Plasma matrix metalloproteinases in neonates having surgery for congenital heart disease

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Abstract

During cardiopulmonary-bypass matrix-metalloproteinases released may contribute to ventricular dysfunction. This study was to determine plasma matrix-metalloproteinases in neonates after cardiopulmonary-bypass and their relation to post-operative course. A prospective observational study included 18 neonates having cardiac surgery. Plasma matrix-metalloproteinases-2 and 9 activities were measured by gelatin-zymography pre-operatively, on starting cardiopulmonary-bypass, 7-8 min after aortic cross-clamp release, and 1h, 4h, 24h, and 3d after cardiopulmonary-bypass. Plasma concentrations of their tissue inhibitors 1 and 2 were determined by enzyme-linked immunosorbent assay. Cardiac function was assessed by serial echocardiography. Paired t-tests and Wilcoxon tests were used to assess temporal changes, and linear correlation with simultaneous clinical and cardiac function parameters were assessed using Pearson's product-moment correlation coefficient. Plasma matrix-metalloproteinases activities and their tissue inhibitor concentrations decreased during cardiopulmonary-bypass. Matrix-metalloproteinase-2 plasma activity increased progressively starting 1h after cardiopulmonary-bypass and returned to pre-operative levels at 24h. Matrix-metalloproteinase-9 plasma activity increased significantly after release of aortic cross-clamp, peaked 7-8min later, and returned to baseline at 24h. Plasma tissue-inhibitor 1 and 2 concentrations increased 1h after cardiopulmonary-bypass. Cardiac function improved from 4h to 3d after surgery ($p<0.05$). There was no evidence of significant correlations between matrix-metalloproteinases or their inhibitors and cardiac function, inotrope scores, organ dysfunction scores, ventilation days, or hospital days. The temporal profile of plasma matrix-metalloproteinases and their inhibitors after cardiopulmonary-bypass in neonates are similar to adults. In neonates, further study should determine whether circulating matrix-metalloproteinases are useful biomarkers of disease activity locally within the myocardium, and hence of clinical outcomes.

Introduction

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases known for their role in tissue remodeling through the degradation of extracellular matrix components,[1] potentially contributing to capillary permeability and neutrophil exudation in inflammatory states.[1,2] Matrix metalloproteinases are inhibited by tissue inhibitors of matrix metalloproteinases (TIMPs), and imbalances between activation and inhibition of MMPs may result in uncontrolled proteolysis. Matrix metalloproteinases-2 and 9 have been shown to be involved in the development of cardiac disease, including congestive heart failure and myocardial infarction.[3-6] In addition to a role in remodeling of extracellular matrix, MMP2 and 9 are involved in acute processes such as the aggregation of platelets[7] and regulation of vascular tone.[8] Recently, MMP2 was found to be activated and to degrade troponin I during reperfusion of isolated rat hearts following ischemia, and this activity was associated with acute left ventricular dysfunction.[9]

Myocardial dysfunction attributed to reperfusion of transiently ischemic myocardium is commonly observed after cardiac surgery with cardiopulmonary bypass (CPB). The activation of MMPs has been suggested as one possible mechanism.[10] In isolated rat hearts subjected to ischemia-reperfusion, MMP2 activity in the coronary effluent was increased, and correlated positively with duration of ischemia and negatively with recovery of mechanical function after reperfusion; moreover, mechanical dysfunction after ischemia-reperfusion was improved by MMP2 antibody and worsened by semi-purified MMP2 infusion.[10] Plasma and myocardial MMP2 and 9 activities in adults having CPB for coronary artery bypass grafting

increase in a specific temporal pattern, [11-14] and myocardial MMP2 and 9 activities correlate negatively with ventricular function after surgery.[15]

To our knowledge, little is known about the temporal profile of MMPs and TIMPs in the plasma of neonates undergoing cardiac surgery for congenital heart disease. We therefore designed this prospective cohort pilot study to examine the temporal changes of plasma MMP2 and 9 activities and plasma TIMP1 and 2 concentrations in neonates with congenital heart disease having intracardiac surgery, and to describe their relationship to post-operative clinical course and cardiac function.

Design and Methods

Patient population and recruitment

Neonates equal to or less than six weeks of age who were to undergo surgery with CPB for congenital heart disease were identified upon admission to the Neonatal Intensive Care Unit. Exclusion criteria included: prior CPB or surgery in the previous two weeks; and documented infection within four days of surgery, defined as a positive blood culture or a clinical diagnosis of pneumonia, urinary tract infection or wound infection requiring a course of antibiotic treatment. The study was approved by the institutional Health Research Ethics Board and was performed in accordance with the ethical standards of the Helsinki Declaration. Following signed informed legal guardian consent, prospective data was collected. Demographic data, pre-operative clinical state, operative details, and post-operative course (including the duration of mechanical ventilation, hospital length of stay, inotrope scores,[16] and Pediatric Logistic Organ Dysfunction Scores[17]) were recorded.

Blood sample collection

Arterial blood samples (0.5 mL) were collected in glass tubes containing 3.15% sodium citrate (9:1, v:v) and immediately centrifuged (3000 g, 10min, 4°C). The plasma fraction was then removed, frozen in liquid nitrogen, and stored at –80°C. Blood samples were obtained from arterial catheters less than 24h prior to surgery (designated as t1). Intra-operative samples were collected at the following times: immediately after cannulation and institution of CPB (baseline, t2), immediately after the release of the aortic cross-clamp (t3), and 7-8min after release of the aortic cross-clamp (t4). After separation from CPB, samples were obtained at 1±0.5h (t5), 4±0.5h (t6), 18-24h (t7) and 66-72h (t8). Unless specified otherwise, measurements were collected at each of these time points for MMPs and TIMPs assays.

Measurement of matrix metalloproteinases-2 and 9 plasma activities

Gelatinolytic activities of plasma MMPs were examined by zymography as previously described.[18] Briefly, samples in sodium dodecylsulphate buffer were subjected to 8% polyacrylamide separating gels containing gelatin (2 mg/mL, Sigma). The supernatant of human fibrosarcoma HT1080 cells (American Type Culture Collection, Rockville, MD, USA) was used as a standard for MMPs. Following electrophoresis, the gels were washed with 2.5% Triton X-100 and incubated overnight at 37°C in 50 mM Tris-HCl buffer (0.15 M NaCl, 5 mM $CaCl_2$, 50 mM Tris HCl and 0.05% NaN_3, pH 7.6). Gelatinolytic activities were visualized as transparent bands against a Coomassie Blue stained background. The gels were scanned and analyzed using SigmaGel measurement software (Jandel Corporation, San Rafael, CA, USA). Matrix metalloproteinase activity was calculated as per amount of protein and expressed in arbitrary units (AU). The plasma protein concentration was assessed by bicichoninic acid assay (Sigma) using bovine serum albumin as the standard.

Quantification of tissue inhibitor of metalloproteinase-1 and 2 plasma concentrations

The plasma concentrations of TIMP1 and 2 were determined by commercially available enzyme immunoassays (Oncogene Research Products, Darmstadt, Germany) using specific human TIMP1 or 2 antibodies, respectively. The manufacturer's instructions for sample preparation and assay were adhered to with minor modifications. The concentration of TIMP1 or 2 was determined by interpolation from a standard curve.

Post-operative assessment of cardiac function

The post-operative cardiac function was assessed by serial transthoracic echocardiography at 4h, day 1-2, and day 5-7 following surgery. Parameters measured included: shortening fraction, velocity of circumference shortening, left ventricular outflow tract velocity, and corrected maximum power. The echocardiographic examination was performed by one of the authors (JD) or his delegate and interpreted by JD with no knowledge of the MMP and TIMP results.

Statistics

Data are expressed in median (mean±standard deviation, range). Paired t-tests and Wilcoxon signed rank tests were used to assess percent changes from CPB baseline (immediately after cannulation; t2). Percent change was only calculated for patients whose baseline measurement was not zero (100%× [final - baseline]/baseline). For the echocardiographic parameters, changes over time were examined using paired t-tests adjusted for multiple testing by the Bonferroni method. Each of the MMP activities and TIMP concentrations were assessed for significant linear correlation with simultaneous clinical and cardiac function parameters using the Pearson's product-moment correlation coefficient (r) and associated t-test adjusted for multiple testing by the Bonferroni method. The Statistical Package for the Social Sciences (SPSS) version 11 was used to perform the majority of analyses.

Results

From February to December 2002, 18 term neonates requiring intracardiac surgery for congenital heart disease were enrolled. There were 11 male and 7 female infants, whose surgery was performed at the age of 9 [12±10, 3-38] days and a weight of 3.4 [3.4±0.6, 2.1-4.9] kilograms. Diagnoses included hypoplastic left heart syndrome (5), transposition of the great arteries (5), interrupted aortic arch (2), total anomalous pulmonary venous drainage (2), tetralogy of Fallot (1), truncus arteriosus,(1) atrial and ventricular septal defects (1), and ventricular septal defect with hypoplastic aortic arch (1). The intraoperative and post-operative clinical features are shown in Table 1. There were no post-operative deaths.

Plasma MMP2 and 9 activities

The gelatinolytic activities of MMP2 and 9 in the plasma were detected at 72 kDa and 92 kDa, respectively (Figure 1A). Following a dramatic decrease immediately after cannulation for CPB (baseline; t2), the plasma MMP2 activity increased at 1h after separation from CPB ($p<0.01$) and progressively recovered to pre-operative levels within the first post-operative 24h (Figure 1B). The plasma MMP9 activity after cannulation for CPB (baseline; t2) decreased significantly, increased immediately after the release of aortic cross-clamp ($p<0.005$), and peaked 7-8min later ($p<0.005$). The plasma MMP9 activity then progressively decreased and by 24h post-operatively, the plasma MMP9 activity was similar to CPB baseline (Figure 1C). The plasma MMP9 activity then remained stable in the subsequent 48h.

Plasma TIMP1 and 2 concentrations

The plasma TIMP1 and 2 concentrations decreased after cannulation for CPB (baseline; t2) and increased at the subsequent times. The increase in plasma TIMP1 concentration was significant immediately after the release of aortic cross-clamp ($p<0.05$) and at 1h after separation from CPB ($p<0.001$) (Figure 2A). The increase in plasma TIMP2 concentration was less dramatic immediately after the

release of aortic cross-clamp (p=0.07), and became significant at 1h after separation from CPB (p<0.0001) (Figure 2B). Both plasma TIMP1 and 2 concentrations reached their pre-operative levels at 1-4h after separation from CPB, and remained stable for the next 72h post-operatively.

Echocardiographic findings post-operatively

Forty-three echocardiographic examinations were performed in these 18 patients. Cardiac function determined by the shortening fraction, velocity of circumference shortening, left ventricular outflow tract velocity, and corrected maximum power improved during the post-operative period. There were statistically significant increases in these functional parameters on post-operative day 1-2 (vs. 4h; Table 2). No evidence of significant changes over time were found in the myocardial performance index and Ea (early diastolic filling phase on tissue doppler). No evidence of further significant improvement in the functional parameters was found on day 5-7 post-operatively (vs. day 1-2, Table 2).

Plasma matrix metalloproteinase activities and TIMP concentrations, cardiac function, and clinical outcomes

For echocardiographic parameters, correlations with simultaneous or earlier MMP activity and TIMP concentrations were determined. There was no evidence of statistically significant correlations between plasma MMP activities or TIMP concentrations and parameters of post-operative cardiac function including shortening fraction, velocity of circumference shortening, left ventricular outflow tract velocity, and corrected maximum power. Among clinical parameters, no evidence was found of statistically significant correlations between the MMP activities and the TIMP concentrations and: heart rate, mean blood pressure, arterial pH, base deficit, lactate, or inotrope scores. In addition, the maximum change in plasma MMP activities and TIMP concentrations did not correlate with intraoperative parameters, the duration of mechanical ventilation, hospital stay, inotrope scores, or Pediatric Logistic Organ Dysfunction Scores. No evidence of significant correlation was found between simultaneous plasma MMP activities and TIMP concentrations.

Discussion

We report the temporal profile of plasma MMP2 and 9 activities and their TIMP concentrations in 18 neonates undergoing cardiac surgery with CPB. Our main findings are: 1) after initially decreasing on institution of CPB, MMP2 plasma activity increased progressively starting at 1h after separation from CPB and returned to pre-operative levels at 24h; 2) MMP9 plasma activity was significantly elevated after release of aortic cross-clamp, peaked 7-8min later, and returned to baseline at 24h; and 3) there was no evidence of statistically significant correlation of MMP2 or 9 plasma activity, or TIMP1 or 2 plasma concentration and clinical outcomes including echocardiographic cardiac function, inotrope scores, Pediatric Logistic Organ Dysfunction Score, ventilation days, or hospital length of stay. Although MMP activity and TIMP levels have been examined in adult patients undergoing CPB, to our knowledge this is the first report of the temporal profile in neonates having cardiac surgery with CPB.

The temporal profile of plasma MMP2 and 9 activities

A consistent finding in adults undergoing

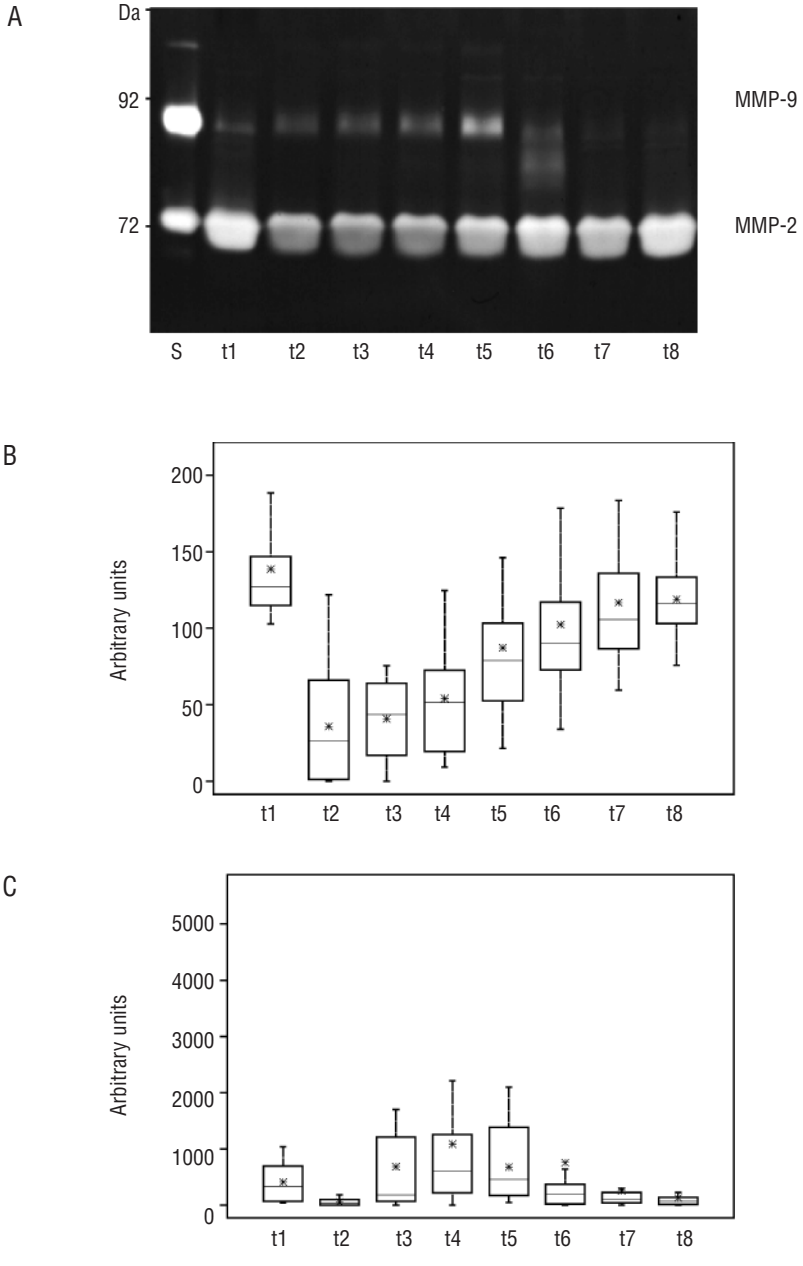

Figure 1. (A) A representative zymogram showing the plasma activities of matrix metalloproteinase-2 (72 kDa) and matrix metalloproteinase-9 (92 kDa). S: standard. (B) The temporal profile of the plasma activity of matrix metalloproteinase-2 and (C) matrix metalloproteinase-9 in 18 neonates undergoing cardiac surgery. Mean (*), median, 25th and 75th percentiles, and extreme values are shown in the boxplots. AU: arbitrary units.

Table 1. Intraoperative and post-operative clinical features of neonates with cardiac surgery.[a]

	Intra-operative	4 hours post-operative	3 days post-operative
Heart rate (beats/min)	89 [83±39, 0-143] (n=10)	165 [164±13, 145-192]	149 [148±17, 105-175]
Mean arterial blood pressure (mmHg)	28 [28±7, 11-40]	58 [58±6, 49-69]	62 [63±10, 35-84]
Arterial pH	7.34 [7.37±0.09, 7.27-7.67]	7.43 [7.43±0.06, 7.33-7.55]	7.41 [7.40±0.08, 7.17-7.52]
Lactate (mmol/l)	2.1 [2.1±0.7, 0.7-3.7]	2.6 [3.5±2.5, 0.8-9.5]	1.0 [1.1±0.5, 0.5-2.7]
Aortic cross-clamp time (min)	50 [56±23, 31-123]	---	---
Deep hypothermic circulatory arrest (min)	20 [26±24, 0-79]	---	---
Cardiopulmonary bypass time (min)	92 [175±214, 61-990]	---	---
Inotrope score	---	10.5 [14+11, 0-40]	12.5 [13.6±10.7, 0-40][b]
Post-operative ventilation (hours)	---	---	156 [196±165, 26-735]
Hospital stay (days)	---	---	29 [34±20, 11-76]

[a]Data reported as median [mean±SD, range]. [b]Data from time 24h post-operatively. There were 16-18 subjects for each variable.

Table 2. Post-operative cardiac function by serial echocardiographic examinations in neonates.

	4 hours post-operative	1-2 days post-operative	5-7 days post-operative
Shortening fraction (%)	36 [30±11, 8-46]	40 [39±8, 23-51][a]	44 [44±12, 20-67][a]
Velocity of circumferential fiber shortening (circ/sec)	1.4 [1.3±0.5, 0.3-2.0]	1.7 [1.7±0.5, 0.8-2.4][a]	1.7 [1.7±0.7, 0.8-3.4]
Left ventricular outflow tract velocity (cm/sec)	50 [53±16, 22-92]	60 [69±22, 45-129][a]	68 [80±41, 41-162]
Myocardial performance index	0.62 [0.73±0.36, 0.24-1.50]	0.62 [0.63±0.16, 0.43-0.92]	0.50 [0.56±0.27, 0.35-1.38]
Ea (cm/sec)	58 [65±23, 33-111]	63 [68±20, 46-111]	61 [67±12, 58-91]
Corrected maximum power	2585 [2784±1551, 1148-6020]	3508 [3788±1942, 1623-8450][a]	3195 [3743±2025, 1526-8000]

[a]$p<0.017$ *vs.* 4h post-operative (paired t-test). Data reported as median [mean±SD, range]. There were 12-15 subjects for each echocardiogram variable.

coronary artery bypass grafting is an increase in MMP9 activity from the CPB baseline after cross-clamp release,[11-14] with a decrease from peak value within 6h.[11,14] The changes in plasma MMP2 activity have been more variable.[11-15] Mayers *et al.* found significant increases in myocardial MMP2, but not plasma MMP2 activity on termination of CPB.[12] Conversely, Watanabe *et al.*[13] and Joffs *et al.*[11] found an initial decrease of plasma MMP2 activity during CPB, followed by a progressive increase in activity over the 24h after discontinuation of CPB. We found temporal changes in plasma MMP activity in neonates remarkably similar to these findings in adults. Plasma MMP2 activity increased post-operatively from CPB baseline, returning to pre-operative levels within 24h. An immediate increase of plasma MMP9 activity soon after aortic cross-clamp release gradually returned to pre-operative levels 4h later. An increase in MMP2 and 9 levels post-operatively was also found in children (age 4-34 months) by Pasnik *et al.*[19]

There are several possible sources for plasma MMP activity after CPB.[6] Neutrophils and platelets can release MMPs into plasma upon activation during exposure to the high oxygen environment and foreign CPB surfaces.[12,14,20] However, Mayers *et al.* found that the increased MMP activity in heart tissue was not caused by an influx of inflammatory cells, and instead was associated with diffuse increase in MMP expression within myocytes.[12] These findings suggest the source of *plasma* and *myocardial* MMPs may be different.

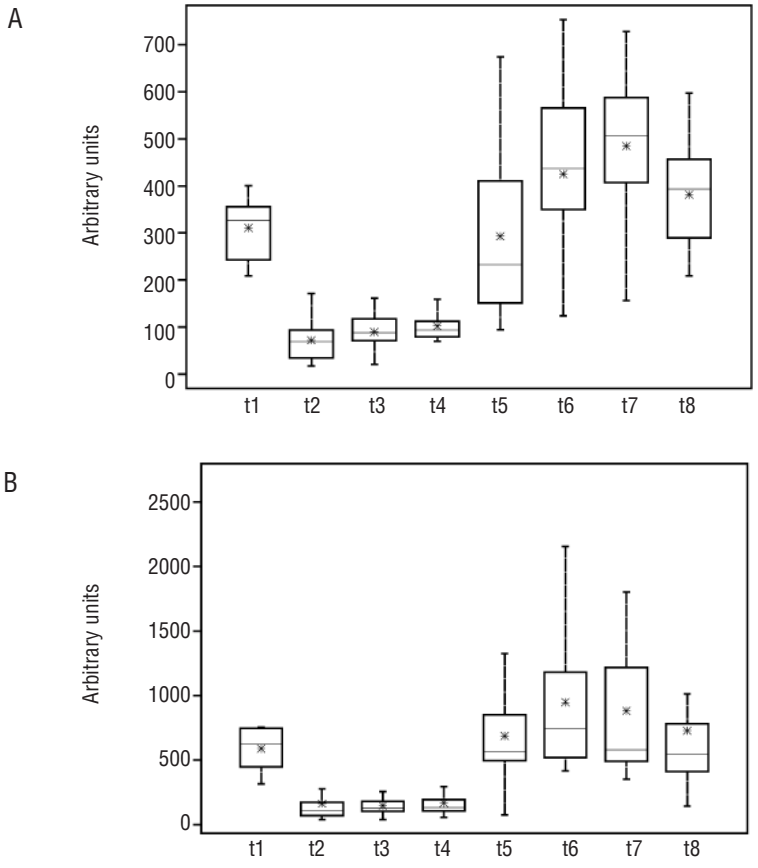

Figure 2. The temporal profile of the plasma concentrations of (A) tissue inhibitor of metalloproteinase-1 and (B) tissue inhibitor of metalloproteinase-2 in 18 neonates undergoing cardiac surgery. Mean (*), median, 25th and 75th percentiles and extreme values are shown in the boxplots.

The functional recovery of cardiac function after cardiopulmonary bypass

To study the cardiac function of complex heart anatomy in the neonate is difficult.[21] We serially measured several echocardiographic parameters of systolic and diastolic functions of the dominant ventricle. We observed a significant improvement in cardiac function assessed by shortening fraction, velocity of circumference shortening, left ventricular outflow tract velocity, and corrected maximum power during the first two days post-operatively, with no further significant increase in the subsequent four days. This improvement occurred with similar inotrope scores (14±11 *vs.* 13.6±10.7, p=ns). This finding of an early nadir in post-operative cardiac function is compatible with the reports of a typical decrease in cardiac index, and increase in systemic vascular resistance and pulmonary vascular resistance in the first 12h, returning to baseline by 24h after cardiac surgery in neonates.[16]

The relationship between plasma matrix metalloproteinase activity and clinical outcomes

Plasma MMP activity theoretically may affect post-operative cardiovascular homeostasis through their effects on platelet aggregatory function, vasomotor control, capillary permeability, and acute lung injury.[2,7,8,22] In addition, changes in plasma MMP2 and TIMP2 concentrations may play a role in left ventricle remodeling after cardiac surgery.[13,23]

We did not find evidence of statistically significant correlations between MMP plasma activity or TIMP concentration and clinical outcomes including post-operative cardiac function, inotrope and Pediatric Logistic Organ Dysfunction Scores, ventilation days and hospital stay. There are several potential explanations for this finding. Although most reports in adults describe changes in plasma MMP activity referenced to CPB baseline,[11,12] this may be misleading in neonates. In neonates with a blood volume of 80 mL/kg, CPB contributes significant extracorporeal blood volume (approximately 500 ml packed red blood cells: saline mixture), diluting plasma and accounting for the dramatic decrease in plasma MMP activity with initiation of CPB. Nevertheless, we believe this dilution effect would be minimal on the *relative* plasma MMP activity over time post-operatively. We did not study myocardial tissue MMP activity due to technical limitations. In children with Kawasaki disease, peripheral blood levels of MMP2 and 9 were not significantly correlated with coronary artery damage, even though coronary *tissue* MMP levels in children and animals are associated with coronary artery damage.[24] Lalu *et al.*, in 15 adults undergoing coronary artery bypass grafting surgery with CPB, found significant negative correlations between left ventricle stroke work index at 3 h following reperfusion and left atrial *tissue* MMP2 and 9 activities (r= -0.56 and -0.89, respectively), but no correlation with the *plasma* MMP activity.[15]

Study limitations

This study has limitations that affect the clinical implications of our findings. The small sample size, statistical correction for multiple testing, and heterogeneous types of congenital heart disease may preclude identifying correlations between some variables. In addition, our patients had very good outcomes, and this may have limited our ability to detect associations between MMP activities, TIMP concentrations, and adverse outcomes. Nevertheless, despite the heterogeneous cardiac lesions, all the neonates had hemodynamically significant congenital heart disease, significant exposure to CPB and aortic cross-clamp times, often with deep hypothermic circulatory arrest, often had tachycardia and high lactate early post-operatively, and significant need for post-operative inotropes and ventilation days (Table 1). In addition, these patients did have a significant nadir in cardiac function early post-operatively (Table 2). As mentioned above, we did not measure myocardial tissue MMP activity, which may better reflect the myocardial environment. Given these significant limitations, our results, particularly regarding the lack of association of MMP activities and TIMP concentrations with clinical outcomes, should be interpreted with caution.

We chose to measure MMP activity, and not concentration, similar to adult studies.[11-15] It is possible that total MMP concentrations may have given different results. However, we believe that it is the activity of the mediators that is important, and not the total level which may include potentially inactive mediators. Furthermore, in the only pediatric study that examined MMP2 and 9 levels after CPB (in 28 children aged a mean 11.3 months, range 4-34 months), the concentrations were generally similar in temporal profile to what we found for activity levels in neonates.[19]

Conclusions

Our findings can be considered the first pilot study of MMPs in neonates after cardiac surgery, and should inform the design of future investigations. In neonates after intracardiac surgery, we describe temporal profiles of MMP2 and 9 activities, and TIMP1 and 2 concentrations in plasma that were similar to those found in adults after CPB. We did not find evidence of a statistically significant association of circulating levels of MMPs and TIMPs with clinical outcomes, and suggest that it is unclear if circulating levels are useful biomarkers of the local myocardial tissue environment. Therefore, the relationship between plasma and myocardial MMP activity in infants should be determined. Given our study limitations, whether there is a lack of association between circulating levels of MMPs and TIMPs with clinical outcomes in neonates after cardiac surgery should be confirmed by future study. This has important implications for future therapeutic intervention.[5,6,25]

References

1. Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodeling. Nature Rev Mol Cell Biol 2007;8:221-33.
2. Shah PK. Targeting the proteolytic arsenal of neutrophils: a promising approach for postpump syndrome and ARDS. Circulation 1999;100:333-4.
3. Nishikawa N, Yamamoto K, Sakata Y, et al. Differential activation of matrix metalloproteinases in heart failure with and without ventricular dilatation. Cardiovascular Research 2003;57:766-74.
4. Squire IB, Evans J, Ng LL, Loftus IM, Thompson MM. Plasma MMP-9 and MMP-2 following acute myocardial infarction in man: correlation with echocardiographic and neurohumoral parameters of left ventricular dysfunction. J Card Fail 2004;10: 328-33.
5. Phatharajaree W, Phrommintikul A, Chattipakorn N. Matrix metalloproteinases and myocardial infarction. Can J Cardiol 2007;23:727-33.
6. Liu P, Sun M, Sader S. Matrix metalloproteinases in cardiovascular disease. Can J Cardiol 2006;22(SupplB):25B-30B.
7. Sawicki G, Salas E, Murat J, Miszta-Lane H, Radomski MW. Release of gelatinase A during platelet activation mediates aggregation. Nature 1997;386:616-9.
8. Fernandez-Patron C, Radomski MW, Davidge ST. Vascular matrix metalloproteinase-2 cleaves big endothelin-1 yielding a novel vasoconstrictor. Circ Res 1999;85: 906-11.
9. Wang W, Schulze C, Suarez-Pinzon W, Dyck J, Sawicki G, Schulz R. Intracellular action of matrix metalloproteinase-2 accounts for acute myocardial ischemia and reperfusion injury. Circulation 2002;106:1543-9.
10. Cheung PY, Sawicki G, Wozniak M, Wang W, Radomski MW, Schulz R. Matrix metalloproteinase-2 contributes to ischemia-reperfusion injury in the heart. Circulation 2000;101:1833-9.
11. Joffs C, Gunasinghe HR, Multani MM, et

al. Cardiopulmonary bypass induces the synthesis and release of matrix metalloproteinases. Ann Thorac Surg 2001;71:1518-23.
12. Mayers I, Hurst T, Puttagunta L, et al. Cardiac surgery increases the activity of matrix metalloproteinases and nitric oxide synthase in human hearts. J Thorac Cardiovasc Surg 2001;122:746-52.
13. Watanabe M, Hasegawa S, Ohshima N, Tanaka H, Sakamoto T, Sunamori M. Differential regulation of MMP-2, TIMP-2 and IL-6 in valve replacement versus CABG patients. Perfusion 2002;17:435-9.
14. Lin TC, Li CY, Tsai CS, et al. Neutrophil-mediated secretion and activation of matrix metalloproteinase-9 during cardiac surgery with cardiopulmonary bypass. Anesth Analg 2005;100:1554-60.
15. Lalu MM, Pasini E, Schulze CJ, et al. Ischaemia-reperfusion injury activates matrix metalloproteinases in the human heart. Eur Heart J 2005;26:27-35.
16. Wernovsky G, Wypij D, Jonas R, et al. Postoperative course and hemodynamic profile after the arterial switch operation in neonates and infants. A comparison of low-flow cardiopulmonary bypass and circulatory arrest. Circulation 1995;92:2226-35.
17. Leteurtre S, Martinot A, Duhamel A, et al. Validation of the paediatric logistic organ dysfunction (PELOD) score: prospective, observational, multicenter study. Lancet 2003;362:192-7.
18. Schulz CG, Sawicki G, Lemke RP, Roeten BM, Schulz R, Cheung PY. MMP-2 and MMP-9 and their tissue inhibitors in the plasma of preterm and term neonates. Pediatr Res 2004;55:794-801.
19. Pasnik J, Moll J, Cywinska-Bernas A, Moll J, Sysa A, Zeman K. Proteolytic and cytokine balance abnormalities in children with congenital heart disease undergoing cardiac surgery with cardiopulmonary bypass. Kardiol Pol 2007;65:1208-14.
20. Cheung PY, Sawicki G, Salas E, Etches PC, Schulz R, Radomski MW. The mechanisms of platelet dysfunction during extracorporeal membrane oxygenation in critically ill neonates. Crit Care Med 2000;28:2584-90.
21. Colan SD, delNido PJ. Left ventricular dysfunction after open repair of simple congenital heart defects in infants and children. J Thorac Cardiovasc Surg 1998;115:74-6.
22. Carney DE, Lutz CJ, Picone AL, et al. Matrix metalloproteinase inhibitor prevents acute lung injury after cardiopulmonary bypass. Circulation 1999;100:400-6.
23. Sakata Y, Yamamoto K, Mano T, et al. Activation of matrix metalloproteinases precedes left ventricular remodeling in hypertensive heart failure rats: its inhibition as a primary effect of angiotensin-converting enzyme inhibitor. Circulation 2004;109:2143-9.
24. Lau AC, Rosenberg H, Duong TT, McCrindle BW, Yeung RSM. Elastolytic matrix metalloproteinases and coronary outcome in children with Kawasaki disease. Pediatr Res 2007;61:710-5.
25. Hu J, Van den Steen PE, Sang QXS, Opdenakker G. Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases. Nature Rev Drug Disc 2007;6:480-98.