoprostane, 0.2 % with prostaglandin (PG) F2, PGF3, PGE1, and PGE2, and 0.1 % with 6-keto PGF1α. The detection limit of the assay was 4 pg/ml.

Demographic, clinical, and surgical data were recorded including age, gender, weight, preoperative oxygen saturation, cardiopulmonary and aortic cross-clamp times, intensive care unit (ICU) and hospital lengths of stay, duration of mechanical ventilation, peak lactate levels in the first 48 postoperative hours, vasoactive-inotropic support (as calculated by peak vasoactive-inotropic score (VIS))[6], and clinically important postoperative events such as arrhythmias, cardiopulmonary resuscitation, cannulation for extracorporeal membrane oxygenation (ECMO), or death.

Statistics

Demographic and clinical data were expressed as frequency with percentage for categorical variables, and as median with interquartile range (IQR) or mean ± standard deviation (SD), as appropriate, for continuous variables. Results for oxidative stress biomarker assays for each patient were compared with the individual patient's baseline sample and expressed as a fold of control. Fold-change from baseline at each time point for individual oxidative stress biomarker assays was compared using one-sample

t test. A P value <0.01 was considered indicative of statistical significance with application of Bonferroni correction for multiple comparisons.

Results

Sixteen patients (mean age 4.7 ± SD 2.0 months) were included in the study. Demographic data and clinical variables are shown in Table 1. Six patients underwent surgical repair of VSD, and ten patients had a complete repair of TOF. Overall hospital survival was 100 %, and four patients had postoperative arrhythmias (all junctional ectopic tachycardia) with an equal distribution among the surgical groups. No patients required cardiopulmonary resuscitation or ECMO. The mean peak lactate level in the first 24 h was 1.6 ± SD 0.5 mmol/l, and the median peak VIS in the first 24 h was 5 (interquartile range [IQR] 1.5–9). The median duration of mechanical ventilation was 16.6 h (IQR 8.3–40.3). Median ICU length of stay was 2.5 days (IQR 1.5–3.5), and median hospital length of stay was 6 days (IQR 5–7).

The magnitude of change and time course of coronary sinus oxidative stress biomarkers are displayed in Table 2 and Fig. 1. Following aortic cross-clamp removal, there was a significant rise in TBARS, 8-isoprostanes, and protein carbonyls from the coronary sinus, with peak measurements generally occurring between 3 and 5 min after myocardial reperfusion (Fig. 1). Although not reaching statistical significance, there was a trend toward a small decrease in myocardial TAC during reperfusion.

Discussion

To our knowledge, this is the first study in pediatric patients to measure myocardial oxidative stress biomarkers directly from the coronary sinus after congenital heart surgery. Our results suggest that there is a rapid, robust, and time-dependent increase in oxidant stress following aortic cross-clamp removal during myocardial reperfusion.

Most surgeries in children with congenital heart disease require cardiopulmonary bypass and cardioplegic arrest of the myocardium. Cardiopulmonary bypass has been shown to cause a systemic inflammatory response due to direct contact of red blood cells with a foreign surface, hemolysis, and hypothermia. During cardiopulmonary bypass, there is activation of the complement cascade as well as activation

Table 1 Patient and clinical characteristics ($N = 16$)

Male sex	8 (50.0)
Age at surgical repair, months	4.7 ± 2.0
Type of surgical repair	
VSD	6 (37.5)
TOF	10 (62.5)
Preoperative oxygen saturation (%)	94.8 ± 5.1
VSD	97.8 ± 1.9
TOF	93.0 ± 5.6
Preoperative weight, kg	6.0 ± 1.6
CPB time, minutes	89.2 ± 34.4
AXC time, minutes	44.3 ± 20.4
Postoperative maximum serum lactate, mmol/l	
0–24 h	1.6 ± 0.5
24–48 h ($N = 11$)	1.5 ± 0.5
Postoperative maximum VIS	
0–24 h	5 (1.5–9)
24–48 h	0 (0–6.5)
Postoperative arrhythmia	4 (25.0)
Duration of initial postoperative ventilation, hours	16.6 (8.3–40.3)
ICU length of stay, days	2.5 (1.5–3.5)
Hospital length of stay, days	6 (5–7)
Survived to hospital discharge	16 (100.0)

VSD ventricular septal defect, *TOF* tetralogy of Fallot, *CPB* cardiopulmonary bypass, *AXC* aortic cross-clamp, *VIS* vasoactive-inotropic score, *ICU* intensive care unit

Table 2 Oxidative stress biomarker assays

Biomarker assays	Fold of control	
Control	Mean ± SD Ref	P value[†]
TBARS assay		
1 min after AXC removal	1.39 ± 0.35	0.002
3 min after AXC removal	1.40 ± 0.27	<.0001
5 min after AXC removal	1.40 ± 0.43	0.003
10 min after AXC removal	1.19 ± 0.16	0.004
8-isoprostane assay		
1 min after AXC removal	3.07 ± 1.81	0.001
3 min after AXC removal	3.09 ± 1.47	<.0001
5 min after AXC removal	2.67 ± 0.82	<.0001
10 min after AXC removal	2.92 ± 1.02	<.0001
Protein carbonyl		
1 min after AXC removal	1.52 ± 0.55	0.005
3 min after AXC removal	1.41 ± 0.50	0.005
5 min after AXC removal	1.63 ± 0.49	0.0002
10 min after AXC removal	1.43 ± 0.44	0.009
Total antioxidant capacity		
1 min after AXC removal	0.88 ± 0.55	0.45
3 min after AXC removal	0.91 ± 0.47	0.43
5 min after AXC removal	0.93 ± 0.50	0.58
10 min after AXC removal	1.04 ± 0.61	0.84

AXC aortic cross-clamp, *ref* reference, *TBARS* thiobarbituric acid-reactive substances

[†] P value from one-sample t test

Fig. 1 Time course of biomarkers of myocardial oxidative stress after myocardial reperfusion. The fold-change for each biomarker as compared to baseline is statistically significant except for the total antioxidant capacity. *TBARS* thiobarbituric acid-reactive substances, *AXC* aortic cross-clamp, *TAC* total antioxidant capacity
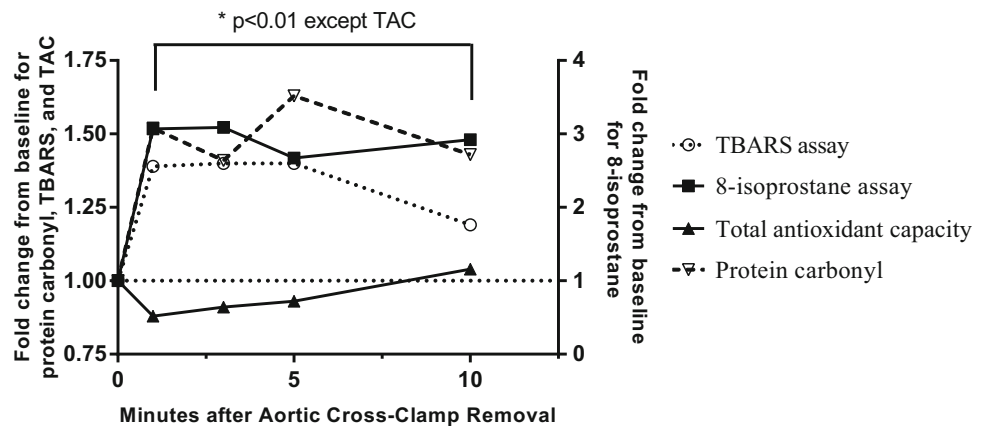
of leukocytes and platelets, leading to cytokine release and endothelial activation. As a result, there can be direct tissue injury and capillary leak, which can eventually lead to myocardial dysfunction. As the previously ischemic myocardium is reperfused with oxygen, there is generation of reactive oxygen species and activation of other inflammatory pathways via cytokines [20]. Reactive oxygen species lead to lipid peroxidation and protein changes which cause myocyte injury and cell death via direct damage or apoptosis [8].

In this study, four different biomarkers were measured from the coronary sinus after ischemia–reperfusion injury, as we hypothesized that differentially affected metabolic pathways may impact the overall oxidative stress response [15]. Oxidation of lipids leads to the generation of lipid peroxides which degrade into malondialdehydes and were detected via a reaction with thiobarbituric acid [17]. Lipid peroxidation was also assessed by measuring 8-isoprostanes, which are a product of non-enzymatic peroxidation of arachidonic acid and have been shown to be reliable measures of lipid peroxidation both in vitro and in vivo [16]. We next assessed protein carbonyls, which are generated upon oxidation of proteins [4]. Finally, we incorporated a total antioxidant assay to assess the amount of remaining antioxidants within the myocardium with the assumption that increased myocardial oxidative stress would deplete the antioxidants within the myocardium. In total, therefore, our data are a reasonable gauge of the overall degree of oxidant stress in this patient cohort.

Studies in adult patients undergoing coronary artery surgery have demonstrated the release of reactive oxygen species and an increase in oxidative stress biomarkers as measured from the coronary sinus [2, 3, 21] during myocardial reperfusion. Other studies in adults have suggested a relationship between the levels of myocardial oxidative stress and postoperative myocardial function, either via reduction in cardiac index [1] or via release of

cardiac enzymes [21]. The only published study to date in pediatrics involved a heterogeneous group of patients and demonstrated that oxidative stress markers measured systemically after surgery did not correlate with the development of low cardiac output syndrome [11]. The study was limited by the heterogeneity of the patients and the fact that oxidative stress measurements were recorded only from peripheral blood and thus were not a true measure of myocardial oxidative stress. Other studies have shown that peripheral measurements did not correlate with samples obtained from the coronary sinus [21].

In conclusion, in this study, we were able to measure accurately and reliably biomarkers of oxidative stress directly from the myocardium, and we were able to demonstrate a rapid increase in oxidative stress during myocardial reperfusion. One technical limitation of the study was that there was no true control sample for comparison with the coronary sinus samples. In addition, we found that it was technically difficult to obtain an accurate sample from the coronary sinus prior to aortic cross-clamp removal. Perhaps most notably, this patient population had a low incidence of low cardiac output syndrome or poor postoperative outcome. Therefore, we were unable to examine an association of levels of oxidative stress with clinical outcomes. In addition, the sample size limited any analysis of the link between preoperative cyanosis and a change in the oxidative stress response. Future studies are needed in a larger cohort of patients with a higher risk of postoperative low cardiac output to be able to define further the link between oxidative stress, ischemia–reperfusion injury, and myocardial dysfunction. Data from these future studies could allow for development of novel therapies to limit the oxidative stress of the myocardium in this patient population.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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