Alternate antegrade/retrograde perfusion: an effective technique to preserve hypertrophied hearts during valvular surgery

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Abstract

Objective: Continuous antegrade perfusion (AP) may interfere with surgical precision. Continuous retrograde perfusion (RP), on the other hand, cannot sustain the empty-beating hypertrophied hearts. Therefore, alternate antegrade/retrograde perfusion (AA/RP) may be a rational technique to preserve the hypertrophied hearts. This study is to determine whether AA/RP could maintain myocardial energy metabolism, oxygenation, and contractile function of the empty-beating hypertrophied hearts. Methods: Sixteen hypertrophied pig hearts were divided into four groups (n = 4 per group). Group I and II underwent an 80-min AA/RP (four 10-min APs and four 10-min RPs), followed by a 20-min reperfusion. Group III and IV were subjected to an 80-min AP and 20-min reperfusion and used as a control. Energy metabolism was evaluated in group I and III using magnetic resonance spectroscopy. Myocardial oxygenation (MO) was assessed in group II and IV using near infrared spectroscopic imaging. Results: During 80-min AA/RP, four episodes of RP resulted in a significant decrease in myocardial phosphocreatine (PCr) and MO. The subsequent AP, however, resulted in complete recovery of the parameters. Moreover, myocardial adenosine triphosphate (ATP) remained at a normal level throughout the 80-min AA/RP. As expected, hearts in groups III and IV showed normal level of myocardial PCr, ATP, and MO throughout protocol. Finally, hearts in all four groups showed similar contractile function during reperfusion. Conclusions: AA/RP with four 10-min intervals of AP and RP sustained normal myocardial energy metabolism, oxygenation, and contractile function of empty-beating hypertrophied hearts. We conclude that AA/RP is an effective technique for preservation of empty-beating hypertrophied hearts during valvular surgery.

Keywords: Hypertrophied heart; Antegrade perfusion; Retrograde perfusion; Empty-beating; Energy metabolism; Myocardial oxygenation

1. Introduction

Traditional cardiopulmonary techniques provide sufficient myocardial protection for most cardiac patients with preserved ventricular function. In patients with compromised heart function, however, conventional cardiopulmonary techniques may not be sufficient, particularly for hypertrophied hearts because the latter have less tolerance to the potential detrimental effects of cardioplegia, such as myocardial edema and overload of potassium and chloride [1,2]. Beating-heart valve surgery has been used as an effective alternative to prevent cardioplegia-associated detrimental effects [3,4]. Preliminary clinical experience suggests that the visualization of operative field during beating-heart valve surgery is comparable to that of cardioplegia-assisted valve surgery [3,4]. Keeping the heart beating did not compromise surgical precision [3,4]. Moreover, dynamic 3-D architecture of a beating heart may facilitate intra-operative examination of aortic and mitral valve [5,6]. Our previous study demonstrated that keeping the heart beating with empty ventricles improved myocardial homeostasis for hypertrophied hearts relative to cardiopulgia arrest [7].

Sufficient homogeneous myocardial perfusion is the prerequisite for adequate myocardial preservation. Antegrade perfusion (AP), a physiological perfusion modality, provides homogeneous perfusion to a heart with normal coronary system. During valvular surgery, however, AP may have to be interrupted for surgical precision. Retrograde perfusion (RP) through the coronary sinus is usually established to protect the myocardium during the interruptions of AP [8,9]. Thus, alternate antegrade/retrograde perfusion (AA/RP) seems a more practical technique for myocardial preservation during valve surgery.
Oxygen consumption rate of the normothermic empty-beating heart is 5–6 ml/100 g/min, while RP is only able to deliver 1.5–2.5 ml oxygen to 100 g of the myocardium [10,11]. Thus, RP alone cannot provide sufficient blood flow to sustain an empty-beating heart, particularly a hypertrophied one. Repetitive and prolonged RP might result in accumulative ischemic injury of the hypertrophied hearts. On the other hand, it has been shown that three episodes of brief coronary artery occlusion (5–15 min each) resulted in reversible depression of contractile function and myocardial energy metabolism with 16% reduction in myocardial adenosine triphosphate (ATP) [12–14]. A short period of reperfusion resulted in complete recovery of cardiac function and energy metabolism [14]. Thus, we hypothesized that AA/ RP with 10 min for each perfusion interval could sustain myocardial energy metabolism and oxygenation for the empty-beating hypertrophied hearts. The present study was designed to test this hypothesis.

2. Material and methods

The animals used in this study received humane care in compliance with Guide to the Care and Use of Experimental Animals formulated by Canadian Council on Animal Care.

2.1. Pig model of pressure-overloaded left ventricular hypertrophy

Sixteen domestic pigs with left ventricular (LV) hypertrophy underwent an open-chest surgery. A heparinized polyethylene catheter was inserted into the LV chamber to measure the intra-ventricular pressure. Detailed method for isolation of the pig hearts has been described in our previous study [7]. In brief, after opening chest, the aorta, pulmonary artery, and inferior and superior vena cave were dissected and clamped. Cardioplegia was infused into the aortic root to arrest the heart. The heart was quickly excised and immersed in cold saline solution for instrumentation. The aorta was cannulated for AP. A 17F retrograde catheter with a balloon at its tip was positioned into the coronary sinus and secured with a purse-string suture for RP. A latex balloon was placed inside the LV to measure cardiac function. A short piece of polyethylene tube was inserted into the LV through the apex to drain thebesian flow.

Since pig blood collected from each animal was not enough to prime our heart perfusion apparatus, pig blood was mixed with Krebs–Henseleit (K–H) solution in a 1:1 ratio. The concentration of potassium in the mixture was adjusted at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol. The hemoglobin concentrations at 3.4 mmol/l to maintain myocardial electromechanical activity throughout protocol.

2.2. Experimental protocol

Sixteen isolated hypertrophied hearts were divided into four groups with four hearts in each group. Hearts in group I and group II underwent a protocol consisting of an 80-min AA/ RP (four 10-min APs and four 10-min RPs in alternate manner) and 20-min reperfusion. Hearts in group III and group IV were subjected to a protocol consisting of an 80-min AP and 20-min reperfusion. During the 80-min AA/ RP and AP, the LV balloon was completely deflated, leaving hearts in an empty-beating condition. The 80-min preservation period was chosen because it was sufficient for most valve surgeries.

Cardiac contractile function is often measured during both control perfusion and reperfusion periods. In this study we did not perform control perfusion prior to 80-min AA/ RP or AP. This was because it was almost impossible to completely drain the LV balloon once it was filled with water. To keep the hearts in a completely empty condition, the LV balloon was not inflated until reperfusion.

Group I and group III were used to determine whether AA/ RP was able to sustain normal myocardial energy metabolism by monitoring myocardial ATP, PCr, and Pi using phosphorus-31 (31P) magnetic resonance spectroscopy. Group II and group IV were to determine whether AA/ RP was able to sustain myocardial oxygenation (MO). In this study, myocardial oxygenated hemoglobin (oxy-Hb), oxygenated myoglobin (oxy-Mb), deoxygenated Hb (deoxy-Hb), and deoxygenated myoglobin (deoxy-Mb) were measured using NIRS imaging. MO was assessed using the ratio of [oxy-Hb + oxy-Mg]/[total-Hb + total-Mb].

At the beginning of the 20-min reperfusion, the LV balloon was inflated to the level that gave rise to a diastolic pressure of 4–5 mmHg. Heart rate, LV systolic pressure, LV diastolic pressure, and the rate of LV pressure increase were measured to assess cardiac contractile function. Throughout protocol, perfusion pressure at the aorta and coronary sinus was maintained at 65 ± 5 mmHg and 40 ± 5 mmHg, respectively. The corresponding antegrade flow was 217 ± 4.7 ml/min, and retrograde flow was 65 ± 4.0 ml/min during AA/ RP. Our previous study showed that this aortic pressure (~60 mmHg) was sufficient to sustain normal myocardial energy metabolism and contractile function [15].

At the end of experiment, heart weight, LV weight (LV free wall and septum) and LV wall thickness were measured. Corresponding data of normal hearts were obtained from the animals in our other studies and used as control for this study. Experimental protocols are illustrated in Fig. 1.

2.3. 31P MR spectroscopy

Myocardial energy metabolism was measured using localized 31P MR spectroscopy on a 7-Tesla magnet equipped with a Biospec spectrometer (Bruker, Karlsruhe, Germany). A home-built solenoid coil surrounding the whole heart was used in group I and III. Thus, the MR signals were acquired from the entire pig heart.

Each spectrum was averaged over a 2-min sampling time. The observed phosphorus compounds included Pi, PCr, and three peaks (α, β and γ) of ATP. The β peak was used for quantifying ATP. The values of energy metabolites were expressed as the percentage of the initial ATP value, which was obtained at beginning of the protocol and was set at 100%. The intracellular pH (pHi) of the hypertrophied hearts was calculated from the chemical shift of the Pi peak [16].
2.4. Near infrared spectroscopic (NIRS) imaging

Both Hb and Mb have two states, oxy- and deoxy-ones. Oxy-Hb and oxy-Mb have the same wavelength of light absorbance (760 nm), which is very different from that (920 nm) of deoxy-Hb and deoxy-Mb [17]. Thus, the two proteins in oxy- and deoxy-states can be readily distinguished and quantified using NIRS imaging. The ratio of \([\text{oxy-Hb + oxy-Mb}] / [\text{total-Hb + total-Mb}]\) can then be readily calculated. In this study, the ratio was used to reflect MO level.

NIRS imaging was performed with an infrared-sensitive charge-coupled device (CCD) camera equipped with a liquid crystal tunable filter. NIRS images were acquired with a field of view of \(12 \times 16 \text{ cm}^2\), covering the entire anterior surface of a pig heart.

2.5. Assessment of contractile function

Heart rate, LV developed pressure (LV peak systolic pressure minus LV end-diastolic pressure), and maximum rate of LV pressure increase and decrease \((\pm dp/dt)\) were continuously measured from the LV balloon during the 20-min reperfusion. Contractile ability of the hypertrophied hearts was also assessed by rate-pressure product (RPP, heart rate \(\times\) developed pressure).

2.6. Data analysis

\(^{31}\text{P}\) MR spectra were analyzed by using the software 1D-WINNER (Bruker, Karlsruhe, Germany). NIRS images were processed with MATLAB (Version 5.3, The Mathworks, Natick, MA).

Statistical analyses were performed using Statistica software (Statsoft Inc., Tulsa, Okla.). All numerical data were expressed as the mean \pm standard deviation. Student’s \(t\) test was used to determine any significant differences in myocardial PCr, Pi, ATP, pHi, MO, RPP, and \(+dp/dt\) between the two groups of the hearts (group I vs group III; group II vs group IV). Within a group, analysis of variance for repeated measurements was used to assess changes in the parameters measured during different perfusion stages.

Sample size was estimated using StatSupport (Dr John Eng, Johns Hopkins University, Baltimore, MD, USA). With minimum expected difference of 60% in PCr level (relative to initial level of ATP) and an average standard deviation of around 30%, a desired power of 0.8, and significance criterion of 0.05 for one tailed test, it was calculated that each group required four animals.

3. Results

3.1. Myocardial hypertrophy

LV end-diastolic pressure was significantly \((p = 0.0001)\) higher in the hypertrophied \((12.9 \pm 2.5 \text{ mmHg})\) than in the normal hearts \((4.9 \pm 1.2 \text{ mmHg})\). The ratio of LV weight to heart weight was also significantly \((p = 0.0008)\) higher in hypertrophied hearts \((0.61 \pm 0.02)\) than in the normal hearts \((0.47 \pm 0.02)\). The LV wall of the hypertrophied hearts was significantly \((p = 0.0008)\) thicker \((2.35 \pm 0.13 \text{ cm})\) than that of the normal hearts \((2.06 \pm 0.12 \text{ cm})\). The results indicate that a 12-week aortic banding resulted in the hypertrophied and failing hearts.

3.2. Effect of AA/RP on myocardial energy metabolism in hypertrophied beating hearts

Representative \(^{31}\text{P}\) MR spectra obtained from a pig heart in group I during 80-min AA/RP are shown in Fig. 2. RPs during AA/RP resulted in a significant decrease in PCr, with a corresponding increase in Pi. The two metabolites recovered to the initial baseline levels in the subsequent AP. ATP remained unchanged throughout 80-min AA/RP.

The changes in PCr, Pi, and ATP of group I hearts were summarized in Fig. 3. It is clear that decrease in PCr and increase in Pi during four 10-min RPs were in a similar level, suggesting that 10-min RP did not cause accumulative irreversible ischemic changes (Table 1). The levels of the metabolites at the end of reperfusion were not statistically different from those measured at the beginning of protocol.
ATP remained relatively unchanged throughout AA/RP (Fig. 3 and Table 1). ATP remained relatively unchanged throughout AA/RP (Fig. 3 and Table 1).

Fig. 4 shows comparison of myocardial PCr, Pi, and ATP between group I (80-min AA/RP) and group III (80-min AP). Both groups of the hearts showed no significant difference in ATP (p = 0.97), PCr (p = 0.28), and Pi (p = 0.78) at the end of each AP interval and reperfusion period (Fig. 4 and Table 1). At end of reperfusion, mean values of ATP in the two groups were 99.07% and 99.93%. Mean values of PCr were 270% and 272%. Mean values of Pi were 47.95% and 49.07%. Ninety-five percent confidence intervals for the mean difference were /C6 for ATP, /C6 for PCr, and /C6 for Pi. The 95% confidence intervals reveal that mean values of the respective parameter between the groups are within a few percentage points of each other. This further demonstrates that empty-beating hypertrophied hearts would only exhibit very insignificant changes in the parameters when preserved with alternate antegrade/retrograde perfusion.

Four RP episodes of AA/RP resulted in an approximately 0.2 unit decrease in pHi. However, there were no significant differences (p = 0.84) among the pHi values obtained at end of the four RPs. More importantly, pHi during reperfusion was comparable to that obtained at the first AP (p = 0.48, Table 1).

3.3. Effect of AA/RP on MO of hypertrophied hearts

Representative NIRS images obtained from a group II heart are shown in Fig. 5. The red area represents a well-oxygenated region with MO ([oxy-Hb + oxy-Mb]/[total-Hb + total-Mb]) above 0.9. Yellow and green areas reflect the less-oxygenated regions. During four RP intervals, several
regions of anterior walls of the heart turned to yellow and green, indicating heterogeneous ischemic changes. Regions and size of the yellow and green areas were comparable in the four NIRS images, suggesting that RP interval-associated ischemic changes were not accumulative. Moreover, during the subsequent APs, the anterior walls of the heart became red, indicating that the ischemic changes were completely reversible.

MO measured in group II and IV is summarized in Fig. 6. As expected, hearts subjected to 80-min AP (group IV) showed a normal level of MO throughout protocol (Fig. 6). In contrast, during the four RP intervals the hearts subjected to 80-min AA/RP (group II) showed a repetitive decrease in MO of both ventricles. MO decrease was significantly (*p = 0.0001) greater in the right ventricular (RV) wall (15 ± 3%) than in the LV wall (10 ± 2%). The subsequent AP perfusion resulted in a complete recovery of MO (Fig. 6 and Table 1).

### 3.4. Efficacy of AA/RP to preserve cardiac function

Fig. 7 shows comparison of maximal rate of LV pressure increase (+dp/dt) and the rate-pressure product (RPP) measured during reperfusion between the hearts subjected to 80-min AA/RP (group II) and those subjected to 80-min AP (group III and IV). No significant differences in the two contractile parameters between the groups, suggesting that 80-min AA/RP was able to preserve myocardial contractile function of hypertrophied hearts.

### 4. Discussion

Beating heart surgery has recently emerged as an alternative technique in valvular surgery [3,4]. This is because beating heart surgery prevents the potential detrimental effects of cardioplegia, such as myocardial edema and overload of potassium and chloride [7]. Development of an effective perfusion technique to protect the hypertrophied beating heart has become an urgent issue. Although AP is a physiological perfusion modality, it may have to be interrupted for surgical precision. RP, on the other hand, cannot fully sustain myocardial energy metabolism of a beating heart. Thus, combination of AP and RP seems a logical technique to protect the beating hypertrophied

### Table 1

<table>
<thead>
<tr>
<th>MO of LV(%)</th>
<th>Initial AP</th>
<th>End of each RP</th>
<th>Reperfusion</th>
</tr>
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<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>MO (%)</td>
<td>91.1 ± 1.3</td>
<td>85.1 ± 2.8</td>
<td>80.9 ± 3.6</td>
</tr>
</tbody>
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AA/RP, alternate antegrade/retrograde perfusion; AP, antegrade perfusion; RP, retrograde perfusion; PCr, phosphocreatine; Pi, inorganic phosphate; ATP, adenosine triphosphate; MO, myocardial oxygenation; LV, left ventricle; RV, right ventricle.

*p > 0.05 for the difference in PCr, Pi, MO of LV or RV compared among the end of each RP.

*p > 0.05 for the difference in ATP level compared among six different phase of AA/RP.

*p > 0.05 for the difference in PCr, Pi, MO of LV or RV compared between initial AP and reperfusion.

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**Fig. 4.** Comparison of myocardial PCr (upper panel), Pi (middle panel), and ATP (lower panel) measured during 80-min AA/RP and 80-min AP. Both groups of the hearts showed no significant difference in ATP, PCr, and Pi at the end of each AP interval and reperfusion period. PCr, phosphocreatine; Pi, inorganic phosphate; ATP, adenosine triphosphate; AA/RP, alternate antegrade/retrograde perfusion; AP, antegrade perfusion.
The present study was therefore to determine whether AA/RP was able to sustain myocardial energy metabolism, oxygenation, and contractile function of hypertrophied hearts under an empty-beating condition. We found that four RPs during AA/RP resulted in a small but significant decrease in PCr, pHi, and MO. This demonstrated that RP alone was not able to sustain hypertrophied beating hearts and should be used in combination with AP. On the other hand, 80-min AA/RP did not lead to significant decrease in ATP level. Also, PCr, pHi, and MO completely returned to normal levels during the subsequent APs, suggesting that 10-min RP interval did not cause severe irreversible ischemic injury. This demonstrated that AA/RP with a 10-min interval for each perfusion can sustain myocardial energy metabolism and oxygenation of hypertrophied hearts. In addition, PCr in the myocytes functions as ATP buffer to maintain ATP at normal levels. Thus, decrease in PCr under ischemic conditions is usually much earlier in decrease of ATP. As a result, we did not observe a significant change in ATP level during 80-min AA/RP although PCr decreased significantly.

In this study, no control perfusion was performed prior to the 80-min empty-beating perfusion. Thus, we did not have control values of cardiac function before myocardial preservation and could not calculate recovery percentage of cardiac contractile function. We found that the LV balloon...
could not be completely drained once it was filled. In order to keep the LV completely empty and prevent the LV performing any external work, we did not perform control perfusion. Our protocol started from the first AP episode of AA/RP or AP. Nevertheless, the hearts subjected to AA/RP (group I and II) and those to AP (group III and IV) showed comparable RPP and maximum pressure increase and decrease (±dp/dt) of the LV. This suggests that AA/RP could sustain normal myocardial contractile function of the hypertrophied hearts.

In this study $^{31}$P MR spectroscopy was used to assess the effects of AA/RP on myocardial energy metabolism. A MR coil surrounding the whole heart was used to acquire $^{31}$P spectra of energy metabolites. With this technique, we could not assess heterogeneity of myocardial energy metabolism in each region. It has been shown that MO ($\text{[(oxy-Hb + oxy-Mb)]/}$ $\text{[total-Hb + total-Mb]}$) is closely related to coronary blood flow and energy metabolism and can be readily monitored with NIRS imaging. The latter has a high spatial resolution. Therefore, NIRS imaging was used in this study to monitor myocardial oxygenation. We found that the four RP episodes of AA/RP resulted in a greater MO decrease in the RV wall than in LV wall due to the inability of RP to perfuse the RV wall [21]. Decrease in RV’s MO was not accumulative (Fig. 6 and Table 1). Moreover, MO in both ventricles returned to normal levels during the subsequent AP episodes (Fig. 6 and Table 1). These results suggest that 10-min RP episodes did not result in severe irreversible injury and was able to sustain RV energy metabolism.

It should be pointed out that the present study focused on the hypertrophied hearts that have no significant coronary stenosis. Patients with severe myocardial hypertrophy may also have coronary abnormalities. In addition, myocardial hypertrophy was induced using supra-coronary banding of the aorta. Increased coronary pressure may have some patho- logical effects on the coronary system. Moreover, this study used isolated (denervated) hypertrophied pig hearts. Coronary perfusion and myocardial energy metabolism of the isolated hearts may be slightly different from those of in vivo human hearts. Thus, it should be with caution to extrapolate the findings of this study to the clinical arena. On the other hand, the hypertrophied hearts were perfused with a mixture of pig blood and K–H solution, which had a lower oxygen-carrying capacity than physiological blood. Thus, it is possible that RP episodes of AA/RP may result in less prominent ischemic changes in human patients than those observed in this study.

In conclusion, 80-min AA/RP can sustain myocardial energy metabolism and contractile function of empty-beating hypertrophied hearts. Thus, normothermic normokalemic AA/RP is an effective technique for protection of the hypertrophied hearts.

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References