Circulating and Urinary miR-210 and miR-16 Increase during Cardiac Surgery Using Cardiopulmonary Bypass – A Pilot Study

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Abstract: A pilot study to measure and compare blood and urine microRNAs miR-210 and miR-16 in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) and off-pump coronary artery bypass grafting surgery. Frequent serial blood and urine samples were taken from patients undergoing cardiac surgery with CPB (n = 10) and undergoing off-pump cardiac surgery (n = 5) before, during, and after surgery. Circulating miR-210 and miR-16 levels were determined by relative quantification real-time polymerase chain reaction. Levels of plasma-free haemoglobin (fHb), troponin-T, creatine kinase, and creatinine were measured. Perioperative serum miR-210 and miR-16 were elevated significantly compared to preoperative levels in patients undergoing cardiac surgery with CPB (CPB vs. Pre Op) and Rewarm vs. Pre Op; p < .05 for both). There were increases of greater than 200% in miR-210 levels during rewarming and immediately postoperatively and a 3,000% increase in miR-16 levels immediately postoperatively in urine normalized to urinary creatinine concentration. Serum levels of miR-16 were relatively constant during off-pump surgery. miR-210 levels increased significantly in off-pump patients perioperatively (p < .05 Octopus on vs. Pre Op); however, the release was less marked when compared to cardiac surgery with CPB. A significant association was observed between both miR-16 and miR-210 and plasma fHb when CPB was used (r = -.549, p < .0001 and r = -.463, p < .0001 respectively). Serum and urine concentrations of hypoxically regulated miR-210 and hemolysis-associated miR-16 increased in cardiac surgery using CPB compared to off-pump surgery. These molecules may have utility in indicating severity of cardiac, red cell, and renal injury during cardiac surgery. Keywords: cardiac surgery, microRNA, cardiopulmonary bypass, hypoxia, hemolysis. J Extra Corpor Technol. 2018;50:19–29

MicroRNAs (miRNAs) are small RNA molecules that can regulate hundreds of genes and are involved in diverse biological processes including cellular differentiation, proliferation, angiogenesis, and apoptosis (1). They are resilient to degradation, stable in blood, urine, and other bodily fluids and are emerging as novel biomarkers reflecting disease states (2).

Differential abundances of specific miRNAs have been reported for many diseases. Several studies have described changes in miRNA expression in myocardial ischemia in relation to coronary artery disease (CAD), myocardial infarction (MI), and ischemia-reperfusion injury. Specific cardiac-expressed circulating miRNAs, including miR-1 and miR-126, increase in patients with CAD and after MI (3–5).

Cardiac surgery and the use of cardiopulmonary bypass (CPB) have been associated with a variable degree of myocardial damage and organ ischemia including acute kidney injury (AKI). The extent to which operative, ischemic, and hypoxic stresses during cardiac surgery using CPB cause alterations in specific or total circulating miRNAs has not been fully explored. Cardiac surgery provides a unique setting to observe levels of circulating
miRNAs in response to a known ischemic and operative insult.

miR-210 is a hypoxia-inducible miRNAs. It has numerous physiological roles including arrest of cell proliferation, repression of mitochondrial respiration, and angiogenesis (6–8). It is aberrantly expressed in a number of diseases such as aortic stenosis and AKI and could potentially serve as a biomarker for prognostic purposes and therapeutic intervention (6,9). Animal studies have shown that intramyocardial injections of plasmid delivering transfected miR-210 after MI improve cardiac function and reduce cellular injury, suggesting a potential therapeutic application for miR-210 in ischemic heart disease (10).

In critically ill patients with AKI, miR-210 levels increased, and miR210 was an independent predictor of mortality, potentially reflecting release from renal cells in response to hypoxia (11). Impairment of renal perfusion and oxygenation during CPB may play a central role in AKI. Circulating biomarkers of renal hypoxia such as miR-210 could provide important insights into the etiology and prediction of AKI after cardiac surgery.

The release of miRNAs including miR-16 from red blood cells during hemolysis has been reported (12). During cardiac surgery, hemolysis is generally attributed to CPB and the CPB-related stresses applied to red blood cells within the perfusion circuit. Venous, arterial and suction cannulae, roller pumps, oxygenators, and the air-to-blood interface in the reservoir cause hemolysis and the intravascular rupture of red blood cells (RBC) with plasmatic release of free hemoglobin (fHb) with a hemolysis peak soon after CPB weaning (13). Increasing evidence suggests that CPB-induced hemolysis may exacerbate kidney injury after cardiac surgery (14,15).

Off-pump coronary artery bypass grafting surgery (OPCABG) was developed in an attempt to ameliorate the perioperative complications associated with CPB. The OPCABG technique allows the heart to continue beating and maintain systemic circulation, using a device to stabilize the heart during coronary grafting, thus offering physiological pulsatile renal perfusion. However, large multi-center trials and meta-analyses suggest that while there may be benefit in short-term outcomes, including reduced cerebrovascular and renal injury, longer-term benefits have not been seen (14,15).

In this pilot study, our aim was to examine whether levels of hypoxically regulated miR-210 and levels of hemolysis susceptible miR-16 increase in the blood and urine of patients before, during, and after cardiac surgery in patients undergoing cardiac surgery with CPB or OPCABG. Investigation of the levels of miRNAs will determine the extent to which the stresses of cardiac surgery causes alterations in miRNAs and the extent to which cardiac surgery using both CPB and OPCABG differentially affect these circulating and urinary miRNAs.

MATERIALS AND METHODS

Patient Inclusion
Ten patients undergoing elective cardiac surgery with CPB were studied (four patients isolated CABG, four isolated valves, and two isolated valve and CABG). Blood and urine samples were obtained at 11 time points including a pre-operative sample (before skin incision), 10 minutes after initiation of CPB, during the rewarming phase of CPB (Rewarm), post-operatively at skin closure, and at 4, 6, 8, 10, 12, 18, and 24 hours in the intensive care unit (ICU).

Five patients undergoing OPCABG were studied with 10 blood and urine samples including pre-operative (before skin incision), 5 minutes after application of Octopus® for 1st graft, post-operatively at skin closure, and at 4, 6, 8, 10, 12, 18, and 24 hours in the ICU. Patient demographic and procedural data are reported in Table 1.

The study was approved by the Southern Adelaide Clinical Human Research Ethics Committee (SAC HREC 202.13), and written informed consent was obtained.

On-Pump Procedure
Intravenous heparin (300 IU/kg) was administered immediately before cannulation for CPB, and additional doses were given to maintain an activated clotting time of 400 seconds or greater. CPB was instituted by cannulation of the distal ascending aorta and insertion of a single two-stage cannula into the right atrium for CABG and aortic valve replacement (AVR) procedures with bicaval venous cannulation used for mitral valve procedures. Roller pumps provided non-pulsatile flow rates of 1.8–2.4 L/min/m², and patient temperature was allowed to drift to maintenance temperature of 34°C and rewarml to maximum of 36.5°C. Open-reservoir membrane oxygenators, cardiotomy suction, or cell salvage was used. Myocardial protection used blood cardioplegia and was delivered intermittently at approximately 20-minute intervals, at a ratio of 4:1 (blood:crystalloid) at 34°C.

OPCABG Procedure
Intravenous heparin (10,000 IU) was administered before grafting. The tissue stabilizer (Octopus® Evolution AS TS2500, Medtronic, Minneapolis, MN) was placed to minimize movement in the arterial territory being grafted.

Collection of Serum, Plasma, and Urine
Arterial blood (10 mL) was collected from the radial arterial catheter. A total of 5 mL was aliquoted into a silicone-coated BD Vacutainer® blood collection tube (Becton, Dickson and Company, Franklin Lakes, NJ) for serum separation and into a 4 mL Vacutainer® Plus blood collection K3EDTA tube (Becton, Dickson and Company) for plasma separation. Blood was allowed to clot at room
with .02 pmol of cel-miR-54 RNA (miRBase database accession # MI000025). The pellet was re-suspended in 20 μL of RNase free water and stored at −80°C.

RNA was extracted from urine (1 mL) using a Urine Exosome RNA Isolation Kit (Cat No 47200, Norgen Biotek Corp, Thorold, ON, Canada). Urine was spiked with .02 pmol of cel-miR-54 RNA. The pellet was re-suspended in 50 μL of RNase free water and stored at −80°C.

After extraction of RNA, 10 μL of sample was pre-treated with .5 μL of the RNase Inhibitor (Cat No M0307L, New England BioLabs®, Inc, Ipswich, MA) and 2 units of Heparinase I (Cat No H2519, Sigma-Aldrich®, St Louis, MO) [in 20 mM Tris-HCl (pH 7.5), 50 mM NaCl, 4 mM CaCl₂] for 1 hour at 25°C to remove heparin (16). Preliminary experiments showed that heparinase does not affect polymerase chain reaction (PCR) analyses in serum and urine from non-heparinized patients, while allowing unaffected PCR amplification in samples potentially contaminated with heparin.

### miRNA Real-Time PCR

TaQMan miRNA assays (ThermoFisher Scientific) were used for expression profiling. A volume of 2.5 μL of RNase- and heparinase-treated serum RNA and 2.5 μL of RNase- and heparinase-treated urine RNA was used for each assay in the 7.5 μL reaction containing 3.5 μL of master mix and 1.5 μL of reverse transcription (RT) primer.

Assay standard curves were prepared using cDNA generated from cell lines. RNA from the renal cell cancer line (RCC4) over expressing von Hippel-Lindau tumor suppressor gene (VHL) + or control VHL − was used as positive controls for miR-16 and miR-210, respectively.

cDNA was synthesized from 2.5 μL of total RNA heparinase-treated serum and urine using miRNA-specific primers according to the TaqMan Assay protocol (hsa-miR-210 Cat #4427975, hsa-miR-16 Cat #4440887, cel-miR-54 Cat #4440887, ThermoFisher Scientific, Waltham, MA). RCC4 VHL− RNA (20 ng μL) was used as a control for qRT-PCR investigating levels of miR-210, 20 ng of RCC4 VHL+ RNA was used as a control for levels of miR-16, and 20 ng of human epidermal keratinocytes (HEK) 293 cell RNA spiked with cel-miR-54 was used as a control. RT was undertaken at 30 minutes of incubation at 42°C, 5 minutes of incubation at 85°C, and incubation at 4°C until ready for use in qRT-PCR.

Real-time PCR was carried out according to the TaqMan miRNA assay protocol (Thermo Fisher Scientific) using triplicate reactions for each biological replicate including 1 μL of RT product, .5 μL miRNA-specific primer and probe assay mix, 5 μL 1X TaqMan universal PCR Master Mix No AmpErase UNG (Cat # 4324018, Applied Biosystems, Thermo Fisher Scientific), and 3.84 μL of water.

### Table 1. Preoperative, procedural and outcome data.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Preoperative</th>
<th>On Pump (n = 10)</th>
<th>OPCABG (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>71 (47–86)</td>
<td>64 (54–77)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
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<td>31 (26–38)</td>
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</tr>
<tr>
<td>COPD</td>
<td>2</td>
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<td></td>
</tr>
<tr>
<td>Previous cardiac surgery</td>
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<td>0</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
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<td>2</td>
<td></td>
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<tr>
<td>Hypertension</td>
<td>6</td>
<td>4</td>
<td></td>
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<td>Congestive heart failure</td>
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<td>2</td>
<td></td>
</tr>
<tr>
<td>Smoking history</td>
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<td>3</td>
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<tr>
<td>Ejection fraction &lt;30%</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
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<td>0</td>
<td></td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
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<td>1</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Preoperative Hb, g/L</td>
<td>143 (79–155)</td>
<td>139 (111–164)</td>
<td></td>
</tr>
<tr>
<td>Preoperative creatinine, μmol/L</td>
<td>91 (65–150)</td>
<td>102 (75–126)</td>
<td></td>
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<tr>
<td>Procedure</td>
<td>CABG 4</td>
<td>Valve repair/replacement 4</td>
<td>Valve + CABG 2</td>
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<td>CPB duration, minutes</td>
<td>94.5 (126–35)</td>
<td>Aortic clamp time, minutes 78.5 (23–95)</td>
<td>CPB Naso temperature, minute °C 34</td>
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<td>CPB MAP, avg mmHg</td>
<td>58</td>
<td>CPB Hb, min g/L 93</td>
<td>CPB Hb, max g/L 96</td>
</tr>
<tr>
<td>CPB blood glucose, min mmol/L</td>
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<td>CPB blood glucose, max mmol/L 9</td>
<td>Outcome</td>
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<tr>
<td>Acute kidney injury</td>
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<td>0</td>
<td>Acute kidney injury 2 0</td>
</tr>
<tr>
<td>Highest post op creatinine</td>
<td>97 (69–237)</td>
<td>99 (78–152)</td>
<td>Highest post op creatinine 97 (69–237) 99 (78–152)</td>
</tr>
<tr>
<td>ICU time (hour)*</td>
<td>43 (23–554)</td>
<td>23 (22–29)</td>
<td>ICU time (hour)* 43 (23–554) 23 (22–29)</td>
</tr>
<tr>
<td>Length of stay (days)*</td>
<td>7 (5–32)</td>
<td>6 (6–7)</td>
<td>Length of stay (days)* 7 (5–32) 6 (6–7)</td>
</tr>
</tbody>
</table>

Data expressed as median and range. No significant differences except for ICU time* and length of stay* (p < .05, unpaired t test). All baseline characteristics defined by the Australian and New Zealand Society of Cardiac and Thoracic Surgeons national database. All CPB-related data accessed from Australian and New Zealand Collaborative Perfusion Registry. COPD, chronic obstructive pulmonary disease; Hb, Haemoglobin; Naso, Nasopharyngeal; MAP, mean arterial pressure.

Temperature for 30 minutes. Samples were centrifuged at 1,200 × g for 10 minutes at room temperature. After transferring serum and plasma supernatants into a 15 mL tube, samples were centrifuged at 1,800 × g for 10 minutes at room temperature to remove debris. Samples were aliquoted into 1.5 mL microcentrifuge tubes and stored at −80°C until RNA preparation. Urine (10 mL) was collected from the sample port of the urinary catheter at each time point and stored at −80°C until RNA preparation.

### RNA Extraction

TRIzol LS Reagent (ThermoFisher Scientific, Waltham, MA) was used to obtain RNA from serum samples spiked with .02 pmol of cel-miR-54 RNA (miRBase database
Thermal cycling was performed using a Rotorgene Q (Qiagen®, Foster City, CA) with 10 minutes of incubation at 95°C, 50 cycles of a 15-second denaturing step at 95°C, and a 60-second annealing/extension step at 60°C. Relative expression levels were calculated from quantification cycle (Cq) values using Q-gene (17).

**Isolation, Measurement, and Quality Control of Small RNA from Serum**

The quantity and quality of the RNA extracted from 250 μL of serum was determined using an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA) with a Small RNA Analysis Kit. RNA was extracted with the miRCURY RNA Isolation Kits for biofluids (Cat #300,112 Exiqon, Copenhagen, Denmark) and re-suspended in 2.5 μL of RNase free water. To concentrate RNA, samples underwent a further precipitation by adding 20 μg of glycogen, 5 μL of 3 M sodium acetate (pH 5.2), and 150 μL of ethanol. After vortexing, samples were incubated at −80°C overnight. The following day, samples were centrifuged at 16,000 × g for 30 minutes at 4°C. After removing the supernatant, 200 μL of 75% ethanol was added, vortexed, and centrifuged at 16,000 × g for 10 minutes at 4°C. After removal of the supernatant, the pellet was resuspended in 2.5 μL of RNase free water and stored at −80°C.

**Plasma-fHb**

Plasma fHb level measurements were routinely carried out as part of a Serum Indices Test to detect hemolysis, bilirubin, and lipaemia on a Roche/Hitachi Modular Analyzer (Hitachi High-Technologies Corp., for Roche Diagnostics GmbH, Tokyo, Germany) in the SA Pathology Biochemistry Laboratory, Flinders Medical Center. Levels of hemolysis were assessed by spectrophotometry with wavelengths scanning from 350 to 650 nm.

**Troponin T, Creatine Kinase, Serum Creatinine**

Routine creatine kinase and troponin T measurements were carried out preoperatively and 6, 12, and 72 hours postoperatively. Serum creatinine levels were determined when clinically assessed.

**Statistical Analysis**

Expression of miR-16 and miR-210 for each individual was described using the mean of the three values determined by qRT-PCR. To adjust for urinary creatinine, each individual value was expressed relative to the levels of exogenous cel-miR-54 using Cq values from Q-gene. Repeated measures analysis of variance (ANOVA) was performed with group as a between-subjects factor and time as a within-subjects factor. Between-group differences at each time point were considered significantly different when the overall time × group effect based on 9 degrees of freedom was significant (p < .05) in addition to a p-value < .05 at each specific time point. The Mann–Whitney U test was used to determine differences in preoperative baseline values between the two groups. The Spearman correlation coefficient was used for assessing correlations between hemoglobin and miRNA expression. Statistical analyses and graph preparation were performed with GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA). Global and multiple comparisons-adjusted p-values of <.05 (95% confidence interval) were considered significant. Descriptive data were presented using Box-plots with the whiskers defined using the 25th and 75th percentile ±1.5 times the inter-quartile range. Outliers, numbers less than the 25th percentile or greater than the 75th percentile by more than 1.5 times the interquartile range are shown as separately plotted points.

**RESULTS**

There were no significant differences in patient demographics or procedural data between the on-pump and OPCABG groups (Table 1). There were significant differences in ICU stay and length of hospital stay in the OPCABG groups (Table 1). There were significant differences in ICU stay and length of hospital stay in the OPCABG groups (Table 1). There were significant differences in ICU stay and length of hospital stay in the OPCABG groups (Table 1). There were significant differences in ICU stay and length of hospital stay in the OPCABG groups (Table 1). There were significant differences in ICU stay and length of hospital stay in the OPCABG groups (Table 1)
Figure 1. (A) Serum miR-210 levels from on-pump patients before, during and after cardiac surgery (*p < .05 vs. pre-op). (B) Serum miR-16 levels from on-pump patients before, during and after cardiac surgery (*p < .05 vs. pre-op). (C) Serum miR-210 levels from off-pump patients before, during and after OPCABG (*p < .05 vs. pre-op). (D) Serum miR-16 levels from off-pump patients before, during and after OPCABG. Data is presented as Box-plots with the whiskers defined using the 25th and 75th percentile ± 1.5 times the inter-quartile range (IQR) (● represent outliers numbers less than the 25th percentile or greater then the 75th percentile by more than 1.5 times the interquartile range).


miR-210 and miR-16 in Urine of Patients Undergoing Cardiac Surgery

Both miR-210 and miR-16 were detectable in the urine of patients undergoing cardiac surgery, but mean concentrations were substantially less than in serum (100-fold less for miR-210 and 700-fold less for miR-16) in on-pump patients (Figures 2A and 3A). Both miR-210 and miR-16 were detectable in the urine of OPCABG patients with the mean concentrations less than in the serum of on-pump patients (45-fold for miR-210 and 1,000-fold less for miR-16) (Figures 2C and 3C). No changes in urine miR-210 or miR-16 levels were statistically significant. After adjusting for urinary concentration by normalizing with urinary creatinine concentration, there was a large increase in normalized miR-210 and miR-16 levels in the urine of on-pump patients. There was a greater than 200% increase in normalized miR-210 levels in both the in rewarming phase of CPB and immediately postoperatively (Figure 2B). miR-16 levels increased by 3,000% in the urine of on-pump patients in the immediate postoperative period (Figure 3B). There were no changes in patients undergoing OPCABG (Figures 2D and 3D).

Plasma fHb Levels

Mean plasma fHb levels showed no significant change in either on-pump or OPCABG surgeries (Figure 4). Plasma fHb did, however, demonstrate a significant positive correlation with levels of miR-16 and miR-210 (Figure 5A). These correlations were not observed in OPCABG patients (Figure 5B).

Injury Markers—Troponin T, Creatine Kinase, and Serum Creatinine

To examine links between organ injury and miRNA release, correlations were sought between indicators of myocardial and renal injury and miRNA levels. There were no significant correlations between miR-16 or miR-210 levels with troponin T, creatine kinase, or serum creatinine.

Comparison of Small RNA Levels: On-Pump vs. Off-Pump

The total concentration of small RNAs demonstrated a 10-fold mean increase during CPB, with a significant rise seen in the immediate postoperative period, returning to preoperative levels from 4 hours postoperatively in on-pump patients (Figure 6A). A substantial increase in total small RNAs was also seen during the application of the Octopus®, with levels returning to preoperative levels in the immediate postoperative period, although the rise was not statistically significant (Figure 6B).

DISCUSSION

Circulating miRNAs were successfully isolated and measured in patients undergoing cardiac surgery during and after CPB. Levels of miR-210, miR-16, and total circulating small RNAs markedly increased in the serum of patients upon initiation of CPB and continued to increase during the rewarming phase of CPB. Levels remained elevated into the immediate postoperative period and returned to preoperative levels after 4 hours postoperatively. Levels of miR-210 and total small RNAs increased in the serum of patients during off-pump cardiac surgery but to a lesser degree than those in the serum of patients on CPB. No changes in circulating levels of miR-16 were observed during off-pump surgery.

The CPB period presents a potentially challenging situation for RNA determination because patients are systemically heparinized (300 IU/kg). Heparin has been shown to influence the results of qRT-PCR analysis interfering with amplification of PCR. Treatment of samples with heparinase degrades the heparin, allowing successful PCR amplification and determination of miRNA levels (16). Furthermore, others have reported that the exposure to heparin does not have an effect on circulating levels of miRNAs (11).

Levels of circulating miR-210 were significantly increased during the CPB period and immediately into the postoperative period. Although the cellular source responsible for the miR-210 release has not been identified, CPB and cardioplegic arrest known to cause hypoxic injury to the myocardium and myocardial cells may be contributing to the increased circulation of miR-210 that was observed (6,18).

Levels of miR-210 were also significantly increased during the period after the application of Octopus® during OPCABG. During off-pump surgery, it had been thought that the constant source of myocardial blood flow offered greater protection than conventional on-pump surgery. However, the surgical and mechanical trauma to the heart during OPCABG is sufficient to activate an inflammatory response in the myocardium with increases in lactate, creatine kinase MB, troponin I, and interleukin 6 during OPCABG (19–21), and the risks of ischemia and renal injury are not eliminated (19). Hence, the release of miR-210 observed during the grafting period of OPCABG surgery might be due to cardiac ischemia or an effect of a decrease in the cardiac output as the beating heart is manipulated, eliciting a hypoxic stress response from other organ systems. To help determine if the insult of surgery itself contributes to the increase observed in circulating miRNAs, samples were also taken after sternotomy, before the administration of heparin and the initiation of CPB. Levels of miRNAs remained unchanged from pre-operative levels (results not shown).
Figure 2. (A) miR-210 levels in the urine of on-pump patients before, during, and after cardiac surgery. (B) miR-210 levels in the urine of on-pump patients before, during, and after cardiac surgery when corrected for dilution of CPB using urine creatinine. (C) miR-210 levels in the urine of off-pump patients before, during, and after cardiac surgery. (D) miR-210 levels in the urine of off-pump patients before, during, and after cardiac surgery when corrected for dilution of fluid administered using urine creatinine.
Figure 3. (A) miR-16 levels in the urine of on-pump patients before, during, and after cardiac surgery. (B) miR-16 levels in the urine of on-pump patients before, during, and after cardiac surgery when corrected for dilution of CPB using urine creatinine. (C) miR-16 levels in the urine of off-pump patients before, during, and after cardiac surgery. (D) miR-16 levels in the urine of off-pump patients before, during, and after cardiac surgery when corrected for dilution of fluid administered using urine creatinine.
During the course of this work, Emanueli et al. (22) reported that the concentration of cardiac-enriched ischemia-responsive miRNAs, including miR-210, increased in the plasma early after CABG surgery. We have confirmed this finding and extended it to demonstrate the release of miRNAs during the perioperative CPB period. In addition, the profile of release of miR-210 in relation to OPCABG has not been elucidated previously.

The major release of miRNAs including miR-16 from red blood cells during hemolysis has been reported, and our findings are consistent with hemolytic release of small RNAs during bypass (12). In pediatric patients, hemolysis induced by CPB has been associated with AKI (23).

Patients with AKI, as defined by the acute kidney injury network, displayed significantly higher plasma fHb levels during surgery compared to non-AKI patients (24). Although this study did not show any significant mean change in plasma fHb during CPB or in the immediate post-CPB period, changes in plasma fHb correlated significantly with changes in detected miR-16 levels.

The correlation between plasma fHb and miR-16 levels observed in the on-pump cohort most plausibly reflects
exposure of the blood to the extracorporeal circuit with resultant hemolysis. Levels of plasma fHb were also associated with changes in miR-210, which may also be attributed to hemolysis (12). The absence of a significant mean increase in fHb during CPB may reflect the poor sensitivity of fHb to detect hemolysis at low levels of red cell damage, in part because of quenching by haptoglobin, and raise the possibility that miR-16 release might be a more sensitive measure of hemolysis or a reflection of sublethal damage to the red blood cell membrane not reflected in plasma fHb levels (19). By contrast, levels of miR-16 and plasma fHb remained relatively constant in the off-pump cohort.

Urine concentration of miR-210 and miR-16 (when corrected for the effects of urine dilution during CPB) was increased in patients during CPB and in the immediate postoperative period, returning to preoperative levels after 4 hours postoperatively. The elevations of miRNA levels during CPB were particularly striking when urine concentration was accounted for by measurement of urinary creatinine (25). Levels of miR-16 detected in the urine of patients undergoing CPB increased almost 3,000%.

Levels of miR-210 showed a similar release in the urine of the OPCABG group; however, when corrected for urinary creatinine concentration, the release was less marked than the on-pump group. This may reflect inadequate perfusion of the kidney during the perioperative period of CPB leading to either elevated miR-210 production by the kidney in response to hypoxia or increased circulating miR-210 being eliminated via the kidneys.

Cardiac surgery is associated with variable degrees of myocardial damage related to the use of CPB and the ischemia-reperfusion insult of cardiologic arrest (22). In this study, we focused on miR-210 because of its established induction by hypoxia. In cardiomyocytes, miR-210 exerts cytoprotective effects during hypoxia (8). In a murine model of MI, miR-210 was shown to improve angiogenesis, inhibit apoptosis, and improve cardiac function (10). Hence, the increase in miR-210 observed during CPB and into the immediate postoperative period could mediate cardioprotective effects and play a role in attenuating the insult of cardioplegic arrest.

These observations raise the question of whether such miRNA release or the associated hemolysis has beneficial or deleterious pathophysiological effects and whether they are predictive of adverse outcomes.

Study Limitations

In this preliminary study, a detailed release profile of changes in miRNA release of two specific miRNAs during cardiac surgery was determined in a modest number of patients undergoing on-pump (10) and OPCABG (5) surgeries. We were unable to determine the cellular source of the observed miRNA release.

CONCLUSIONS

Levels of miR-210 and miR-16 are increased in the serum and urine of patients undergoing cardiac surgery, with CPB displaying different release profiles to patients undergoing
off-pump surgery. Hemolysis may partially account for such release. These results also indicate an independent accumulation of hypoxically induced miR-210 during on-pump surgery with a less release observed during off-pump procedures. Levels of miR-210 and miR-16 were also increased in the urine of on-pump patients compared to off-pump patients, suggesting the release of specific miRNAs during surgery potentially related to the operative, ischemic, and hypoxic insult attributed to CPB.

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