Evaluation of ATP-PostC protective effect on myocardial function: A speckle tracking echocardiography study in ischemia-reperfusion injury

Min Ren

Tongji University School of Medicine

Keting Li

Tongji University School of Medicine

Xuemei Liu

Shuguang hospital affiliated to Shanghai University of Traditional Chinese Medicine

Hongyu Yang

The second affiliated Hospital of Harbin Medical University

Zhonghui Jiang

The second affiliated hospital of Harbin Medical University

Xinyu Wu

The second affiliated hospital of Harbin Medical University

Yujie Liu (✉ liuyujie@163.com)

The second affiliated hospital of Harbin Medical University <https://orcid.org/0000-0001-6805-0440>

Research

Keywords: speckle tracking echocardiography, ischemia-reperfusion injury, ATP, ischemia postconditioning, pharmacologic postconditioning

Posted Date: March 13th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-17130/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background: Ischemia-reperfusion injury (IRI) seriously affects the prognosis of patients. We sought to use speckle tracking echocardiography (STE) to accurately evaluate the effect of drugs.

Methods: In this study, STE was used to quantitatively evaluate the changes of myocardial function before and after reperfusion in myocardial ischemia rabbits with ischemia postconditioning (I-PostC) or ATP postconditioning. The variations of the left ventricular (LV) longitudinal, circumferential and radial myocardium were detected by STE technique. Meanwhile, a series of biochemical experiments were performed including myocardial enzymes assay, myocardial infarction size assay and TUNEL assay.

Results: After ligation of coronary artery for 45 min, the strain and strain rate curves of left ventricular myocardium was disordered. The most STE parameters were significantly diminished and the time of reaching peak was delayed. After reperfusion for 120 min, the parameters (longitudinal, circumferential, radial strain and strain rate, as well as ventricular torsion function) were obvious recovery in I-PostC and ATP postconditioning groups. The sensitivity and specificity of global circumferential peak strain (GCSp), global longitudinal peak strain (GLSp), peak twist (Ptw) and peak twisting velocity (PTV) to diagnose myocardial infarction rabbits were 86.1% and 75%, 79.2% and 72.5%, 79.2% and 75%, 66.7% and 70.8%, respectively. The levels of aspartate aminotransferase (AST) and creatine phosphokinase isozyme (CKMB) were up-regulated in I/R group. AST and lactate dehydrogenase (LDH) values significantly reduced in the other three groups. The Bax expression was decreased, simultaneously, the Bcl-2 expression was increased after I-PostC and ATP postconditioning treatment.

Conclusions: The application of STE in the assessment of IRI has high accuracy and reproducibility. Therefore, ATP has an important clinical value as a pharmacologic postconditioning drug.

Introduction

There are annual 17 million to 19 million people dying from cardiovascular disease around the world. Among them, about 50% of people died of coronary heart disease, especially acute myocardial ischemia or infarction (MI). Early open occlusion of the coronary artery, ischemic myocardium can effectively restore blood reperfusion and save the dying myocardium. However, revascularization, such as percutaneous transluminal balloon angioplasty and coronary stenting, can cause ischemia-reperfusion injury (IRI), including cardiac dysfunction and metabolic ultrastructure, further affecting the prognosis. Therefore, it is significant to look for the new method to treat IRI [1].

Ischemia preconditioning (IPC) [2], ischemia postconditioning (I-PostC) [3] and pharmacologic postconditioning (P-PostC) are the three methods that can alleviate IRI [4, 5]. IPC needs to be implemented before occurrence, while myocardial ischemic events are often unpredictable in clinic, which makes it significantly limited. I-PostC is applied after ischemia by the short recovery perfusion in many times. Although the use scope of I-PostC is expanded, the mechanical damage it brings is inevitable. P-PostC therapy is predictable and can be performed under the control of doctors, which provides a simpler strategy for MI. However, the specific clinical and biological mechanisms are unclear, and this may be the key to the application of drugs in IRI.

ATP is a commonly used drug in clinical practice because it is inexpensive and can be metabolized rapidly to the adenosine in the body to function as the adenosine [6, 7]. The mechanism of the adenosine protecting ischemia-reperfusion may be related to scavenging oxygen free radicals and the adenosine receptors. The latest myocardial function quantification technique, speckle tracking echocardiography (STE) [8], was used to evaluate the changes of left ventricular (LV) global and regional myocardial function before and after infarction in each model group. STE [9] can track echo space motion in myocardium in real time, tracking the direction of the long axis of the myocardium (from the bottom of the heart to the apex). It also can track the movement of the radial (central motion) and axial (rotary motion) of the myocardium. Combined with pathological results, the value of early localization and quantification of regional myocardial dysfunction in STE was evaluated. The myocardial protective effects of I-PostC and adenosine triphosphate (ATP)-PostC were analyzed and the pathophysiological mechanism of IRI was explored. The molecular biological mechanism of ATP-PostC was discussed in order to lay a good foundation for the clinical application of the technology and method.

Methods

Animal preparation and experimental protocol

All animal studies were performed in accordance with the National regulations on the Administration of Laboratory Animals. Forty healthy Japanese white rabbits, age 5–6 months, weight 2.00–2.75 kg, which were raised in a single cage at room temperature.

The rabbits were anesthetized via intravenously injecting 2.5% pentobarbital sodium (30 mg/kg) from the auricular margin. To expose the heart, the skin, subcutaneous tissue and sternum were cut along the middle of the sternum. The circular needle passed through the superficial layer of the myocardium about 2 mm under the left atrial appendage, then the ligation line was retained to make the myocardial infarction model. Immediately after ligation, the color of the tissue at the ligation line of the anterior wall of the LV was observed. The regional myocardial movement was weakened and ST segment was obviously elevated on two or more leads of limbs or chest, which indicated that ligation was successful.

The rabbits were randomly divided into 4 groups (10 rabbits in each group), including I/R, I-postC, ATP-V-PostC, and ATP-A-PostC, respectively. The LV branches of rabbits in I/R group were occluded for 45 min followed by 120 min reperfusion. Those in the I-postC group were occluded for 45 min followed by four cycles of 30 s reocclusion and 30 s reperfusion, and then reperfusion lasted 120 min. The LV branches of rabbits in the ATP-V-PostC group were occluded for 45 min, afterwards, the rabbits were injected ATP ($300 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) by ear vein 5 min before reperfusion. The time for reperfusion was 120 minutes. The

rabbits in the ATP-A-PostC group were subjected the similar experimental operation with those in the ATP-V-PostC group. The only difference was that ATP was administered differently. The rabbits in the ATP-A-PostC group were injected ATP by aortic root.

Acquisition And Post-processing Of STE Images

Echocardiography was performed using a Vivid 7 color Doppler ultrasound instrument (GE, Boston, USA). The shape, echo and motion of each segment of the LV wall were observed by conventional two-dimensional echocardiography, and the atrioventricular lumen size was measured. The LV end-diastolic diameter (LVEDD), LV ejection fraction (LVEF), end-diastolic volume (EDV) and end-systolic volume (ESV) were measured by biplane Simpson's method, following the stroke output (stroke volume, SV) was calculated. Two-dimensional gray-scale dynamic images of three consecutive cardiac cycles on the long axis of the apical axis (four-chamber, two-chamber and LV long axis) and a series of short axis of the LV (mitral valve, papillary muscle and apical level) were collected.

The STE images were analyzed via the EchoPAC 7.0 external workstation (GE Healthcare, Horten, Norway). The parameters included longitudinal, circumferential, radial strain rate and strain, and LV torsion function indexes: torsion angle and peak time, peak torsion velocity and peak time, and calculation of resolution and half rotation time. The degree of left ventricular unwinding [(AVC point torsion angle-MVO point torsion angle) / AVC point torsion angle] / IVRT × 100%, half of the time of unwinding: the time from the peak of R wave to the peak of torsion angle at the end of contraction.

Longitudinal speckle tracking imaging indicators: global longitudinal systolic strain (GLSsys) and the peak time (T-GLSsys); global longitudinal peak strain (GLSp) and the peak time (T-GLSp); longitudinal systolic strain (LSsys) and the peak time (T-LSsys); longitudinal peak strain (LSp) and the peak time (T-LSp).

Circumferential speckle tracking imaging indicators: global circumferential contraction strain (GCSsys) and the peak time (T-GCSsys); global circumferential peak strain (GCSp) and the peak time (T-GCSp); circumferential systolic strain (CSsys) and the peak time (T-CSsys); circumferential peak strain (CSp) and the peak time (T-CSp).

Radial speckle tracking imaging parameters: radial systolic strain (RSSys) and the peak time (T-RSSys); radial peak strain (RSp) and the peak time (T-RSp).

Torsion function indicators: peak twist (Ptw), time to peak twist (TPtw); peak twisting velocity (PTV), time to peak twisting velocity (TPTV); peak untwisting velocity (PUV), time of peak untwisting velocity (TPUV); half time of untwisting (HTU); calculation of untwisting rate (untw R).

Myocardial Enzymes Assay

Myocardial enzymes refer to a variety of enzymes in the myocardium, including aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and isoenzymes, creatine phosphokinase (CK) and isozyme (CKMB). The levels of AST, CK, CKMB and LDH were detected at three time points (before ligation, 45 min after ligation, and 120 min after reperfusion) using kits.

Measurement Of Myocardial Infarction Size

The rabbits were killed by air embolization of auricular vein, the hearts were removed, and the LV branches were re-ligated. Evans blue dye (10 ml) was injected into the coronary artery through the aorta, then the heart was cut horizontally into approximately 2 mm slices. The thick slices corresponding to the level of the short axis papillary muscle of the heart were taken out and incubated with 2% TTC phosphate buffer (pH = 7.4) at 37 °C for 20 min. Myocardial infarct area was grayish white, ischemic area was brick red, and non-ischemic area was blue. The total area of the circumferential myocardium (TA) and the area of the obstruction segment (A) were measured. The value (A/TA) was calculated to estimate the percentage of myocardial infarction area.

TUNEL Assay

Briefly, the tissue mass was placed in xylene for 30 min. The dissolved paraffin wax was poured into metal frame and paraffin was cut to 5 µm thin slice after cooling in slicing machine. TUNEL Kit was used according to the kit instruction. The cells were permeated by 0.1% Triton X-100, following 3% H₂O₂ was added to seal the cells at room temperature for 10 min. The samples were incubated with TUNEL reaction solution at 37 °C for 60 min, followed by added Converter-POD for 30 min. The samples were stained with hematoxylin. The staining results were observed under microscope (200×). Each slide was randomly selected 10 regions and counted the number of apoptotic cells per 100 cardiomyocytes in each region.

Real-time PCR

Total RNA was extracted by Trizol one-step method. Real-time PCR (ABI Prism 7500, USA) was performed according to the instruction of SYBR Premix Ex Taq™. The cDNA sequences of Bcl-2, Bax and β-actin were obtained from Genbank. The primer sequences were listed: The upstream primer sequence of Bcl-2 forward, 5'-TTTCTCTCTTTGGGCCGTG-3'; Bcl-2 reverse, 5'-TATCCCACTCGTAGCCCTC-3'; Bax forward, 5'-GCGTTGTCGCCCTGTTTAC-3'; Bax reverse, 5'-CCCAACCACTGCTGTTG-3'; β-actin forward, 5'-CCAACTGGGACGACATGGA-3'; β-actin reverse, 5'-TTGAACGTCTCGAACATGATCTG-3'.

Statistical analysis

The data were expressed as mean ± SD. One-way analysis of variance (ANOVA) using GraphPad Prism 5.0 (La Jolla, California, USA) was used for multiple comparisons followed by Bonferroni's post-hoc test. A value of P < 0.05 was considered statistically significant.

Results

Conventional echocardiography

The conventional echocardiography parameters of the rabbits before and after coronary artery ligation as well as after reperfusion were shown in table 1. LVEF decreased significantly after ligation, and there were no significant differences in the remaining parameters.

STE

Longitudinal Strain

The normal longitudinal strain curve was mainly a negative wave below the baseline, arranged neatly, and the peak time was concentrated (Fig. 1A). After 45 min ligation, the longitudinal strain was distorted. GLSsys and GLSp decreased significantly, and the peak was shifted back (Fig. 1B). After 120 min reperfusion, GLSsys and GLSp increased to varying degrees in I-PostC group, ATP-V-PostC group and ATP-A-PostC group (Fig. 1C). The results were shown in Table 2.

After 45 min ligation, the curves of strain and strain rate in the segments with LV dysfunction of rabbits were disordered, LSsys and LSp decreased significantly, and the peak shifted. After 120 min reperfusion, the parameters increased significantly in segments with LV dysfunction in I-PostC group, ATP-V-PostC group and ATP-A-PostC group. The peak time moved forward. LSp increased significantly in ATP-V-PostC group compared with that in I-PostC group ($p < 0.01$). The peak time of LSsys and LSp increased significantly in ATP-A-PostC group ($p < 0.01$).

Circumferential Strain

Circumferential strain curves of normal rabbits were wide negative single peak, the strain curves of every segment were arranged neatly, the peak time was concentrated, and the shape of curves was consistent (Fig. 2A). After 45 min ligation, the circumferential strain curves were flat, the form of the waves was disordered, and the peak times were not concentrated (Fig. 2B). After 120 min reperfusion, the parameters were recovered to varying degrees. The results were shown in Table 3.

After 45 min ligation, GCSsys and GCSp decreased significantly. Most of the peak times shifted. The curves of strain and strain rate in the segments with LV dysfunction of rabbits were low and most of them were distributed in the middle and apical segments of each chamber wall. GCSsys and GCSp increased to some extent in I-PostC group, ATP-V-PostC group and ATP-A-PostC group after 120 min reperfusion. Peak time moved forward. Parameters were increased slightly in I/R group (Table 3). Both the strain peak and the strain rate rose to varying degrees, and the reverse motion of the systolic and diastolic compressive strain curves disappeared (Fig. 2C and 2D).

Radial Strain

The normal radial strain curve was a wide single-peak forward wave. The strain rate curve was a positive wave during the systolic phase and the diastolic phase was a negative wave. The peak time of each curve was concentrated and arranged neatly (Fig. 3A). After 45 min ligation, the curves of radial strain and strain rate in dysfunced segments were low and disordered (Fig. 3B). The absolute value of RSsys and RSp of every segment decreased significantly and the peak time delayed distinctly. After 120 min reperfusion, the reverse motion of RSsys curve disappeared in I-PostC group, ATP-V-PostC group, and ATP-A-PostC group. RSsys and RSp increased significantly in ATP-V-PostC group and ATP-A-PostC group after reperfusion ($p < 0.01$) and the delay phenomenon in peak time of systolic strain relieved (Fig. 3C). RSp was higher in I-PostC group and ATP-PostC group than that in I/R group (Table 4).

Evaluation Of Torsion Function

Rotation curves of normal rabbits LV (ROT) were wide and counterclockwise with single peak, arrived peak at the end of systolic phase and descended in diastolic phase (Fig. 4A). Rotation rate curves of normal rabbits LV (ROTR) were positive rotation peak in systolic phase and high negative untwISTEng peak in diastolic phase (Fig. 4B). After 45 min ligation, the absolute values of Ptw, PTV and PUV of LV decreased significantly ($p < 0.01$), the peak time delayed, HTU increased (Fig. 4C), ROTR curve in diastolic period had a notch (Fig. 4D). After 120 min reperfusion, the reverse motion of the torsion curves disappeared in I-PostC group, ATP-V-PostC group and ATP-A-PostC group. Ptw, PTV, and PUV significantly increased, and the peak time delay of the peak of the torsion angle was alleviated (Fig. 4E and 4F). The results were shown in Table 5.

Sensitivity and specificity of STE technique in detecting MI rabbits

The area under the ROC curve AUC, cutoff value, sensitivity and specificity results were shown in Table 6. For LSp, the sensitivity and the specificity of the MI rabbits were 89.3% and 85.7%, respectively. The AUC of RSp was 0.858, and the cutoff value of 22.67%. The sensitivity and specificity were 88.1% and 81.5%, respectively. The AUC of CSp and GLSp were 0.856 and 0.848. The sensitivity was – 15.33% and – 13.89%, respectively. The AUC of Ptw was 0.810, and the sensitivity and specificity were 79.2% and 75%, respectively. The sensitivity and specificity of PTV in detecting MI rabbits were lower and statistically significant.

Myocardial Enzymology

As shown in the table 7, AST, CK, CK-MB and LDH increased significantly in all groups after ligation. After reperfusion, AST and CK-MB further increased in I/R group, and other parameters had no changes. After reperfusion, myocardial enzyme values in I-PostC group, ATP-V-PostC group and ATP-A-PostC group were lower than those in I/R group to varying degrees.

Myocardial Infarct Size

The myocardium supplied by the occluded LV branch of LAD exhibited an irregular dark area distributed mainly over the lateral, posterior, and inferior walls of the LV and the apex. The percentage of AR (AR/LV) had no difference among the four groups before reperfusion. The percentage of AN (AN/AR) was obviously larger in I/R group than that in the other three groups ($p < 0.05$) after reperfusion. The infarct size in I/R group were higher than those in other three groups (Table 8).

The Changes In Cell Apoptosis

After staining with the TUNEL assay kit, the normal nucleus was blue and the nucleus of the apoptotic cells was brown. The cells were arranged disorderly and the nucleus was unevenly deformed in I/R group (Fig. 5A). The cardiomyocyte nuclei were all in blue in I-PostC group, the cells were arranged neatly, and the nucleus was elliptical. Few brown nuclei were in the ATP-PostC group. The percentages of apoptotic cells in I/R group were higher than those in other three groups (Table 8). The Bax content in the normal tissues of four experimental groups was basically the same. After ischemia and reperfusion, the Bax in the ischemic tissue and infarcted tissue increased. The Bax content decreased in the I-PostC group and APT-PostC group (Fig. 5B). All the levels of Bcl-2 in the normal tissues were basically the same. After ischemia and reperfusion, the content of Bcl-2 in the ischemic and infarcted tissues decreased, the Bcl-2 increased after ischemic postconditioning and post-drug adaptation treatments (Fig. 5C).

Discussion

The accurate evaluation of myocardial function after reperfusion in patients with coronary heart disease and MI exerts great significance for further clinical treatment and prognosis estimation. At present, coronary angiography is still the gold standard for the diagnosis of coronary heart disease. However, coronary angiography is a traumatic examination, and there must be certain indications for this examination, so it is not suitable as a method of postoperative evaluation. Echocardiography always plays an important role in the aspect of diagnosing coronary heart disease and evaluation of postoperative efficacy because of its characteristics of non-invasive, simple, rapid, inexpensive, and repeatable follow-up. Especially in recent years, the development of new ultrasound technologies has provided more clinical information on myocardial function evaluation. The STE technology [10] is based on using high frame rate to produce two-dimensional gray-scale ultrasound images. The STE technology is without angle dependence, without interference from surrounding myocardial traction and overall cardiac motion. This study uses STE technology to observe the protective effect of ischemic preconditioning and ischemic postconditioning on IRI. Therefore, a more manageable myocardial protection strategy for the prevention of clinical ischemia-reperfusion injury is provided.

Some studies have shown that longitudinal myocardial fiber relaxation and contraction exercise is very important to maintain normal heart function [11]. It even shows more practical significance in patients with coronary heart disease. When MI occurs, the longitudinal strain and the strain rate are more sensitive than the radial and hoop strain and strain rate. The reason for that may be related to the myocardial structure. The circumferential motion and the longitudinal movement is mainly determined by the endocardial surface myocardial fibers, while the radial and circumferential directions exercise is mainly related to the middle ring muscle [12]. The early myocardial ischemia may be limited to the subendocardial myocardium, the middle layer of the myocardium and the epicardial layer are not involved. Hence, the radial and circumferential movements is not very sensitive comparing the longitudinal movement. Ischemia is not very sensitive. Therefore, detecting function of changes in long-axis longitudinal strain can detect left ventricular regional myocardial dysfunction earlier and more accurate [13].

Our data indicated that the protective effect of P-PostC on the myocardium is equivalent to the effect of I-PostC, and P-PostC can replace the I-PostC to some extent. Compared with the overall strain index, the improvement of local function is more obvious. Previous animal experiments and this experimental study have confirmed [14]. Segmental motor abnormalities that occur during myocardial ischemia caused by insufficient blood supply to the heart are earlier than changes in overall motion, ventricular contractility, hemodynamics, and ECG signals. The reason for the analysis is that the coronary arteries ligation has a metabolic disorder for a few seconds in the ventricular wall. Therefore, the detection of regional myocardial motor dysfunction is more important than the overall function, and myocardial ischemia or infarction can be assessed earlier and sensitively, and its location and extent are confirmed.

Multiple studies have shown that STE evaluate MI have higher sensitivity and specificity. Kukulski [15] studied the accuracy of ultrasound speckle tracking in the evaluation of acute myocardial ischemia in patients with coronary heart disease. The results showed that the sensitivity of MI was 86% and the specificity was 83% through the peak strain and strain rate in longitudinal systole. In addition, the results showed that the changes in strain index of the ischemic myocardium were measured [16]. Our results were consistent with the literature reports.

Popovic [17] showed that the radial and tangential strains were relatively higher LV ejection fraction sensitivity. In addition, our results indicated that the effect of drug administration in the aortic root might be superior to that in the auricle venous administration. Winter and Asanuma [18, 19] exhibited the same results in circumferential strain. Treguer [20] also found that the accuracy of short-axis strain was higher than that of the long axis during myocardial ischemia reperfusion in rats. Studies have shown that the realization of systolic function is related to the shortening of myocardial contraction and the overall myocardial torsion movement [21]. The kinematic forces of the myocardium in each layer offset each other, and the resultant force produces the torsional

deformation movement of the left ventricle [22]. This helical structure of the heart plays an important role in the maintenance of LV systolic function. STE can evaluate the left ventricular rotation by torsion and torsion speed curve.

There was no significant difference between the ATP arterial injection and the intravenous injection. Only the short-axis circumferential strain index showed that the arterial administration was slightly better than the intravenous administration. The intravenous administration was convenient and safe, which further explained the broad application prospect of ATP for post-drug adaptation.

The limitations of STE mainly include the following aspects. The image quality can affect the data accuracy and repeatability due to some myocardial infarction cases are not ideal. Two-dimensional torsion angle calculation needs to fit the information of the two short-axis section of mitral valve level and apical level, which cannot be calculated in the same cardiac cycle. The severe arrhythmias can affect the results of the analysis. However, STE combined with three-dimensional imaging, myocardial contrast, transesophageal echocardiography and other technologies can open up a new field of quantitative evaluation of myocardial torsional deformation under physiological and pathological conditions.

Conclusions

This study indicates that the ATP-postC can significantly improve the LV long-axis and short-axis myocardial function damage caused by IRI. STE can evaluate the protective effect of ATP-PostC on IRI objectively, accurately, and quantitatively.

Declarations

Ethics approval and consent to participate

All animal studies were performed in accordance with the National regulations on the Administration of Laboratory Animals.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declared that there are no conflicts of interest.

Funding

This study was supported by grants from the National Natural Science Foundation of China (No. 81571680 and 81701720) and the Post-doctoral Science Foundation of Heilongjiang Province (No. LBH-Q17124).

Authors' contributions

Min Ren, Keting Li and Xuemei Liu performed the experiments and wrote this manuscript. Hongyu Yang, Zhonghui Jiang and Xinyu Wu performed the experiments. Yujie Liu designed this study and polished the manuscript.

Acknowledgements

None.

References

1. Grebenchikov OA, Likhvantsev VV, Plotnikov E, Silachev DN, Pevzner IB, Zorova LD, Zorov DB: **Molecular mechanisms of ischemic-reperfusion syndrome and its personalized therapy.** *Anesteziologiya i reanimatologiya* 2014(3):59-67.
2. Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, Vinten-Johansen J: **Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning.** *American journal of physiology Heart and circulatory physiology* 2003, **285**(2):H579-588.
3. Skyschally A, Walter B, Heusch G: **Coronary microembolization during early reperfusion: infarct extension, but protection by ischaemic postconditioning.** *European heart journal* 2013, **34**(42):3314-3321.
4. Tsang A, Hausenloy DJ, Mocanu MM, Yellon DM: **Postconditioning: a form of "modified reperfusion" protects the myocardium by activating the phosphatidylinositol 3-kinase-Akt pathway.** *Circulation research* 2004, **95**(3):230-232.
5. Sivaraman V, Mudalagiri NR, Di Salvo C, Kolvekar S, Hayward M, Yap J, Keogh B, Hausenloy DJ, Yellon DM: **Postconditioning protects human atrial muscle through the activation of the RISK pathway.** *Basic research in cardiology* 2007, **102**(5):453-459.
6. Kin H, Zatta AJ, Lofye MT, Amerson BS, Halkos ME, Kerendi F, Zhao ZQ, Guyton RA, Headrick JP, Vinten-Johansen J: **Postconditioning reduces infarct size via adenosine receptor activation by endogenous adenosine.** *Cardiovascular research* 2005, **67**(1):124-133.

7. Morrison RR, Tan XL, Ledent C, Mustafa SJ, Hofmann PA: **Targeted deletion of A2A adenosine receptors attenuates the protective effects of myocardial postconditioning.** *American journal of physiology Heart and circulatory physiology* 2007, **293**(4):H2523-2529.
8. Liu YJ, Leng XP, Du GQ, Wang XD, Tian JW, Ren M: **Two-dimensional longitudinal strains and torsion analysis to assess the protective effects of ischemic postconditioning on myocardial function: a speckle tracking echocardiography study in rabbits.** *Ultrasonics* 2015, **56**:344-353.
9. Korinek J, Kjaergaard J, Sengupta PP, Yoshifuku S, McMahon EM, Cha SS, Khandheria BK, Belohlavek M: **High spatial resolution speckle tracking improves accuracy of 2-dimensional strain measurements: an update on a new method in functional echocardiography.** *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography* 2007, **20**(2):165-170.
10. Abduch MC, Alencar AM, Mathias W, Jr., Vieira ML: **Cardiac mechanics evaluated by speckle tracking echocardiography.** *Arquivos brasileiros de cardiologia* 2014, **102**(4):403-412.
11. Greenbaum RA, Ho SY, Gibson DG, Becker AE, Anderson RH: **Left ventricular fibre architecture in man.** *British heart journal* 1981, **45**(3):248-263.
12. Kim HK, Sohn DW, Lee SE, Choi SY, Park JS, Kim YJ, Oh BH, Park YB, Choi YS: **Assessment of left ventricular rotation and torsion with two-dimensional speckle tracking echocardiography.** *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography* 2007, **20**(1):45-53.
13. Henson RE, Song SK, Pastorek JS, Ackerman JJ, Lorenz CH: **Left ventricular torsion is equal in mice and humans.** *American journal of physiology Heart and circulatory physiology* 2000, **278**(4):H1117-1123.
14. Hausenloy DJ, Yellon DM: **New directions for protecting the heart against ischaemia-reperfusion injury: targeting the Reperfusion Injury Salvage Kinase (RISK)-pathway.** *Cardiovascular research* 2004, **61**(3):448-460.
15. Kukulski T, Jamal F, Herbots L, D'Hooge J, Bijmens B, Hatle L, De Scheerder I, Sutherland GR: **Identification of acutely ischemic myocardium using ultrasonic strain measurements. A clinical study in patients undergoing coronary angioplasty.** *Journal of the American College of Cardiology* 2003, **41**(5):810-819.
16. Anwar AM: **Accuracy of two-dimensional speckle tracking echocardiography for the detection of significant coronary stenosis.** *Journal of cardiovascular ultrasound* 2013, **21**(4):177-182.
17. Popovic ZB, Kwon DH, Mishra M, Buakhamsri A, Greenberg NL, Thamilarasan M, Flamm SD, Thomas JD, Lever HM, Desai MY: **Association between regional ventricular function and myocardial fibrosis in hypertrophic cardiomyopathy assessed by speckle tracking echocardiography and delayed hyperenhancement magnetic resonance imaging.** *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography* 2008, **21**(12):1299-1305.
18. Winter R, Jussila R, Nowak J, Brodin LA: **Speckle tracking echocardiography is a sensitive tool for the detection of myocardial ischemia: a pilot study from the catheterization laboratory during percutaneous coronary intervention.** *Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography* 2007, **20**(8):974-981.
19. Asanuma T, Fukuta Y, Masuda K, Hioki A, Iwasaki M, Nakatani S: **Assessment of myocardial ischemic memory using speckle tracking echocardiography.** *JACC Cardiovascular imaging* 2012, **5**(1):1-11.
20. Treguer F, Donal E, Tamarelle S, Ghaboura N, Derumeaux G, Furber A, Prunier F: **Speckle tracking imaging improves in vivo assessment of EPO-induced myocardial salvage early after ischemia-reperfusion in rats.** *American journal of physiology Heart and circulatory physiology* 2010, **298**(6):H1679-1686.
21. Spotnitz HM: **Macro design, structure, and mechanics of the left ventricle.** *The Journal of thoracic and cardiovascular surgery* 2000, **119**(5):1053-1077.
22. Buckberg GD: **Basic science review: the helix and the heart.** *The Journal of thoracic and cardiovascular surgery* 2002, **124**(5):863-883.

Tables

Table-1 Conventional echocardiographic index.

	LAD (cm)	LVEDD (cm)	LVEF (%)	SV (ml)
Before ligation	0.78±0.08	1.55±0.39	73.02±4.46	3.16±0.59
45 min after ligation	0.74±0.07	1.45±0.30	67.52±3.65*	2.64±0.68
120 min after reperfusion				
I/R	0.75±0.05	1.57±0.45	71.18±5.02	2.82±0.54
I-PostC	0.80± 0.09	1.51±0.48	65.89±4.15	2.77±0.55
ATP-V-PostC	0.78±0.10	1.59±0.44	71.25±5.12	3.29±0.56
ATP-A-PostC	0.82±0.11	1.53±0.32	68.19±3.96	3.09±0.66

*p < 0.05 vs. before ligation. LAD: left atrial diameter; LVEDD: left ventricular end-diastolic diameter; LVEF: left ventricular ejection fraction; SV: stroke volume.

Table-2 Left ventricular longitudinal strain and the time index.

	GLSsys (%)	T-GLSsys (ms)	GLSp (%)	T-GLSp (ms)	LSsys (%)	T-LSsys (ms)	LSp (%)	T-LSp (ms)
Before ligation	-15.87±2.69	147.63±31.28	-17.75±2.58	144.60±27.63	-15.11±1.57	144.21±25.74	-17.88±0.93	150.38±19.95
45min after ligation	-7.77±1.35**	185.54±35.02*	-8.08±2.12**	175.26±38.94*	-6.21±0.98**	183.76±25.62*	-6.34±0.69**	205.18±20.58
120 min after reperfusion								
I/R	-8.84±1.95	171.26±38.57	-9.98±1.74*	170.10±30.87	-8.14±2.87	165.57±29.59	-8.55±1.87	184.17±16.60
I-PostC	-13.56±2.32▲#	168.52±33.53	-14.87±2.69▲#	161.26±36.54	-12.10±1.80▲▲#	157.45±30.88▲	-12.73±1.53▲#	164.44±30.87
ATP-V-PostC	-11.89±1.86▲#	174.13±36.56	-13.58±2.08▲#	168.25±29.66	-10.98±1.28▲▲#	151.12±29.85▲#	-11.78±0.81▲▲#	167.72±17.09
ATP-A-PostC	-12.03±2.11▲#	171.45±39.51	-14.15±2.53▲#	166.87±34.56	-11.23±1.52▲▲#	154.09±26.99▲	-12.50±0.75▲▲##	160.33±18.71

*p < 0.05, **p < 0.01 vs. before ligation; ▲p < 0.05, ▲▲p < 0.01 vs. after ligation; #p < 0.05, ##p < 0.01 vs. I/R group. GLSsys: global longitudinal systolic strain; T-GLSsys: time to GLSsys; GLSp: global longitudinal peak systolic strain; T-GLSp: time to GLSp; LSsys: longitudinal systolic strain; T-LSsys: time to LSsys; LSp: longitudinal peak strain; T-LSp: time to LSp.

Table-3 Left ventricular circumferential strain and the time index.

	GCSsys (%)	T-GCSsys (ms)	GCSp (%)	T-GCSp (ms)	CSsys (%)	T-CSsys (ms)	CSp (%)	T-CSp (ms)
Before ligation	-17.55±3.25	111.21±25.24	-21.17±4.16	115.45±21.28	-15.38±2.26	103.57±18.54	-19.97±3.18	113.35
45 min after ligation	-7.83±2.21**	141.52±24.14*	-8.43±3.21**	176.69±44.99*	-8.93±1.86**	185.48±38.41*	-9.44±2.53**	134.18
120 min after reperfusion								
I/R	-8.68±2.56	132.58±30.11	-9.69±3.36	148.77±38.52	-10.28±1.95	164.56±36.88	-12.86±3.08▲	136.55
1. PostC	-10.32±2.59	128.16±28.53	-16.19±4.59▲▲#	130.23±36.65▲#	-14.00±2.91▲▲#	125.41±17.74▲#	-15.96±3.15▲▲##	112.68
ATP-V-PostC	-11.60±3.27	124.78±27.55	-15.31±4.26▲▲#	141.45±35.58▲#	-12.66±2.51▲#	124.98±23.16▲#	-16.14±2.87▲▲##	124.78
ATP-A-PostC	-11.94±3.95	127.66±29.57	-14.17±3.99▲▲#	136.62±36.16▲#	-13.78±2.85▲▲#&	126.35±25.43▲#	-17.02±3.36▲▲###	128.37

*p < 0.05, **p < 0.01 vs. before ligation; ▲p < 0.05, ▲▲p < 0.01 vs. after ligation; #p < 0.05, ##p < 0.01 vs. I/R group; &p < 0.05 vs. ATP-V-PostC group. GCSsys: global circumferential systolic strain; T-GCSsys: time to GCSsys; GCSp: global circumferential peak strain; T-GCSp: time to GCSp; CSsys: circumferential systolic strain; T-CSsys: time to CSsys; CSp: circumferential peak strain; T-CSp: time to CSp.

Table-4 Left ventricular radial strain and the time index.

	RSsys (%)	T-RSsys (ms)	RSp (%)	T-RSp (ms)
Before ligation	28.39±5.24	106.25±18.24	29.56±5.24	119.56±26.26
45 min after ligation	19.63±4.85**	144.89±22.58*	15.03±5.12**	140.09±25.72*
120 min after reperfusion				
I/R	21.07±4.32	137.85±29.88	18.82±4.36	138.53±30.18
I-PostC	25.13±4.46▲▲#	118.41±13.61▲	25.88±4.12▲▲#	128.51±23.91
ATP-V-PostC	27.50±4.29▲▲##	126.87±23.44	27.55±4.61▲▲#	131.23±20.89
ATP-A-PostC	26.34±5.36▲▲#	127.02±27.55	27.48±4.53▲▲##	124.48±31.75

*p < 0.05, **p < 0.01 vs. before ligation; ▲p < 0.05, ▲▲p < 0.01 vs. after ligation; #p < 0.05, ##p < 0.01 vs. I/R group. RSsys: radial systolic strain; T-RSsys: time to RSsys; RSp: radial peak strain; T-RSp: time to RSp.

Table-5 Left ventricular twist function and time index.

	Ptw (°)	TPtw (ms)	untw R	HTU (ms)	PTV (°/s)	TPTV (ms)	PUV (°/s)	TPUV (ms)
Before ligation	11.84±2.15	123.24±19.88	2.63±0.41	161.19±34.12	190.59±38.16	65.72±11.63	-183.32±30.42	165.89±34.24
45 min after ligation	7.13±1.89**	176.62±30.13*	1.12±0.35*	205.46±43.16*	115.31±20.45**	98.47±16.61	-114.30±23.88 **	211.54±35.77*
120 min after reperfusion								
I/R	8.78±1.85	160.53±33.87	1.54±0.38	196.25±33.28	129.33±22.71	80.56±17.23▲	120.40±26.53	205.14±43.05
I-PostC	10.92±2.33▲#	135.50±22.89	2.13±0.37▲▲#	178.65±37.58	173.88±31.95▲#	79.71±15.93▲	150.27±24.11▲#	187.48±35.56
ATP-V-PostC	9.67±1.78▲	138.12±29.83	2.02±0.47▲#	180.23±29.95	166.96±29.08▲#	75.88±16.37▲	-130.47±22.08▲	190.71±37.27
ATP-A-PostC	11.01±1.94▲#	131.54±28.01	2.06±0.46▲▲#	173.53±32.50	171.76±28.64▲#	81.45±15.66▲	-143.52±23.67▲#	183.85±39.59

*p < 0.05, **p < 0.01 vs. before ligation; ▲p < 0.05, ▲▲p < 0.01 vs. after ligation; #p < 0.05, ##p < 0.01 vs. I/R group. Ptw: peak twist; TPtw: time to peak twist; untw R: untwiSTEng rate; HTU: half time of untwiSTEng; PTV: peak twiSTEng velocity; TPTV: time to peak twiSTEng velocity; PUV: peak untwiSTEng velocity; TPUV: time to peak untwiSTEng velocity.

Table-6 AUC, cutoff value, sensitivity and specificity of 2DS-STE parameters.

Parameter	ACU	Stanard error	P	95% confidence interval		Cutoff value	Sensitivity (%)	Specificity (%)
				Lower limit	Upper limit			
GLSsys	0.769	0.050	0.000	0.671	0.868	-12.11	77.8	70.8
GLSp	0.815	0.049	0.000	0.719	0.910	-12.42	79.2	72.5
LSSys	0.805	0.047	0.000	0.751	0.891	-13.14	81.9	75.6
LSp	0.896	0.045	0.000	0.719	0.910	-13.55	89.3	85.7
GCSsys	0.812	0.046	0.000	0.722	0.902	-13.42	81.9	72.2
GCSp	0.848	0.057	0.000	0.736	0.959	-13.89	82.1	73.2
CSsys	0.823	0.052	0.000	0.765	0.936	-14.05	85.9	79.2
CSp	0.856	0.055	0.000	0.774	0.962	-15.33	86.1	75.5
RSsys	0.802	0.037	0.000	0.743	0.896	21.22	87.7	80.2
RSp	0.858	0.043	0.000	0.795	0.921	22.67	88.1	81.5
Ptw	0.810	0.047	0.000	0.719	0.902	9.41	79.2	75.6
PTV	0.703	0.059	0.003	0.587	0.819	151.02	66.7	70.8

Table-7 Myocardial enzymology.

	AST (U/L)	CK (IU/L)	CKMB (IU/L)	LDH (IU/L)
Before ligation	39.66±12.58	1648.21±278.56	1993.53±95.45	279.38±98.47
45 min after ligation	62.48±19.21 [#]	1898.52±405.28 [#]	22.58.82±186.32 [#]	339.54±70.38 [#]
120 min after reperfusion				
I/R	115.55±31.63 ^{##}	1996.84±395.68 [#]	2104.19±125.59 [#]	346.81±90.27
I-PostC	45.60 ±8.58 ^{*#}	1731.12±189.57 ^{*#}	2536.28±199.68 [*]	286.18±42.81 ^{*#}
ATP-V-PostC	38.79±10.23 ^{*#}	1867.35±208.86 ^{*#}	2638.96±175.67 [*]	288.42±35.28 [*]
ATP-A-PostC	40.15±9.07 ^{*#}	1795.89±191.44 ^{*#}	2585.57±259.21 [*]	295.56±44.32 ^{*##}

[#]p < 0.05, ^{##}p < 0.01 vs. before ligation; ^{*}p < 0.05 vs. I/R group. AST: aspartate aminotransferase; CK: creatine phosphokinase; CK-MB: creatine phosphokinase isozyme; LDH: lactate dehydrogenase.

Table-8 Myocardial infarct size and apoptosis cell quantity.

Groups	Infarct area percentage (%)	Percentage of apoptotic cells (%)
I/R	27.49±4.15	16.91±2.38
I-PostC	21.74±4.53	13.46±2.25
ATP-V-PostC	22.31±3.46	13.94±2.10
ATP-A-PostC	20.39±2.30	12.94±1.93

Figures

Figure 1

Longitudinal strain curves. (A) The longitudinal strain curve characteristics of normal rabbits: wide negative waves decreased in systolic phase and rose in diastolic phase and arrived peak at the end of systolic phase. (B) Longitudinal strain curve characteristics of rabbits after 45 min ligation (C) Longitudinal strain curve characteristics of rabbits after 120 min reperfusion.

Figure 2

Circumferential strain curves. (A) Circumferential strain curves of normal rabbits: wide negative waves rose from 0-strain, arrived peak at the end of systolic phase and fell around to 0-strain in diastolic phase. The strain curves of each segment were neat with concentrated peak and consistent shapes. (B) Circumferential strain curves of rabbits after ligation: the shapes of curves were disorder. (C) Circumferential strain curves of rabbits after intravenous injection ATP: CSsys and CSp were increased compared with those of pre-reperfusion at lateral and posterior walls. (D) Circumferential strain curves of rabbit after arterial injection of ATP: CSsys and CSp were increased compared with those in ATP-V-PostC group.

Figure 3

Radial strain curves. (A) Radial strain curves of normal rabbits: wide positive waves rose from 0-strain, arrived peak at the end of systolic phase, and fell around to 0-strain in diastolic phase. Each curve was neatly arranged with concentrated peak. (B) Radial strain curves of rabbits after ligation: the shapes of curves were low and flat. RSp values decreased significantly at anterior and lateral walls. Inverse movement existed in part of walls and T-RSsys delayed. (C) Radial strain curves of rabbits after arterial injection ATP: RSp values increased. Inverse movements disappeared and T-RSsys recovered.

Figure 4

Evaluation of torsion function. (A) Rotation curves of normal rabbits: the ROT curves were wide and counterclockwise with single peak, and arrived peak at the end of systolic phase and reduced in diastolic phase. (B) Rotation rate curves of normal rabbits: positive rotation peak in systolic phase and high negative untwisting peak in diastolic phase. (C) Rotation curves of rabbits after ligation: long time clockwise rotation came out at the beginning of systolic phase. Ptw decreased and T-Ptw delayed. (D) Rotation rate curves of rabbits after ligation: PTV decreased and the diastolic notch was formatted in diastolic phase. PTV decreased and T-PUTV delayed. (E) Rotation curves of rabbits after intravenous injection ATP: the shape of curves and Ptw appeared to be normal, however, T-Ptw STEII delayed. (F) Rotation rate curves of rabbits after intravenous injection ATP: the values of PTV were increased compared with those of pre-perfusion.

Figure 5

The expression levels of apoptosis factors. (A) The apoptosis of cardiomyocytes was detected by TUNEL assay. Bax (B) and Bcl-2 (C) expression levels were analyzed via Real-time PCR.