Displacement of the Beating Heart Induces an Immediate and Sustained Increase in Myocardial Reactive Oxygen Species

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Background Heart manipulation and displacement are common maneuvers during beating heart surgery to expose coronary arteries for revascularization. Effects of heart displacement on free radical generation, reactive oxygen species (ROS) have not been previously described.

Methods and Results Seven adult male dogs were anesthetized, a left lateral thoracotomy performed to expose the heart, and the coronary sinus cannulated for ROS sampling during different manipulation protocols: (1) heart in normal position; (2) 90 degree manual heart displacement; (3) Trendelenburg position while the heart displaced 90 degrees and (4) return heart to normal resting anatomical position and plus the operating table returned to horizontal. Heart displacement followed by anatomical re-positioning significantly increased the ROS signal as measured by EPR (50-fold compared with control values; p<0.01).

Conclusion Trendelenburg positioning and/or repositioning the heart during cardiac surgery may induce acute reperfusion injury and increase ROS. (Circ J 2006; 70: 1226–1228)

Key Words: Dogs; Free radicals; Heart displacement; Hemodynamics; Trendelenburg position

Displacement of the beating heart during beating heart surgery can reduce coronary blood flow and cardiac output (CO) and to normalize hemodynamics the Trendelenburg position is often employed with or without fluid and pharmacological intervention! Methods to re-establish coronary flow could be deemed reperfusion therapy and cardiac reperfusion after a brief ischemic insult can increase reactive oxygen species (ROS), which may promote cardiac dysfunction and/or arrhythmias. However, no studies have investigated the effects of dislocating the beating heart on possible associated reperfusion and free radical production. Thus, the aim of the present study was to examine heart dislocation and its re-positioning on myocardial ROS generation in dogs.

The AEC National University of Singapore approved the experimental protocols. Seven male dogs (17–21 kg) were fasted overnight and anesthetized via intramuscular injection of ketamine (20 mg/kg) and intravenous thiopentone (30 mg/kg), intubated and ventilated. Anesthesia was maintained via 1.5 L oxygen/air and 1.5–2% isoflurane. Ventilation rate (20 strokes/min), tidal volume and FiO2 were adjusted to maintain arterial PO2 >100 mmHg. A left thoracotomy was performed in the fifth intercostal space and the heart was suspended in a pericardial cradle. A catheter was inserted into the coronary sinus for blood sampling of venous effluent.

After 20-min stabilization, the heart was displaced by 90 degrees (dislocation) manually until the apex pointed upward to expose the left circumflex coronary artery (LCx). After 10 min each dog was placed in the Trendelenburg position (20 degrees head down) for 15 min without changing the position of the displaced heart. The operating table was then returned to horizontal and the heart returned to its anatomical position. ROS measurements began immediately after the heart was replaced in its normal position.

Dogs were instrumented for hemodynamic measurements during heart displacement. Intra-aortic pressure was measured by inserting a fluid-filled 20 g Huber point needle cannula into the ascending aorta. CO and flow for the left anterior descending coronary artery (LAD), right coronary artery (RCA) and LCx was measured via ultrasonic probes (Power Lab/4S).

ROS were measured in blood samples prior to heart dislocation procedures (control) and at 3, 50 and 60 min after the heart had been replaced in its anatomical position. A 2-ml sample of venous blood from the coronary sinus was collected in a 5-ml syringe pre-filled with 1 ml of D-phenyl-N-nitroso-butylnitrone (PBN, 50 mmol/L) and gently mixed with brief periods of exposure to room air for 1 min and then immediately frozen in liquid nitrogen. For EPR measurements, samples were thawed on ice and transferred into glass tubes containing 1.5 ml spectroscopic-grade toluene, gently shaken and centrifuged for 5 min at 4,000 rpm.

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Fig 1. Histogram showing (a) the percent levels of coronary blood flow of the right coronary artery (RCA), left circumflex coronary artery (LCx) and left anterior descending coronary artery (LAD) and (b) cardiac output (CO) at the following time points: baseline (ie, prior to heart displacement), dislocated heart, during heart displacement plus Trendelenburg, and 30 min post repositioning of the heart with the operating table in the horizontal position. Note that coronary blood flow and CO are statistically significantly reduced during heart displacement compared to baseline. Bars indicate mean ± SD. *p<0.01 vs baseline.

Fig 2. (a) Examples of EPR spectra that are a result of reactive oxygen species (ROS) released into the blood following heart dislocation and returning it to its anatomical position. Samples for the EPR spectra of D-phenyl-N-tert-butylnitrone (PBN) signals detected in the cardiac venous efflux at 3, 30 and 60 min post heart re-positioning are shown. (b) Histogram depicting calculated area under the curve of the EPR spectra of the PBN signal for ROS (arbitrary units). PBN signal was detected in the cardiac venous efflux at baseline (ie, prior to heart displacement), and at 3, 30 and 60 min after dislocation and replacement of the heart in its correct anatomical position. Bars indicate mean ± SD. *p<0.01 vs baseline. These EPR spectra were obtained with a Bruker (Bruker BioSpin GmbH, Rheinstetten/Karlsruhe, Germany) Elexys Series E500 CW-EPR X-band (9–10 GHz) spectrometer. The spectra were recorded with microwave power 50 mW, microwave frequency ~9.83 GHz, modulation amplitude ~1 Gauss, modulation frequency ~100 kHz, center field 3,448 G, field sweep 110 G, sampling time 40.96 ms, receiver gain 60 dB, receiver time constant 163.84 ms with 20 averaged scans.
at 4°C. The upper phase was collected on ice and used for EPR analysis.

Student's t-test was used to compare 2 groups or ANOVA and posthoc Tukey test for multiple comparisons. A p-value of <0.05 was considered statistically significant.

Initial vertical heart displacement, for 10 min, induced a significant decline in coronary blood flow (LCx: 42%, p<0.01; LAD: 55%, p<0.01; RCA: 65%, p<0.01) and CO (65%, p<0.01) (Fig 1). Within 5 min of Trendelenburg positioning, with continued heart displacement, there was an improvement in coronary blood flow (Fig 1). After returning the operating table to the horizontal position and repositioning the heart, coronary flow returned to baseline values and CO to 95% of pre-dislocation values (Fig 1). From the time of repositioning the heart, serial blood samples were taken to measure ROS. Fig 2a depicts the EPR spectra for ROS at 3, 30 and 60 min after returning the heart to its anatomical position. The greatest increase in ROS was immediately after repositioning the heart and was more than 50-fold greater in its signal intensity than baseline (Fig 2b).

Displacement of the heart affects right heart contractility, resulting in reduced venous return to the right ventricle and consequently, decreases in left ventricular output and coronary blood flow! We found that heart displacement had a generally negative effect on myocardial ROS when the heart was returned to its anatomical position. ROS induction may have negative consequences on cardiac contractile function (myocardial stunning) when superimposed on myocardial ischemia (myocardial stunning) when superimposed on myocardial ischemia, although in the present study coronary blood flow was reduced enough to induce ROS yet not enough to induce stunning. Hence, we found that returning the heart to its normal position resulted in normal coronary flow and contractile function, despite a 4-fold increase in the ROS signal intensity at 60 min compared with baseline.

We suggest that the reduction in coronary flow in our study was not due to ROS, but rather to anatomical right ventricular compression reducing venous return when the heart was elevated. It is possible that Trendelenburg positioning and/or repositioning the heart may induce acute reperfusion injury and thus explain the significant increase in ROS levels in our study. Furthermore, this finding of increased ROS may help to explain arrhythmia generation after beating heart bypass, in particular, for multivessel revascularization, when significant manipulation of the heart is undertaken.

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