Circulating matrix metalloproteinase levels after ventricular septal defect repair in infants

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Background: Surgery for congenital heart disease initiates a complex inflammatory response that can influence the postoperative course. However, broad integration of the cytokine and proteolytic cascades (matrix metalloproteinases: MMPs), which may contribute to postoperative outcomes, has not been performed.

Methods and Results: Using a low-volume (50–60 μL), high-sensitivity, multiplex approach, we serially measured a panel of cytokines (interleukins 2, 4, 6, 8, and 10, tumor necrosis factor alpha, interleukin 1β, and granulocyte-macrophage colony stimulating factor) and matrix metalloproteinases (matrix metalloproteinases 2, 3, 7, 8, 9, 12, and 13) in patients (n = 9) preoperatively and after repair of ventricular septal defect. Results were correlated with outcomes such as inotropic requirement, oxygenation, and fluid balance. Serial changes in perioperative plasma levels of the cytokines and matrix metalloproteinases exhibited distinct temporal profiles. Plasma levels of interleukins 2, 8, and 10 and matrix metalloproteinase 9 peaked within 4 hours, whereas levels of matrix metalloproteinase 3 and 8 remained elevated at 24 and 48 hours after cross-clamp removal. Area-under-the-curve analysis of early cytokine levels were associated with major clinical variables, including inverse correlations between early interleukin 10 levels and cumulative inotrope requirement at 48 hours (r: –0.85; $P < .005$) and late matrix metalloproteinase 7 levels and cumulative fluid balance (r: –0.90; $P < .001$).

Conclusions: The unique findings of this study were that serial profiling a large array of cytokines and proteolytic enzymes after surgery for congenital heart disease can provide insight into relationships between changes in bioactive molecules to early postoperative outcomes. Specific patterns of cytokine and matrix metalloproteinase release may hold significance as biomarkers for predicting and managing the postoperative course after surgery for congenital heart disease. (J Thorac Cardiovasc Surg 2010;140:1257-65)

The importance of post–cardiopulmonary bypass (CPB) inflammation in pediatric cardiac surgery is reflected in the many interventions directed at its reduction. Steroid administration, modification of pump circuit surfaces by heparin bonding, ultrafiltration strategies, leukocyte trapping filters, reduced post-CPB oxygen exposure, and monoclonal antibody administration have all been described to modify features of the post-CPB inflammatory response in newborns, infants, and children.[1] Despite this, a survey of 36 centers performing pediatric CPB[1] revealed that no anti-inflammatory strategy achieved the level of standard practice in the pediatric CPB population, reflecting absence of convincing data to guide therapies. CPB engenders inflammation through multiple mechanisms, including direct tissue injury, myocardial ischemia/reperfusion, neutrophil/platelet activation from the CPB circuit, and lipopolysaccharide exposure.[2] The molecular events of inflammation include synthesis and release of cytokines, which in turn induce complex and context-dependent cellular and molecular events, including release of matrix metalloproteinases (MMPs).[3] The characteristic pleiotropy, redundancy, and complexity of the inflammatory response have complicated efforts to define this process in infants owing to the number of inflammatory biomarkers to consider in relation to relative volume of blood available for sampling. This study was performed to address this issue through the use of high-sensitivity multiplex assays to allow serial measurement of cytokine and MMP levels from repeated small-volume blood sampling in a population of infants undergoing repair of ventricular septal defect (VSD). This study provided the first opportunity to perform a comprehensive cytokine/MMP profile and begin to identify potential relationships between the inflammatory response and clinical outcomes.

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Abbreviations and Acronyms

AUC = area under the curve
BSA = body surface area
CPB = cardiopulmonary bypass
GM-CSF = granulocyte-macrophage colony stimulating factor
IL = interleukin
INF-γ = interferon gamma
MMP = matrix metalloproteinase
Th = T-helper
TNFα = tumor necrosis factor alpha
VSD = ventricular septal defect

METHODS

Patients

This study was approved by the Medical University of South Carolina Institutional Review Board (HR 16017 and HR 17161). Entry criteria for the VSD group were planned complete surgical repair of the anatomic defect and age from 1 to 9 months. Exclusion criteria were recognized chromosomal anomalies, prior intracardiac surgery, or complex heart disease. Enrollment was from January 2006 through June 2007, and the cardiac surgeons remained constant during this interval (F.A.C., S.M.B.). The conduct of CPB was performed in identical fashion and aprotinin was used in all cases. Standard nonpulsatile CPB was used, and the circuit was primed with electrolyte solution (PlasmaLyte A; Baxter Healthcare Corporation, Deerfield, Ill) and 1 unit of fresh-frozen plasma. Banked, packed red blood cells were added to achieve a hematocrit value of approximately 28% to 30% during CPB. No steroids were administered preoperatively or in the CPB prime. Moderate (25°C–28°C) hypothermia was used, and myocardial preservation was obtained with cold blood cardioplegia at 20-minutes intervals. The pH-stat regimen was used during cooling and alpha-stat for rewarming. Modified ultrafiltration was performed after separation from CPB. Protamine was given at a 0.6:1 protamine/heparin ratio. Blood product transfusions after CPB were administered as necessary to achieve satisfactory hemostasis and a target hematocrit value of more than 30%. Standard transatrial closure of the VSD was used in all patients. The aprotinin dose consisted of both an intravenous and pump prime load of 240 mg/m^2 body surface area (BSA) (1.7×10^6 kIU/m^2 BSA) and a continuous infusion at 56 mg/m^2 BSA/h (4×10^5 kIU/m^2 BSA/h) until the completion of the primary procedure. For the purposes of providing a referent control range, a group of 10 infants were recruited from patients undergoing ambulatory noncardiac surgery. In this group, a 1-time venous blood collection (1 mL) was performed at the time of intravenous cannulation.

Clinical Data Collection

Clinical characteristics such as age, weight, and BSA, as well as z-score for weight, were recorded. Data recorded or calculated during the 48-hour study period included CPB and crossclamp times, arterial blood gases, blood counts, and arterial–alveolar oxygen gradients at 4 hours. Inotrope score was calculated as dopamine ([μg · kg^{-1} · min^{-1}] × h) + epinephrine ([μg · kg^{-1} · min^{-1}] × h × 100) + milrinone ([μg · kg^{-1} · min^{-1}] × h × 10). Fluid balance was calculated as total fluid administered (all sources) minus total fluid out (all sources). Inotrope score, fluid balance, and near-infrared spectroscopy were analyzed as cumulative values from time of arrival in the postoperative care unit. In addition, blood product use was recorded during the operative and perioperative periods.

Blood Collection and Processing

Baseline blood (1 mL) was collected in the operating room before the incision into a chilled ethylenediaminetetraacetic acid tube. Plasma was isolated by centrifugation, decanted into aliquots, and stored at –70°C until processed for immunoassays. Subsequent 1-mL samples were obtained after modified ultrafiltration and 4, 12, 24, and 48 hours after crossclamp removal and processed identically. Crossclamp removal (onset of myocardial reperfusion injury) was the reference time 0 for postoperative measurements.

Quantitative Measures of Cytokines and MMPs

Plasma levels of cytokines and MMPs were determined by multiplex suspension array using calibrated and validated combinatorial immunoassays (R&D Systems, Minneapolis, Minn). For cytokines, measurements were made from undiluted plasma. For MMP analysis, plasma was diluted 1:10 for MMP-3, -7, -8, -12, and -13 and 1:100 for MMP-2 and -9. The identification and quantification of the analyte/bead complexes were determined by flow cytometry with dual excitation lasers (the excitation and emission wavelengths are 532 nm and 575 nm, respectively; Bio-Plex Suspension Array Workstation; Bio-Rad, Hercules, Calif). Each analyte concentration was calculated from an analyte-specific 5-parameter logistic calibration equation (Bio-Plex Manager Software 4.1.1). Average sensitivities for cytokines were 0.3 pg/mL and 5 pg/mL for MMP/tissue inhibitors of metalloproteinases. The cytokine and MMP multiplex assays have less than 0.5% cross-reactivity and interference with the other measured analytes. Plasma values were corrected for hemodilution using simultaneously obtained hematocrit values.

Data Analysis

Patient demographics and preoperative values for plasma levels of cytokines and MMPs were compared between the control and VSD groups by the Kruskal–Wallis test of medians. Comparisons were withheld if the analyte failed to be detectable in at least 25% of the samples for each group. For postoperative analysis, 2 area-under-the-curve (AUC) values were derived from the time course of expression for each analyte. Early AUC was determined as the dose × time product (summation of the areas of individual trapezoids) for each analyte from baseline to the 4-hour post-crossclamp time point. Late AUC was computed in the same fashion for the 4- to 48-hour interval. For AUC determinations, an undetectable level was set at 0. Correlations between AUCs for each cytokine/MMP and clinical outcomes, as well as between early and late AUCs, were examined by the Spearman correlation analysis. Statistical tests were performed using the Stata software package (Stata Intercooled, v8.0) or SAS (SAS, Inc, Cary, NC).

RESULTS

Demographics

The patients undergoing VSD closure were 4.3 ± 0.5 months old, whereas the referent normal control subjects were older (7.8 ± 1.3 months; $P < .05$), but nevertheless could be used for the purposes of generating a referent normal range for the cytokine and MMP assays. The male/female ratio in the VSD group was 4:5 and similar in the referent control group (5:5). Preoperative white blood counts, platelet counts, and serum measurements of renal function (electrolytes, blood urea nitrogen, and creatinine, not shown) were all within the normal range for the VSD patients. Crossclamp time, CPB time, intubation times, cumulative introtrope score, fluid balance, near infrared spectroscopy, and blood product use for the VSD patients are summarized in Table 1.