Effect of ischemic postconditioning in adult valve replacement

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Abstract

Objective: Ischemic postconditioning (POC) by brief episodes of ischemia performed just at the time of reperfusion can reduce infarct size in animal models and clinical settings of percutaneous coronary intervention. However, the clinical applicability of postconditioning in cardiac surgery remains to be determined. We investigated the effect of postconditioning on myocardial protection in patients undergoing valve replacement. Methods: Fifty adult patients scheduled for elective valve replacement under cold blood cardioplegic arrest were randomly assigned to postconditioning (n = 25) or control treatment (n = 25). Postconditioning was performed by three cycles of 30 s ischemia and 30 s reperfusion using aortic re-clamping and de-clamping started 30 s after cardioplegic arrest. The creatine kinase-MB, troponin I, transcardiac release of lactate were assayed. Measurements of clinical results were recorded during the study. Results: The types of procedure, age, bypass and aortic cross-clamping times were similar in both groups. The postoperative peak creatine kinase-MB was lower after aortic de-clamping in the postconditioning patients compared with the control group (66 ± 24 U/l vs 84 ± 20 U/l, p = 0.02) and peak cTnI was similar in both groups. The required inotropes were reduced in postconditioning group compared with the control group (2.3 ± 1.8 mmol/l vs 4.1 ± 2.2 μg/min/kg, p = 0.03). There were reduction trends with regard to transcardiac release of lactate in postconditioning group compared with the control group (0.10 ± 0.17 mmol/l vs 0.24 ± 0.16 mmol/l, p = 0.08). The transcardiac neutrophil count during reperfusion was less in POC group compared with the control group (7.8 ± 6.3% vs 14.0 ± 8.7%, p = 0.04). Conclusions: The present study demonstrated that postconditioning may protect adult myocardium undergoing cold blood cardioplegic arrest. These data support the need for a further clinical trial of postconditioning in cardiac surgery.

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Keywords: Myocardial protection; Cardiac surgery; Postconditioning; Ischemia; Reperfusion

1. Introduction

Although major advances have been made in the field of adult cardiac surgery, inappropriate myocardial protection is still considered the main cause of mortality and morbidity in adult cardiac surgery. For myocardial protection, the cardioplegia solutions are popular worldwide with cardiac surgeons. Although the application of cardioplegia is an effective strategy, it does not completely eradicate myocardial ischemia—reperfusion injury. The myocardium stunning, necrosis and apoptosis remain to be one of the major issues in cardiac surgery [1,2]. The protection of myocardium during a cardiac operation has two aspects: one is to reduce ischemia injury by using cardioplegia and hypothermia during cardiac arrest; the other is to decrease reperfusion injury by using modified reperfusion.

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The phenomenon of ischemic postconditioning (POC) referring to the multiple brief periods of ischemia—reperfusion performed just after the prolonged ischemic insult was described firstly by Zhao et al. [3] in the dog model and has proved to have strong endogenous myocardial protection by reducing reperfusion injury [4]. The phenomenon of POC has also been proved to exist in the beating heart of humans during the setting of percutaneous coronary intervention for acute myocardial infarction [5,6]. These results of the study on POC in animals and in human are very encouraging. However, whether POC has an additive effect to cardioplegia in the setting of cardiac surgery, one of the few controlled models of human myocardial ischemia, remains to be determined. Until now, the effect of POC on myocardial protection has not been investigated in the field of adult cardiac surgery. We hypothesized that ischemic postconditioning would provide protection against myocardial ischemia–reperfusion in adult patients undergoing cardiopulmonary bypass (CPB) for valve replacement and therefore conducted a controlled trial to evaluate the effect of ischemic postconditioning on myocardial protection in patients undergoing valve replacement.
2. Methods

2.1. Patients and operation

Fifty adult patients aged between 18 and 60 with rheumatic heart valve disease undergoing elective valve replacement were randomly divided into two groups of 25 each. The patients signed a consent form approved by our hospital research committee before operation. The patients were selected by a randomized number table. The hospital staff caring for postoperative patients was blinded for group allocation. Exclusion criteria for this study included infective valve disease, valve disease with coronary artery disease, hypertension and previous valve repair. None of the patients received aspirin, corticosteroids, or statin preoperatively. In addition, none of the patients had received preoperative inotropic support.

During the surgical procedure, anesthesia was induced with midazolam and vecuronium bromide intravenously, followed by the use of intravenous fentanyl (15 \( \mu \)g/kg/h) and intermittent inhalation of isoflurane to maintain anesthesia. The operations were performed by same surgeon team. The operations were performed using standard hypothermic cardiopulmonary bypass (28–31 °C) with bicaval cannulation and left vent via right superior pulmonary vein. The myocardium was protected using intermittent perfusion of cold blood cardioplegia. The cardioplegia solution was mixed through the delivery system of the heart—lung machine with autologous blood obtained from the extracorporeal circuit at a ratio of 1:4 (crystalloid cardioplegia:autologous blood) while the patient is on bypass prior to its application. The cold blood cardioplegia at 4 °C (components: NaCl, 132 mmol/l; KCl, 16 mmol/l; CaCl2, 1.8 mmol/l; MgSO4, 15 mmol/l; procaine, 0.05 mmol/l; NaHCO3 19 mmol/l) was infused into the aortic root if the aortic valve was competent; otherwise, it was infused directly into the orifices of the coronary arteries. The initial dose was 20 ml/kg body weight at a flow rate of 200–250 ml/min. Thereafter, the cardioplegia was reinfused every 20–30 min, at a dose of 10 ml/kg, after initial infusion. The operative technique has been described previously [7]. All patients received similar standard postoperative care in our hospital. The dopamine and/or dobutamine were selected as the first-line inotropes and epinephrine or noradrenine as second-line inotropes for hemodynamic support if needed.

2.2. Postconditioning protocol

Postconditioning was started at 30 s after aortic cross-declamping, and the aorta was re-clamped for 30 s rendering global myocardial ischemia. Meanwhile aortic root suction was established during aortic re-clamping, and thereafter, the aortic clamp was released for 30 s for full myocardial reperfusion. The cycle was repeated three times after cardioplegic arrest. In the control group, the same protocol was performed as in postconditioning patients except the repeated re-clamping aorta was not used after cardioplegic arrest (Fig. 1).

2.3. Blood sampling and biochemical analysis

Blood samples were obtained preoperatively (T1), 4 (T2), 8 (T3), 20 (T4) and 48 h (T5) after aortic de-clamping for determination of creatine kinase-MB (CK-MB), cardiac troponin I (cTnI). The CK-MB was determined immediately after sampling using immunoturbidimetric assay (CK-MB Kit from Jidan Biotechnology Company, Nanjing, China) with analyzer of HITACHI 7170A. The value was expressed as U/l (normal reference value in our hospital: ≤24 U/l). For cTnI measurement, the plasma was transferred to a sterile polypropylene tube and stored at –20 °C until assayed. The cTnI were measured by means of commercially available enzyme-linked immunosorbent assays according to the supplier’s recommendations (cTnI Kit from Jidan Biotechnology Company, Nanjing, China) with an analyzer of ELX800 (BIO-TEK Instruments, Inc., USA). The value was expressed as ng/ml (normal reference value in our hospital is <0.15 ng/ml).

2.4. Transcardiac white blood count

Blood samples were drawn simultaneously from the aorta and coronary sinus, respectively, just before aortic cross-clamping and at 5 min after aortic de-clamping to obtain the routine white blood cell (WBC) count. The WBC count was determined using analyzer of CD-3700. The difference of WBC count between the blood from the aorta and from the coronary sinus was calculated as follows: WBC sequestration = (WBCa – WBCcs) × 100/WBCa and represents as percentage of WBC in the aorta.

2.5. Transcardiac lactate

The same blood samples as taken for white blood cell count were analyzed for blood gases and lactate with an analyzer (GEM Premier 3000, Instrumentation Laboratory, USA). The difference of lactate between arterial and coronary sinus was calculated as follows: the lactate level in the coronary sinus minus lactate level in the artery. Positive values of lactate were defined as release from myocardium, and negative values were defined as uptake by myocardium.

2.6. Hemodynamic measurement and clinical data

Intraoperative and postoperative clinical data were prospectively collected. Mean arterial pressure, heart rhythm and rate, central venous pressure, ventilation time, intensive care unit and hospital stay were routinely recorded during the experiment. The first postoperative 24 h quantitative measures of inotropes were evaluated by inotropic score with the formula [8]: (dopamine + dobutamine)
atrial fibrillation (AF) in either group. The operative and gender, weight, NYHA class, ejective fraction (EF) and significant differences were noted with regard to age, 3.1. Clinical outcomes

3. Results

3.2. CK-MB activity

Preoperative plasma concentrations of CK-MB activity were within normal limits for all patients and were not different between the groups. A significant postoperative increase in CK-MB activity was observed in the control and postconditioning groups (time effect, \( p = 0.00 \) and \( p = 0.01 \), respectively). There was a significant difference over time between groups (group effect, \( p = 0.03 \)), and the significant differences between the groups were found at 4 and 8 h intervals after aortic de-clamping (Fig. 3; \( p = 0.02 \) and \( p = 0.04 \), respectively).

3.3. The levels of plasma cTnl

The levels of serum cTnl, collected at several sampling times in both groups of patients, are presented in Fig. 4. The preoperative plasma levels of cTnl were similar in both groups. A significant postoperative increase in cTnl activity was observed in both groups (time effect, \( p = 0.00 \) for POC and \( p = 0.01 \) for control, respectively). Repeated measures of ANOVA revealed a trend to less release of cTnl in POC patients but not to reach statistically significant difference compared with the control group (group effect, \( p = 0.09 \)).

3.4. Transcardiac white blood count

The white blood cell count difference (aorta—coronary sinus) was similar before aortic clamping in both groups (3.1 ± 0.8% vs 2.8 ± 1.0%, \( p = 0.56 \)). The difference was significant in both groups at 5 min after aortic de-clamping.

### Table 1
Patient demographics and preoperative characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control ((n = 25))</th>
<th>Postconditioning ((n = 25))</th>
<th>( p )-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40 ± 12</td>
<td>42 ± 10</td>
<td>0.49</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>14/11</td>
<td>13/12</td>
<td>0.61</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>54 ± 10</td>
<td>56 ± 11</td>
<td>0.58</td>
</tr>
<tr>
<td>NYHA class ((1/2/3/4))</td>
<td>1/10/14/0</td>
<td>0/7/17/1</td>
<td>0.38</td>
</tr>
<tr>
<td>MVR (n)</td>
<td>15</td>
<td>13</td>
<td>0.67</td>
</tr>
<tr>
<td>DVR (n)</td>
<td>14</td>
<td>13</td>
<td>0.71</td>
</tr>
<tr>
<td>AF (n)</td>
<td>18</td>
<td>19</td>
<td>0.72</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>56 ± 6</td>
<td>57 ± 9</td>
<td>0.70</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>84 ± 9</td>
<td>87 ± 11</td>
<td>0.63</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>86 ± 7</td>
<td>85 ± 5</td>
<td>0.73</td>
</tr>
<tr>
<td>CVP (mmHg)</td>
<td>7.8 ± 1.7</td>
<td>7.6 ± 1.5</td>
<td>0.52</td>
</tr>
</tbody>
</table>

NYHA = New York Heart Association; MVR = mitral valve replacement; DVR = double valve replacement; AF = atrial fibrillation; LVEF = left ventricular ejection fraction; HR = heart rate; MAP = mean arterial pressure; CVP = central venous pressure.

× 1) + ([epinephrine + norepinephrine + isoproterenol] × 100). All patients were followed up for at least 30 days after operation.

2.7. Statistical analysis

If not otherwise indicated, values were expressed as a mean ± SD. Statistical analysis was performed with the SPSS13.0 software (SPSS Inc., Chicago, IL). The differences were assessed by unpaired Student’s t-test for parametric data and Mann–Whitney test for non-normally distributed data. Two-factor repeated-measures analysis of variance (ANOVA) was used to evaluate differences over time between groups for CK-MB, cTnl and hemodynamic parameters and post hoc tests (Bonferroni test) were used to compare these parameters at each time point between the groups. Categorical data were analyzed using the two-tailed Fisher exact test or chi-square test as appropriate. A \( p \)-value less than 0.05 was considered significant.

### Table 2
Intraoperative and postoperative characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control ((n = 25))</th>
<th>Postconditioning ((n = 25))</th>
<th>( p )-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPB time (min)</td>
<td>78 ± 23</td>
<td>81 ± 24</td>
<td>0.47</td>
</tr>
<tr>
<td>ACC time (min)</td>
<td>54 ± 20</td>
<td>58 ± 21</td>
<td>0.46</td>
</tr>
<tr>
<td>Postoperative hospital stay (days)</td>
<td>9 (6—13)</td>
<td>8 (6—11)</td>
<td>0.73</td>
</tr>
<tr>
<td>Ventilation time (h)</td>
<td>11 ± 6</td>
<td>10 ± 5</td>
<td>0.69</td>
</tr>
<tr>
<td>ICU stay (h)</td>
<td>28 ± 10</td>
<td>29 ± 13</td>
<td>0.83</td>
</tr>
<tr>
<td>Inotrope score ((\mu)g/min/kg)</td>
<td>4.1 ± 2.2</td>
<td>2.3 ± 1.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Transfusion of PRBC ((\mu)l)</td>
<td>2.0 ± 1.5</td>
<td>2.2 ± 1.8</td>
<td>0.62</td>
</tr>
<tr>
<td>Drainage of first 24 h (ml)</td>
<td>330 ± 91</td>
<td>345 ± 101</td>
<td>0.67</td>
</tr>
</tbody>
</table>

CPB = cardiopulmonary bypass; ACC = aortic cross-clamping; ICU = intensive care unit; PRBC = packed red blood cell.
(7.3 ± 6.3% for postconditioning vs 14.8 ± 8.7% for control, p = 0.043; Fig. 5). Further analysis revealed that sequestration of WBC in myocardium at 5 min during reperfusion was significantly correlated with postoperative inotropes score (r = 0.504, p = 0.026).

### 3.5. Transcardiac lactate release

Before cardioplegic arrest, the transcardiac lactate values were all positive (uptake) in both groups without significant difference between groups. At 5 min after aortic de-clamping, there were reduction trends with regard to transcardiac release of lactate in the postconditioning group compared with the control group (0.10 ± 0.17 mmol/l vs 0.24 ± 0.16 mmol/l, p = 0.08).

### 4. Discussion

The major finding of this study is that three cycles of intermittent brief episodes of global myocardial ischemia induced by repeated aortic clamp after cardioplegic arrest significantly reduced the postoperative inotropic requirement by 40% in adults undergoing valve replacement. In the postconditioning group, only 16% of patients required doses of dopamine greater than 5 µg/kg/min, whereas in the control group 40% patients received such doses of dopamine to support hemodynamics. The total inotropes score of postoperative 24 h in postconditioning was also significantly reduced compared with the control group. This result interestingly was consistent with our previous pilot study on ischemic postconditioning in patients with tetralogy in which postoperative inotropic requirement (score) was reduced by 40% in the POC group [9], and also consistent with a recent study using adenosine as pharmacologic postconditioning in valve replacement patients with approximately a 30% reduction of inotropes use [10]. In the present study, the postoperative release of CK-MB was also significantly decreased in POC patients. Based on the calculated area under the curve of CK-MB release, the estimated reduction of myocardial necrosis in the postconditioning group is approximately 20% when compared with that in the control group. The study suggested that ischemic postconditioning may provide, to some extent, myocardial protection in adult patients. To our knowledge, the present study is the first controlled and randomized clinical trial evaluating the effect of ischemic...
postconditioning on myocardial protection in the setting of adult cardiac surgery. So far, the majority of studies on both animal models and in humans demonstrated that ischemic postconditioning reduced myocardial necrosis in the setting of acute myocardial ischemia/reperfusion without using cardioplegia [5,6,11–13]. Serviddio et al. [14] reported hypoxia postconditioning could reduce myocardial injury in an isolated rat heart undergoing cold cardioplegic arrest. Although in the setting of cardiac surgery, myocardial stunning, not necrosis, after cardioplegic arrest is a major issue, clinical evidences showed myocardial necrosis after cardiac surgery using cardioplegia still plays an important role in patients’ postoperative recovery and even prognosis [15,16]. Although larger controlled clinical trials are needed to determine the effect of ischemic postconditioning on the reduction of myocardial necrosis in cardiac surgery, our results provide support for ischemic postconditioning as an adjunct to cardioplegia in reduction of myocardial necrosis in adult cardiac surgery. As to whether ischemic postconditioning decreases myocardial stunning after cardioplegia arrest in humans, this needs further study. In present study, the majority of patients in both groups were in a low-risk condition with short aortic cross-clamping time. It remains to be determined if high-risk patients such as those with prolonged aortic cross-clamping may benefit more from ischemic postconditioning.

Another finding of this study is that POC decreases WBC sequestration in myocardium assessed by measurement of transcardiac difference of WBC as an index for neutrophil sequestration in the myocardium during early reperfusion. This finding is consistent with previous results from animal models [17]. The present study also showed that the transcardiac difference of WBC during reperfusion is correlated significantly with inotropes requirement suggesting that WBC sequestration to some extent may be related to myocardial injury. The different pathophysiologic processes have been proposed to explain the beneficial effect of POC. It has been reported that POC reduces the inflammatory response and activates cardioprotective signaling pathways [13,18,19]. Other mechanisms may also contribute to gradual reperfusion [20]. In the setting of cardiac surgery, both aspects with regard to mechanism of POC may be involved. However, the final conclusion about precise mechanisms of POC in humans remains to be answered.

The cardioplegic arrest heart in the setting of cardiac surgery is one of the few controlled models of myocardial ischemia and reperfusion injury in which postconditioning as a simple procedure could be applicable at the early time of reperfusion by using intermittent aortic cross-clamping. Unlike preconditioning induced before ischemia, POC was induced after ischemia insult, which was easily performed in all cardiac operations such as in those with severe aortic insufficiency or aortic stenosis where preconditioning often is difficult to be carried out before an operation due to possible heart arrest and distension induced during repeated aortic clamping.

There are several limitations of our present study. Firstly, the relatively small sample size of patients in our study is not clinically sufficient to evaluate the effects on the reduction of morbidity and mortality. A final determination of effect would need a larger sample size and involve a multicenter study. Secondly, the objective assessments of cardiac output, such as thermodilution measurements of cardiac index, were not performed. Third, this study did not intensively explore the precise mechanisms of postconditioning of humans; clinically relevant evidences related to the mechanism of postconditioning need further study. However, ischemic postconditioning is an attractive strategy for cardiac surgeons due to its simplicity, cost-effectiveness, and ease of performance in the setting of cardiac surgery [21].

In conclusion, the present study suggests for the first time that ischemic postconditioning adjunct to cold blood cardioplegia is related to less inotropic use and less CK-MB release. A larger clinical trial is needed to determine the clinical effect of ischemic postconditioning in adult cardiac operation.

References

Appendix A. Conference discussion

Dr W. Flameng (Leuven, Belgium): Postconditioning is, as is preconditioning, a very, very intriguing phenomenon. When we look at myocardial ischemia in the setting of cardiac surgery, as you presented here, we can recognize two phases; the phase of real myocardial ischemia due to the cessation of coronary flow, and the next phase is the reperfusion injury. To a certain degree, these two phases can end up in myocardial necrosis, and postconditioning is meant to prevent this myocardial necrosis to a certain extent. Now, when you compare preconditioning and postconditioning, pre-conditioning can trigger some cellular protective mechanisms in the myocardium during this first ischemic phase as well as during the reperfusion phase. When you look at postconditioning, this can do it only by protecting or triggering the protective cellular effects in the reperfusion phase.

So my question is, is there a similarity between the two basic mechanisms of postconditioning and preconditioning? And I refer to the publication of your colleagues from Xi’an. We all know that publication. It appeared in this year’s Annals of Thoracic Surgery, presenting a very similar protocol, same surgical setting, valve replacement, but they used as postconditioning an adenosine infusion in the aortic root instead of this intermittent clamping, or re-clamping, as you call it. So my first question is, what is the basic mechanism of this postconditioning in your mind?

The second question is more a question about the robustness of your data, and for this, I want to go back to what Robert Dion previously mentioned. It’s confusing to me that you don’t see any difference in enzyme release based on troponin assessment, which is much more specific than the CK-MB release. Where do you find the significant effect? Also, in the CK-MB release, when you study CK-MB release in this reperfusion phase after cardiopulmonary bypass, usually you see two phases: a first release phase with the peak at 6 h and then a second release phase with the peak at 24 h. So my question is, did you study the area under the curve, the complete release?

We also know that CK-MB release and the concentration in the serum are dependent on urinary function. Were the urinary function and the kidney function in your two populations the same?

The last question is about the positive inotropic support. You have less positive inotropic support required in the postconditioning group, but the need for positive inotropic support does not necessarily reflect myocardial necrosis. Can we be that you are just looking at myocardial stunning which is less pronounced in the postconditioning group as it is in the control group?

Dr Luo: I’m sorry, my English is limited, so I beg your pardon. The first question, do you mean are the preconditioning and postconditioning similar?

Dr Menasche (Paris, France): Similar mechanisms.

Dr Luo: Yes. That’s the first question. The second question is preconditioning? We have performed this study before and used the preconditioning, and those results were published in the Annals of Thoracic Surgery maybe 7 years ago. The major finding was reduction of reperfusion injury, yes, sir. The secondary was the reduction of the CK-MB release. Now our surgeons are not waiting to do the preconditioning before checking the time before the cardiac operation, about 10 min of CPB. So we prefer the postconditioning after the cardiac operation is finished. It’s easy to perform. We didn’t notice measures and mechanisms, like the enzymes, so we didn’t understand what the precise mechanism is of postconditioning. We have other ways to test this mechanism of postconditioning now.

The third question?

Dr Menasche: The question about stunning, do you think that postconditioning may reduce postoperative stunning?

Dr Luo: Stunning?

Dr Menasche: Stunning, transient left ventricular dysfunction. I think that’s what Dr Flameng asked. Do you think that postconditioning may improve left ventricular function, which would be by targeting cell signaling pathways. J Am Coll Cardiol 2004;44:1103—10.

Dr Luo: We didn’t measure their heart function. Maybe there was dysfunction by the catheter, Swan-Ganz. We have no extra money to fund this study, so we didn’t measure the cardiac output.

Dr J. Vøge (Oslo, Norway): Yes, I’m sure there is stunning in this. I will try to be very short, even though there are many, many things to discuss on this study. First, I compliment you. As far as I know, this is the first study in heart surgery on postconditioning.

I will only ask you one question. You just call this valve operations. Did you include all kinds of valves or was it only aortic valves? Was it mitral and aortic? And was there any difference in the distribution of the different valves in the two groups?

Dr Luo: You mean what?

Dr Menasche: Different valve groups, between mitral and aortic, between the two groups.

Dr Luo: No difference.