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Effects of Leukocyte Depletion Filters on Matrix Metalloproteinase Activation in an Extracorporeal Circulation Circuit

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Abstract: Matrix metalloproteinases (MMPs) are a family of endopeptidases that function to remodel tissue during both normal physiology and pathology. Research performed by the Medical University of South Carolina found an increased release of several MMP species during cardiopulmonary bypass (CPB), including the subtype MMP-9, but whether and to what degree the extracorporeal circulation circuit (ECC) induces the release of MMPs has yet to be determined. Human bank whole blood scheduled for discard was obtained and exposed to an ECC. The first set of studies ($N = 8$) was performed with a loop circuit using a standard arterial line filter. A leukoreduction filter was incorporated during the first 30 min of the pump run for the second set of trials; the leukoreduction filter was then bypassed and a standard arterial filter used for the remaining 60 minutes on pump ($N = 8$). Blood samples were drawn at four time points for analysis (baseline, 30, 60, and 90 min). Data were analyzed using repeated measures analysis of variance with between-subjects factors, and a p value of less than .1 was considered statistically significant. The MMP-9 level increased by 130.44% at 90 min on pump in the standard arterial filter group and decreased by 34.62% at 90 min on pump in the leukoreduction group. There was a significant difference between the baseline MMP-9 level and the MMP-9 concentrations at 30, 60, and 90 min for both groups ($p = .0348$); there was a significant difference in MMP-9 levels between the two filter groups ($p = .0611$). The present study found a significant increase in MMP-9 levels when blood was exposed to an ECC with a standard arterial filter. The use of a leukoreduction filter significantly reduced MMP-9 concentrations as compared to baseline levels in this study. Leukocyte depletion filtration may serve to benefit CPB patients by attenuating the inflammatory response and disrupting pathways that govern such mediators as the MMPs. **Keywords:** matrix metalloproteinases, MMP-9, leukocyte depletion, cardiopulmonary bypass, inflammatory response. *JECT. 2003; 35:139–142*

Matrix metalloproteinases (MMPs) are a family of endopeptidases that have roles in both normal physiology and pathology. MMPs have the ability to remodel the extracellular matrix via enzymatic degradation of many substances; subtypes in the MMP family are named for substrate specificity (1,2). Under normal conditions, a balance exists between the degradation and reconstructive functions of these enzymes. When this balance is disrupted, pathological pathways may be developed or enhanced as the levels of MMPs rise. The activity of these enzymes is a focus of research in many areas of medical science because their mechanisms and interactions are not clearly understood.

Investigators have linked the interaction between the MMP cascade and the inflammatory response to adverse outcomes for cardiac patients. A subtype of the MMP family known as gelatinase-B, or MMP-9, degrades collagen, gelatin, proteoglycan, and elastin. One consequence of increased MMP-9 levels is degradation of the basement membrane, which results in compromise of the endothelial vascular barrier. Once the integrity of this barrier is disrupted, fluid egression into the extravascular space occurs. Potential sequelae of cardiopulmonary bypass (CPB) include edema, reperfusion injury, and associated tissue dysfunction of many organ systems in the postoperative period (3).

Research by the Medical University of South Carolina (MUSC) found that there is an increased release of several

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MMP species during CPB, including MMP-9 (4). These data were obtained from an in vivo study of CPB patients and possible multifactorial causes of increased MMP concentrations existed. The present study's design limits the number of factors contributing to MMP activation by using an extracorporeal circulation circuit (ECC) for in vitro trials.

Leukocytes are capable of MMP production and release associated with the inflammatory response. Earlier research has shown that leukocyte-depleting filters selectively reduce the circulating levels of activated neutrophils without removing lymphocytes or platelets (5,6). The purpose of this study was to evaluate MMP-9 activity in whole blood exposed to an ECC and to determine if the interposition of a leukoreduction filter would modify the response.

METHODS

Blood Source

Human bank whole blood scheduled for discard was obtained for use in this study from Transfusion Medicine Laboratory Services at MUSC. This study qualifies under the exempt research review status determined by the Institutional Review Board of MUSC.

Circuit Specifications

The ECC design consisted of a pediatric membrane oxygenator with integral heat exchanger (Micro, Cobe Cardiovascular, Inc., Arvada, CO), an arterial filter for the control circuit (Adult Extracorporeal Filter, Pall Corporation, Ann Arbor, MI), a leukocyte depletion filter (LeukoGuard, Pall Corporation, Ann Arbor, MI) for the treatment circuit, and ¼″ heparin-coated tubing (Edwards Lifesciences, Irvine, CA). Total priming volume for the circuit was 500 mL (±50 mL); a balanced electrolyte prime solution containing 5000 iu of heparin was used. All trials were performed with a positive displacement pump (Stockert Instrumente, Munich, Germany).

Experimental Design

Sixteen independent samples were exposed in vitro to an ECC for 90 min per trial. The first set of studies ($N = 8$) was performed with a loop circuit using a standard arterial line filter. A leukoreduction filter was incorporated during the first 30 min of the pump run for the second set of studies; the leukoreduction filter was bypassed, and a standard arterial filter was used for the remaining 60 min in these trials ($N = 8$). Normothermic (37°C) temperature was maintained. Blood samples were drawn at four time points for analysis (baseline, 30, 60, and 90 min) during each trial. Collected blood samples underwent centrifugation and plasma from each sample was stored at –20°C in individual aliquots of 500 μL each.

MMP-9 Analysis

The concentrations of MMP-9 were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Amersham Pharmacia Biotech, Buckingamshire, England).

Data Analysis

The MMP-9 concentrations were corrected for hemodilution (Appendix A). Because of the age of the blood samples used, baseline MMP-9 levels varied among the subjects. To compare trends of MMP-9 activity for the two filter groups, values are reported as the mean percentage change from baseline MMP-9 level (7).

Data were analyzed using repeated measures analysis of variance (ANOVA) with between-subjects factors, and a p value of less than .1 was considered statistically significant. Repeated measures ANOVA takes into account dependent variability within subject samples collected over the 90-min time period. This test is able to separate variability within the subjects of both groups from variability between the two filter groups. A repeated measures ANOVA approach avoids underestimation of variance among the dependent variables and false elevation of power for the study because the correlation between repeated measures is modeled. Mauchly's sphericity test indicated that a multivariate-repeated measures ANOVA was appropriate for these data.

RESULTS

The MMP-9 level increased by 130.44% at 90 min on pump in the standard arterial filter group and decreased by 34.62% at 90 min on pump in the leukoreduction group (Figure 1). There was a significant difference between the baseline MMP-9 level and the MMP-9 concentrations at 30, 60, and 90 min on pump for both of the groups ($p = .0348$); there was a significant difference in MMP-9 levels between the two filter groups ($p = .0611$; Table 1).

DISCUSSION

Previous investigation in this institution found that specific MMP species function during and after CPB, but whether and to what degree the ECC induces the release of MMPs from endogenous circulating cells in the blood has yet to be determined. Using bank blood and an ECC for this study, it was possible to look at MMP-9 expression caused by contact activation during a pump run. The present study found a significant increase in MMP-9 levels when blood was exposed to an ECC with a standard arterial filter. The contact activation that occurs during CPB attributable to such physical factors as shear force leads to disruption of endothelial cells and neutrophils, causing them to release proteases, free radicals, and other harmful

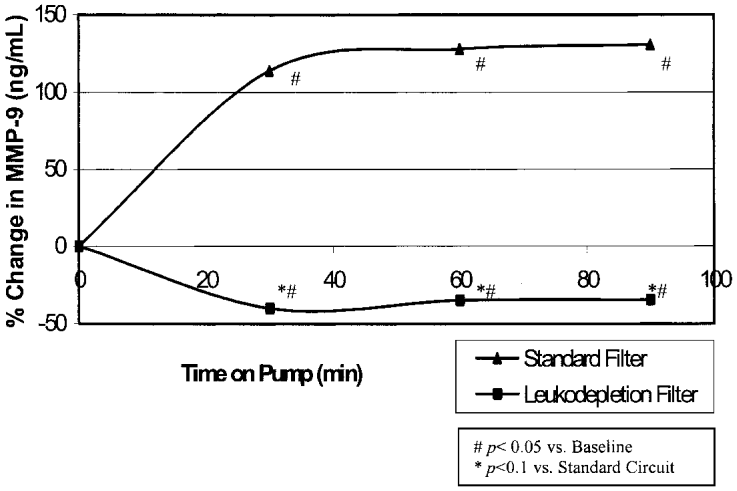

Figure 1. MMP-9 concentration percentage change from baseline. (min = minutes; MMP-9 = matrix metalloproteinase 9; ng/mL = nanograms per milliter; % = percentage; vs. = versus).

Table 1. Repeated measures analysis of variance

Effects	F value	*p* value
Between subjects (filter type)	4.15	0.0611
Within subjects (time interaction)	4.81	0.0348
Within subjects (time ∗ group)	3.96	0.0546

substances. Research by Galt, Lindemann, Medd, et al. found that interactions between activated platelets and leukocytes with collagen further induce MMP-9 expression (8). Once MMP-9 expression is initiated in a damaged region of tissue, platelet and leukocyte aggregation in the area may be enhanced as MMP-9 continues to degrade the membrane matrix, and more MMP-9 is recruited.

It is widely acknowledged that the systemic inflammatory response resulting from CPB factors in intraoperative and postoperative outcomes for cardiac patients. MMPs comprise a complex enzyme cascade that is initiated and upregulated by both pro-enzymes within the pathway and extrinsic factors. Once signaling results in expression of these enzymes, individual MMP species are capable of degrading many substrates. Each MMP may serve multiple roles in the regulation and performance of tissue remodeling processes, cell migration, angiogenesis, and extravasation actions.

Attenuation of the inflammatory response induced by CPB may be achieved by selectively disrupting the pathways that govern mediators like the MMPs. Leukocyte reduction technology may be advantageous in reducing MMP-related dysfunction both during and after CPB procedures. The use of a leukoreduction filter significantly reduced MMP-9 concentrations as compared to baseline levels in this study. This technology has been shown to lessen harmful effects of reperfusion injury, cardioplegia delivery, circulatory arrest, blood transfusion, and possibly other aspects of CPB (9–11). Leukocyte depletion has been shown to provide protection to the pulmonary system of patients undergoing CPB (12). Although Steinberg, Fink, Picone, et al. were unable to correlate increased MMP concentration during CPB with decreased pulmonary function, other research has shown attenuation of respiratory dysfunction via reduction of sequestered neutrophils using specific tissue inhibitors of MMP expression (TIMPs) (13–15).

Because it is released from platelets, MMP-2 activity has been studied during extracorporeal circulation procedures to determine its effects on platelet aggregation, dysfunction, and inhibition (16). Vascular remodeling of vein grafts and instability of the pseudointima in prosthetic vascular implants has been connected with MMP expression as well (17,18). In a study of Marfan's syndrome patients, thoracic aortic aneurysms and aortic valve insufficiency were evaluated for evidence of MMP-induced damage (19). Abnormal matrix components, such as an altered form of fibrillin-1, may predispose these patients to higher levels of MMP degradation. These studies reveal the importance of understanding the array of interactions that involve MMPs to target attenuation of pathological activity in the perioperative period for cardiac surgery patients.

A better understanding of the molecular mechanisms functioning during the perioperative period is essential for preventing such adverse effects of CPB as postoperative organ dysfunction and circulatory instability. In a recent study, increased MMP-9 concentration was linked with the inflammatory response occurring in cerebral tissue following ischemic injury (20). The authors found that inhibition of MMPs could decrease infarct size and minimize damage caused by leukocyte infiltration associated with reperfusion injury.

A similar mechanism may function during reperfusion of the myocardium following ischemic injury. The myocardial extracellular matrix is composed of collagen, proteoglycan, and other molecules known to interact with MMPs. Changes that occur in the collagen structure of

the myocardial matrix may be responsible for left ventricle (LV) dysfunction associated with reperfusion injury during CPB. Early mechanisms of LV remodeling in congestive heart failure have been related to MMP activity (21,22).

Future study of MMP activity during CPB should focus on finding efficient methods of modifying the response. Natural or synthetic TIMPs and such mechanisms of protein binding inactivation as the alpha-2 macroglobulin perhaps could be investigated for use in the bypass circuit to inhibit selectively MMPs known to function in the myocardium. Heparin-coating has also been used during CPB to decrease release complement, selectin, lactoferrin, elastase, interleukins, tumor necrosis factor, and other mediators of the inflammatory response that may contribute to upregulation of MMPs (23–25). Filtration devices may serve to benefit patients by eliminating the neutrophils, cytokines, growth factors, and enzymes in circulation that are responsible for activating these degradative proteolytic enzymes.

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APPENDIX A

Correction for Hemodilution Calculation:

$$\frac{\text{MMP-9 level at time point}}{((\text{baseline HCT} - \text{HCT at time point})/\text{baseline HCT})}$$