International Journal of Physical Medicine & Rehabilitation

Ni et al., Int J Phys Med Rehabil 2017, 5:5
DOI: 10.4172/2329-9096.1000430

Research Article Open Access

Physiologic Remote Ischemic Training Offers a Cardioprotective Effect against Myocardial

Jun Ni[1], Qingyun Peng[2], Chengyao Mei[1], Minhui Jiang[3*], Xiao Lu[4], Jianan Li[4*], Jianhua Zhu[3]

[1]*Department of Rehabilitation Medicine, The Affiliated Hospital of Nantong University, China*

[2]*Department of Intensive Care Unit, The Affiliated Hospital of Nantong University, China*

[3] *Department of Cardiology, The Affiliated Hospital of Nantong University, China*

[4]*Department of Rehabilitation Medicine, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China*

[*]*Corresponding author: Jun Ni, Department of Rehabilitation Medicine, The First Affiliated Hospital of Nanjing Medical University , Nanjing, China, T el: +86-13705161766; E-mail: lijianan@carm.org.cn*

Minhui Jiang, Department of Cardiology, The Affiliated Hospital of Nantong University, China, Tel: +86-13906290505; E-mail: jiangminhui@medmail.com.cn

[#]These authors contributed equally to this work.

Received date: September 07, 2017; **Accepted date:** September 11, 2017; **Published date:** September 14, 2017

Abstract

Aims: The aim of this study was to investigate the effectiveness of Physiologic remote ischemic training (PRIT) to second MI and the difference of variable durations of PRIT against myocardial infarction.

Methods: A myocardial infarction model was first established in 64 male Sprague-Dawley (SD) rats, and after one week the modeled animals were equally randomized into two groups: the PRIT group, which was further divided into1-, 2-, 4- and 6-week PRIT subgroups as 1w, 2w, 4w and 6wPRIT, and the pure myocardial infarction group, which were further divided into 1-, 2-, 4- and 6-week myocardial infarction groups as 1wMI, 2wMI, 4wMI and 6wMI as controls.. At the end of scheduled time points, all rats received the second MI. Myocardial infarct size, vascular endothelial growth factor (VEGF), capillary density and were determined.

Results: The infarct size in PRIT groups was reduced significantly compared to control MI groups (p<0.05).VEGF protein level and capillary density of the myocardium were significantly higher in PRIT groups than those in control MI groups (p<0.05).

Conclusions: PRIT could induce a protective effect against myocardial infarction and these trends became more pronounced with the prolonging of the training time.

Keywords: Physiologic remote ischemic training (PRIT); Myocardial infarction; vascular endothelial growth factor (VEGF)

Introduction

Myocardial ischemia remains a common and potentially devastating clinical problem despite improvements in medical, surgical, and endovascular therapies [1]. Similarly, myocardial infarction (MI) remains a major cause of death, accounting for about one-third of heart failure cases worldwide [2,3]. Sudden occlusion of a major coronary artery can result in acute myocardial ischemia (AMI) and rapid apoptosis of cardiomyocytes, leading to progressive fibrous replacement of the myocardium [3].

Many studies [4,5] reported that coronary heart disease (CHD) patients with new-onset prodromal angina had a significantly smaller infarct size compared with myocardial ischemia patients without prodromal symptoms and myocardial ischemia improved the development of coronary collateral circulation. Efficient coronary collateral circulation formation in the myocardial ischemia zone of CHD patients is the self-protection mechanism of the ischemic myocardium, and also an important mechanism in the treatment of myocardial ischemia. One successful approach in the experimental setting is ischemic preconditioning (IPC), suggesting that previous repeated ischemia followed by reperfusion can delay injury to cardiac cells and protect against myocardial damage [6]. However, the requirement to perform the ischemic stimulus before onset of AMI limits its clinical application because it is obviously impossible in clinical settings [7]. Some studies demonstrated that remote ischemic preconditioning (RIPC) could overcome the aforementioned problem associated with IPC in that and it was still cardioprotective when applied to an organ or tissue away from the heart [7]. Further research demonstrated that remote muscle trainings could facilitate coronary collateral circulation formation, and therefore more attention has been paid to such trainings because they are easily accessible and can be manipulated without major risks in the clinical setting, should this method prove to be of therapeutic value [8]. Exercise training does not seem to accelerate the development of coronary collaterals with normal coronary arteries.

Many experimental studies [9,10] have suggested a kind of ephemeral and appropriate ischemic insults of skeletal muscles called physiologic ischemic training that could decrease the infarct size after coronary artery ligation and induce a protective effect against myocardial infarction. Even though physiologic remote ischemic training can provide a protective effect against myocardial infarction, how long should the training be sustained to achieve the desired effect, and is it the longer the better? To answer this question, we designed this experiment to investigate the difference in the cardioprotective effect of time-related physiologic remote ischemic training on myocardial infarction in rats.

Methods

Animals

Sixty four 8-week-old male Sprague-Dawley (SD) rats weighing 250-270 g (Experimental Animal Center of Nantong University, Nantong, China) were housed six per cage in a climate controlled environment and received an artificial 12 h light/dark cycle with free access to pellet food and tap water. The experimental procedures were performed in accordance with the National Institutes of Health "Guide for the Care of Use of Laboratory Animals" (NIH Pub. No.85-23, revised 1996) and approved by the ethics committee of Nantong University and Affiliated Hospital of Nantong University (The approval number: 20130712-01).

Experimental design and animal grouping

The 64 SD rats were equally randomized to two big groups: the remote ischemic training (PRIT) group, which were further divided into1-, 2-, 4- and 6-week PRIT subgroups as 1wPRIT, 2wPRIT, 4wPRIT and 6wPRIT, and the pure myocardial infarction group, which were further divided into 1-, 2-, 4- and 6-week myocardial infarction groups as 1wMI, 2wMI, 4wMi and 6wMI as controls. The experimental protocols are illustrated in Figure 1.

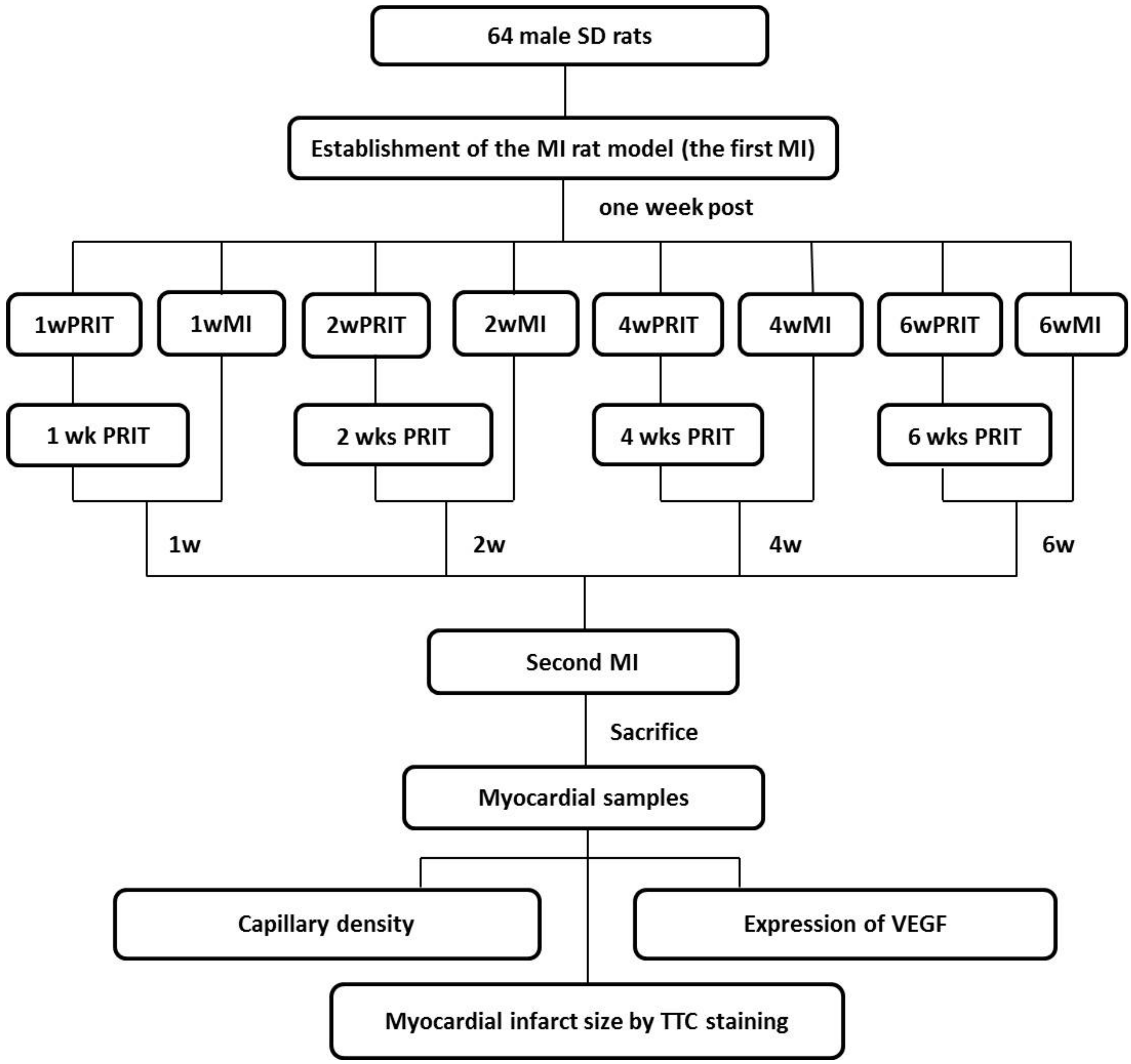

Figure 1: Establishment of the MI rat model (the first MI).

The MI rat model was established by ligating the left anterior descending (LAD) branch of the coronary artery [11]. Rats were first anesthetized with an intraperitoneal injection of 10% chloral hydrate (0.3 ml/100 g of body weight, Merck). Tracheal intubation was then performed with the changed 16-GA trocar (BD) to effect mechanical ventilation, and finally the needle-shaped electrodes were attached under the four limbs to record electrocardiograms (ECGs) using a multipurpose polygraph. After disinfecting the surgical area, the thoracic cavity was opened in the third or fourth intercostal muscles, and the LAD branch of the coronary artery was ligated with a 6-0 suture about 4-5 mm near its origin between the pulmonary artery cone and the left atrial appendage [12]. ST elevation in two or more leads was considered as the evidence of induced infarction.

Induction of physiologic remote ischemic training (PRIT)

After the successful establishment of the MI rat model, the rats were randomly assigned to eight experimental procedures as illustrated in Figure 1. Animals in the PRIT groups were trained for 1, 2, 4 and 6 weeks, while those in the MI control groups were housed in the cages without receiving any training. PRIT was initiated one week after the first MI. PRIT stimulation was performed using an external tourniquet that was bilaterally applied around the upper hind limb joint for 5 min, followed by 5-min reperfusion for a total of 6 cycles, once daily for five days a week [13]. Circulatory arrest in the limb was confirmed by vascular Doppler ultrasound [14]. A rat in 6wPRIT subgroup died during PRIT.

Induction of myocardial infarction (the second MI)

The second MI was performed in all rats at the end of training, and the main difference was that the place of ligation of the LAD branch of the coronary artery was about 2 mm near its origin compared with the first MI [15]. A rat in 1wMI subgroup, a rat in 2wMI subgroup and a rat in 6wMI subgroup died during surgery.

Western blot analysis

The protein expression was measured by Western blotting. The hearts from the remaining 60 surviving rats were excised and kept at -80 (n=7 for the 6wPRIT group and 1wMI group and 2wMI group and 6wMI group, n=8 for the other groups). With the aid of a tissue grinder, the frozen non-infarct LV tissue (100 mg) was homogenized in 400 µl buffer (50 mmol/L Tris base, 150 mmol/L NaCl, 1.0 mmol/L EDTA, 0.1% SDS, 1% TritonX 100, 1% sodium deoxycholate, 1mmol/L phenylmethylsulfony fluoride, pH 7.4) complete with protease inhibitors (Leupeptin 0.1 mmol/L and phenylmethylsulfony fluoride 0.3 mmol/L) and stirred for 30 min at 4 (16). The homogenates were centrifuged for 5 min at 12, 000 rpm in 4. 50µg total protein was separated on 10% sodium dodecyl sulfate polyacrylamide gel and then transferred to nitrocellulose membranes (Bio-Rad). Membranes were blocked with 5% non-fat milk in TBS-0.05% Tween 20 for 1 h at room temperature, and then incubated with primary rabbit anti-VEGF antibody (1:500 Santa Cruz Biotechnology, Inc.) or rabbit anti-GAPDH (1:500, Beyotime Inc., China) antibody overnight at 4. After being washed three times for 10 min each in TBS-0.05% Tween 20, the membranes were incubated with goat ant rabbit IgG HRP secondary antibodies (1:500, Beyotime Inc., China) for 2 h at room temperature. Immunoreactive bands were visualized with enhanced chemiluminescence luminol reagent (ECL) (Beyotime, Inc, China) and exposed to films, which were then analyzed with Quantity One Software (Bio Rad Laboratories).

Measurement of capillary density

For capillary density measurement, endothelial cells were stained with CD31, which is often used as a biological marker to represent capillary vessels in the myocardium [17]. Immunohistochemistry was performed using rabbit anti-rat CD31 antibody (Santa Cruz Biotechnology, USA, 1:100), and the staining was visualized by reaction with DAB (Sigma Chemical Co., USA, 1:20). Capillaries were identified by a brown round structure with a central lumen and a diameter <20µm and a layer of endothelial cells without smooth muscle cells in the myocardium under a light microscope (magnification, 400X) (17) . Five fields on the slide were randomly chosen for counting the stained capillaries.

Infarct size measurement

The heart was excised 72 h after ligation and frozen at -20°C for 30 min, then quickly sliced into 2-mm sections, incubated in 1% 2, 3, 5-triphenyltetrazolium chloride (TTC, Sigma)in phosphate buffer (pH 7.4) for 30 min at 37, and fixed in 4% formalin for 24 h. By this method, the living tissue was displayed red, and the infarcted tissue remained a pale tan color. Next, the sections were placed on a glass slide, photographed with a digital camera using the ImageJ software (NIH, Boston, MA), and analyzed [18].

Statistical analysis

All values are expressed as the mean ± SD. All statistical analyses were performed using SPSS software (ver. 17.0 for Windows, SPSS Inc., Chicago, IL, USA). The differences between more than two groups were analyzed by one-way ANOVA followed by Turkey post-hoc test, and compared between two groups using paired t-test. Statistical significance was defined as P <0.05 (Figures 2 and 3).

Results

Myocardial infarct size

Permanent ligation of LAD caused infarction of the LV myocardium. To measure the myocardial infarct size, TTC staining was performed. Representative images of the heart sections stained with TTC are shown in Figure 4. The areas of infarct sizes were significantly reduced in the LV after PRIT compared with that in control MI subgroups (2wPRIT 52.47±2.41% *vs.* 2wMI 62.00 ± 3.70%, p=0.0024wPRIT 39.77 ± 4.84% *vs.*4wMI 60.23 ± 5.82%, p<0.0016wPRIT 29.13 ± 3.67% *vs.* 6wMI 62.99 ± 1.67%, p<0.001). The effect became more pronounced with the time of RIT prolonging (2wPRIT 52.47 ± 2.41% *vs.* 1wPRIT 61.05±5.58%, p=0.0014wPRIT 39.77±4.84% *vs.* 2wPRIT 52.47±2.41%,p<0.0016wPRIT 29.13 ± 3.67% *vs.* 4wPRIT 39.77 ± 4.84%, p<0.001), indicating that PRIT could reduce the size of myocardial infarction and provide a protective effect on the rat heart (Table 1).

Group	N	Infarct size (% of TTC)
1wPRIT	8	61.05 ± 5.58
1wMI	7	66.11 ± 4.68
2wPRIT	8	52.47 ± 2.41*a
2wMI	7	62.00 ± 3.70
4wPRIT	8	39.77 ± 4.84#b

Citation: Ni J, Peng Q, Mei C, Jiang M, Lu X, et al. (2017) Physiologic Remote Ischemic Training Offers a Cardioprotective Effect against Myocardial Infarction in a Time-Dependent Manner. Int J Phys Med Rehabil 5: 430. doi:10.4172/2329-9096.1000430

4wMI	8	60.23 ± 5.82
6wPRIT	7	29.13 ± 3.67&c
6wMI	7	62.99 ± 1.67

Table 1: Myocardial infarct size.

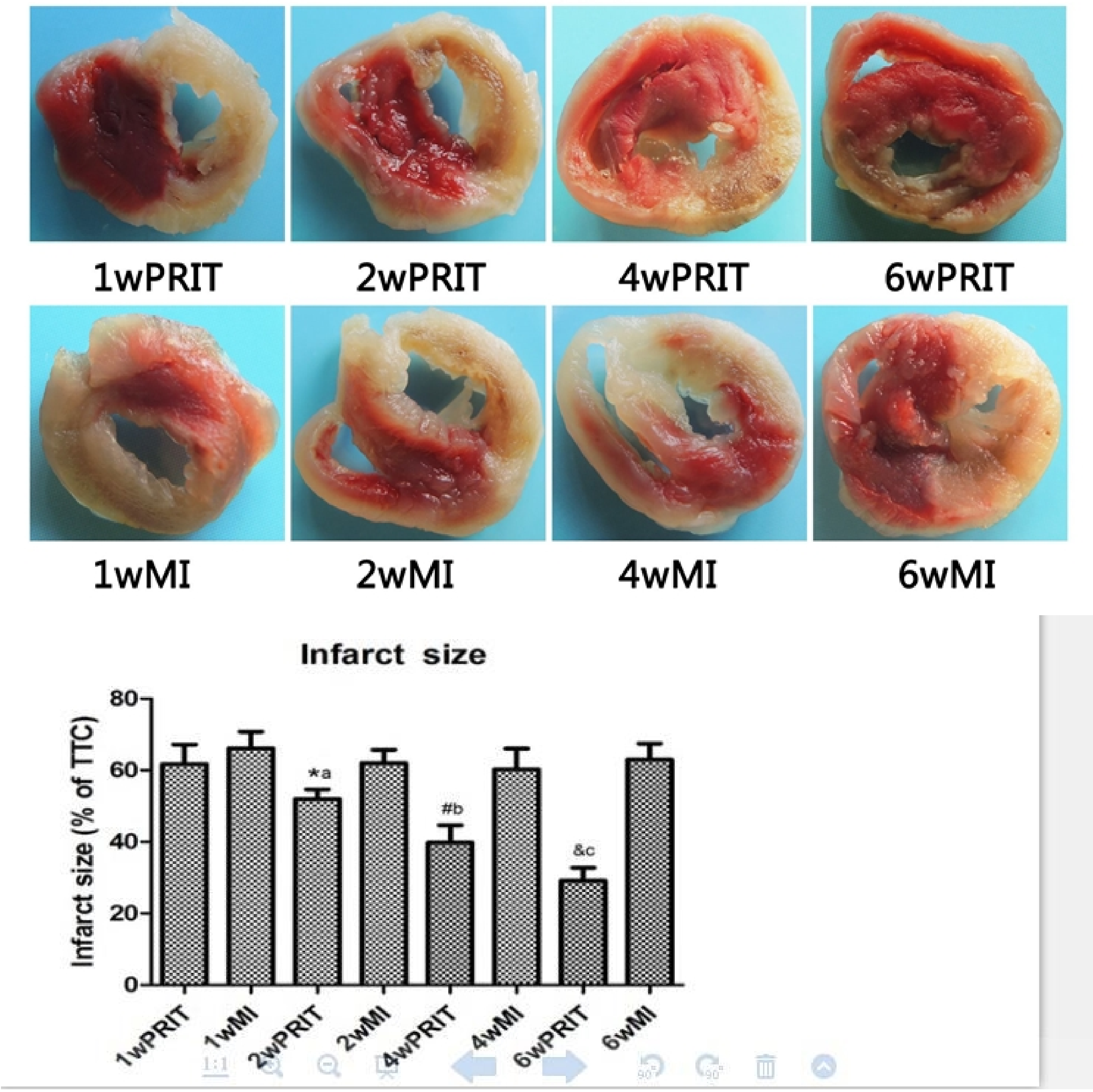

Figure 2: Images of the TTC-stained heart sections.

Effect of PRIT on VEGF expression

After the second MI, the border-zone myocardium was collected for Western blot analysis. To elucidate the mechanism of angiogenesis, VEGF protein levels were evaluated (Figure 3).The protein levels of VEGF in PRIT subgroups were elevated significantly compared with those in the control MI subgroups ($p<0.05$), and VEGF protein levels in PRIT subgroups increased with the training time prolonging ($p<0.05$). In contrast, there was no statistically significant difference between the control MI subgroups, indicating that PRIT could promote the regeneration of vessels.

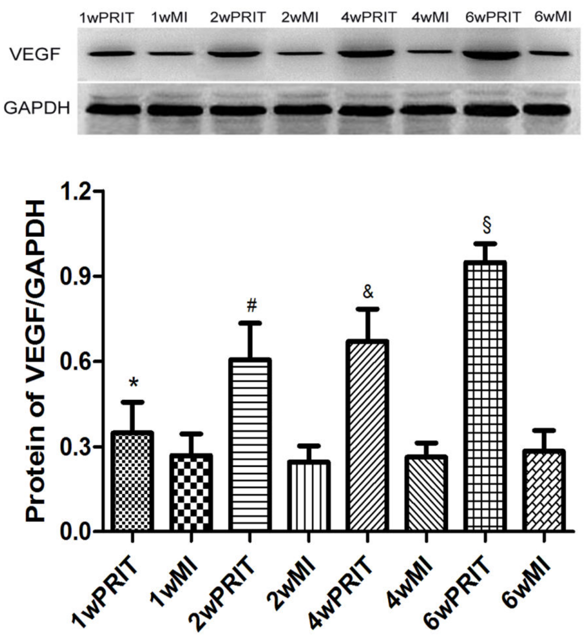

Figure 3: PRIT western blot.

Capillary density

Capillary density was measured by endothelial cells stained with CD31 (Figure 4). Quantitative analysis showed that induction of PRIT significantly promoted cardiac capillary density compared with that in the control MI subgroups (p<0.05) (Table 2, Figure 4), but there was no statistically significant difference between the control MI subgroups. With the training time prolonging, a better effect was also seen in PRIT subgroups (p<0.05).These findings indicate that PRIT could promote capillary density of the myocardium.

Group	N	Capillary density(N/mm2)
1wPRIT	40	484 ± 181
1wMI	35	445 ± 178
2wPRIT	40	637 ± 211*
2wMI	35	432 ± 176
4wPRIT	40	755 ± 250*
4wMI	40	428 ± 161
6wPRIT	35	915 ± 221*#&
6wMI	35	430 ± 181

Table 2: Capillary density.

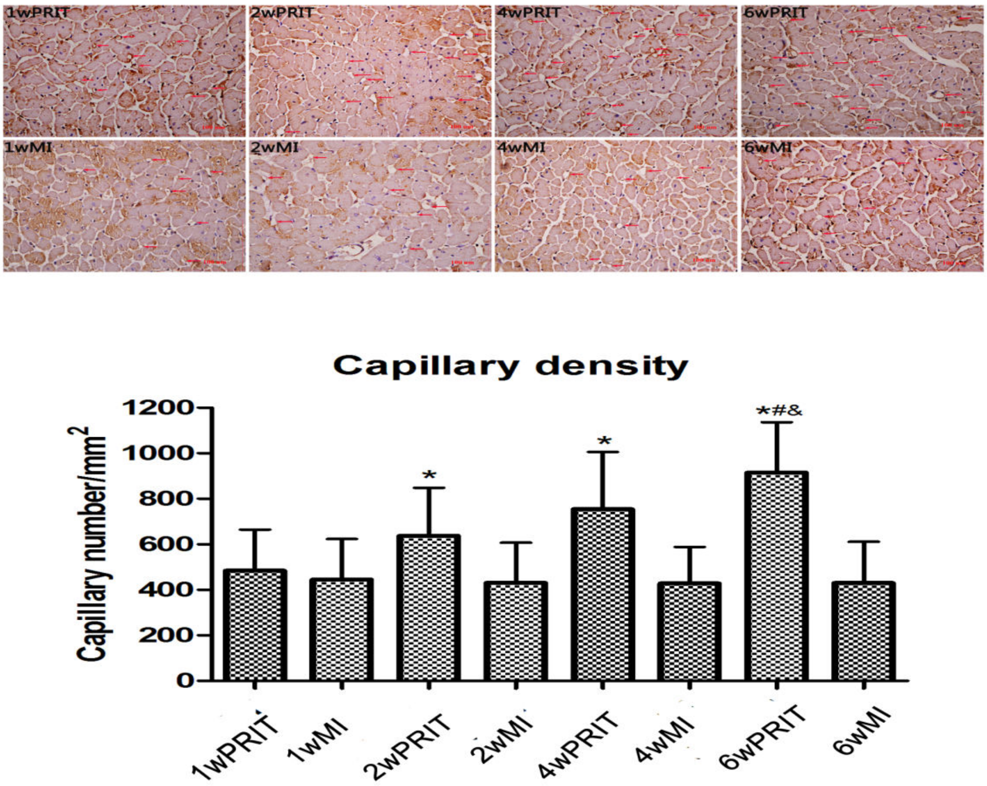

Figure 4: Immunostaining of CD31 in endothelial cell and PRIT promotes capillary density of the myocardium.

Discussion

The present study has demonstrated that PRIT could decrease the infarct size after MI without reperfusion, increase the capillary density and elevate the VEGF protein level in the myocardium after MI, thus facilitating coronary collateral formation of the myocardium.

The concept of PRIT is different from IPC initially mentioned by Murry et al. [19]. IPC refers to a prior brief period of ischemia/reperfusion in the myocardium that may delay cell death after coronary occlusion. Unlike IPC, PRIT has a more remote effect by facilitating coronary collateral formation of the myocardium by repeated short-term skeletal muscle ischemia. The cardioprotective effect of short-term skeletal muscle ischemia has been previously evaluated in experimental [20] and clinical [21] studies and the beneficial effect on the ventricular myocardium is not specific for a particular species [8]. PRIT is reversible non-invasive ischemia of normal skeletal muscles caused by tourniquet or isometric contraction, induce collateral circulation development in the myocardium (9). Most related studies [9,22] have demonstrated that physiologic ischemic training or chronic skeletal muscle ischemia could produce a cardioprotective effect at a certain time, for example, four weeks. However, no study has provided a clear picture about whether the ischemic training time was a significant factor contributing to the cardioprotective effect generated by PRIT, or whether prolonging the training time could produce a better result should this be the case.

To answer these questions, we established a rat model of myocardial ischemia/infarction by ligating the lower segment of the LAD at 4-5 mm from the origin, knowing that LAD ligation can introduce myocardial infarction in the region vessel distributed, and in the border zone of myocardial ischemia. This procedure can simulate the pathologic status of MI [23]. Our preliminary experiment showed that the suitable intensity of training was very important, and that high-intensity training could stiffen the limbs of the rats, or even disable the walking ability of the animals. A appropriate training protocol as suggested by previous study [24] should be proceeded by applying a bilateral external tourniquet around the upper hind limb joint for 5 min, followed by 5 min reperfusion for a total of 6 cycles, once a day and five days a week, so as to achieve a beneficial cardioprotective effect without damaging the function of skeletal muscles. To judge the degree of MI, different test methods were used to evaluate cardiac changes of the heart comprehensively. Infarct sizes in PRIT subgroups were significant smaller than those in the control MI subgroups, and this post-MI reduction in infarct size was time dependent (Figure 4). Sudden occlusion of a major coronary artery can result in AMI and rapid apoptosis of cardiomyocytes, leading to progressive fibrous replacement in the myocardium and LV dilatation [3,25]. Previous studies [26-28] demonstrated that physiologic RIT could promote coronary collateral formation in the ischemic myocardium. Results of capillary density and VEGF protein level in this study also demonstrated it. However, the exact mechanism underlying physiologic RIT in promoting coronary collateral formation in the pathologically ischemic myocardium remains unclearly understood. Coronary collateral formation is reported to be mediated by the release of several growth factors, of which VEGF is the most important [23]. It was found in this study that high expression of VEGF was closely related to coronary collateral formation, and that the level of VEGF protein expression in the myocardium was up regulated in the PRIT subgroups compared with the control MI subgroups, and this phenomenon was more obvious when the training time was prolonged (Figure 3). And the capillary density was also consistent with the result of Western blotting analysis on VEGF protein level. Capillary densities in this study were promoted with the training time prolonging in the RIT subgroups, indicating that physiologic RIT could promote coronary collateral formation in the myocardium, thereby offering a cardioprotective effect, and this effect was more pronounced in the 6wRIT subgroup.

There are some limitations in this study. First, the number of rats in each group was not large enough. And the additional factors could influence the reliability of the results such as failure of operation, disease and malignant arrhythmia after operations. In addition, the training time designed in the study was limited, and therefore we were unable to know whether there would be any change in the result beyond six weeks. Secondly, we failed to set a blank control group. Further studies with larger sample capacities and longer training time are needed to confirm the results of the present study.

Conclusion

In conclusion, Remote ischemic training of skeletal muscles could induce a protective effect against myocardial infarction, and this protective effect may become better with the training time prolonging.

Funding

This work was supported by grant from Jiangsu Education Department, China (overseas Research & Training Program).

References

1. Vartanian SM, Sarkar R (2007) Therapeutic angiogenesis. Vasc Endovascular Surg 241: 173-185.
2. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, et al. (2013) Executive summary: heart disease and stroke statistics--2013 update: a report from the American Heart Association. Circulation 127: 143-152.
3. Bai WW, Xing YF, Wang B, Lu XT, Wang YB, et al. (2013) Tongxinluo Improves Cardiac Function and Ameliorates Ventricular Remodeling in Mice Model of Myocardial Infarction through Enhancing Angiogenesis. Evid Based Complement Alternat Med :813247.
4. Ottani F, Galvani M, Ferrini D, Sorbello F, Limonetti P, et al. (1995) Prodromal angina limits infarct size. A role for ischemic preconditioning. Circulation 91: 291-297.
5. Kobayashi Y, Miyazaki S, Itoh A, Daikoku S, Morii I, et al. (1998) Previous angina reduces in-hospital death in patients with acute myocardial infarction. Am J Cardiol 81: 117-122.
6. Bailey TG, Birk GK, Cable NT, Atkinson G, Green DJ, et al. (2012) Remote ischemic preconditioning prevents reduction in brachial artery flow-mediated dilation after strenuous exercise. Am J Physiol Heart Circ Physiol 303: H533-538.
7. Hausenloy DJ, Yellon DM (2011) The therapeutic potential of ischemic conditioning: an update. Nat Rev Cardiol 8: 619-629.
8. Varnavas VC, Kontaras K, Glava C, Maniotis CD, Koutouzis M, et al. (2011) Chronic skeletal muscle ischemia preserves coronary flow in the ischemic rat heart. Am J Physiol Heart Circ Physiol 301: H1229-1235.
9. Lin A, Li J, Zhao Y, Xiao M, Xiao B, Lu X, et al. Effect of physiologic ischemic training on protection of myocardial infarction in rabbits. Am J Phys Med Rehabil 90: 97-105.
10. Gao J, Shen M, Guo X, Li X, Li J (2011) Proteomic mechanism of myocardial angiogenesis augmented by remote ischemic training of skeletal muscle in rabbit. Cardiovasc Ther 29: 199-210.
11. Selye H, Bajusz E, Grasso S, Mendell P (1960) Simple techniques for the surgical occlusion of coronary vessels in the rat. Angiology 11: 398-407.
12. Yu S, Zhu Y, Li F, Zhang Y, Xia C (2014) Differentiation of human embryonic germ cells and transplantation in rats with acute myocardial infarction. Exp Ther Med 27: 615-620.
13. Li G, Labruto F, Sirsjo A, Chen F, Vaage J, Valen G (2004) Myocardial protection by remote preconditioning: the role of nuclear factor kappa-B p105 and inducible nitric oxide synthase. European J Cardio-Thoracic Surg 26: 968-973.
14. Schmidt MR, Smerup M, Konstantinov IE, Shimizu M, Li J, et al. (2007) Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction through a KATP-dependent mechanism: first demonstration of remote ischemic perconditioning. Am J Physiol Heart Circ Physiol 292: H1883-90.
15. Samsamshariat SA, Samsamshariat ZA, Movahed MR (2005) A novel method for safe and accurate left anterior descending coronary artery ligation for research in rats. Cardiovasc Revasc Med 6: 121-123.
16. Gui L, Bao Z, Jia Y, Qin X, Cheng ZJ, et al. (2013) Ventricular tachyarrhythmias in rats with acute myocardial infarction involves activation of small-conductance Ca2+-activated K+ channels. Am J Physiol Heart Circ Physiol 304: H118-30.
17. Lu J, Yao YY, Dai QM, Ma GS, Zhang SF, et al. (2012) Erythropoietin attenuates cardiac dysfunction by increasing myocardial angiogenesis and inhibiting interstitial fibrosis in diabetic rats. Cardiovasc Diabetol 11: 105.
18. Huang C, Kan J, Liu X, Ma F, Tran BH, et al. (2013) Cardioprotective effects of a novel hydrogen sulfide agent-controlled release formulation of S-propargyl-cysteine on heart failure rats and molecular mechanisms. PloS one. 8: e69205.
19. Murry CE, Jennings RB, Reimer KA (1986) Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circ 74: 1124-1136.
20. Kristiansen SB, Henning O, Kharbanda RK, Nielsen-Kudsk JE, Schmidt MR, et al. (2005) Remote preconditioning reduces ischemic injury in the explanted heart by a KATP channel-dependent mechanism. Am J Physiol Heart Circ Physiol 288: H1252-256.
21. Thielmann M, Kottenberg E, Boengler K, Raffelsieper C, Neuhaeuser M, et al. (2010) Remote ischemic preconditioning reduces myocardial injury after coronary artery bypass surgery with crystalloid cardioplegic arrest. Basic Res Cardiol 105: 657-664.
22. Shen M, Gao J, Li J, Su J (2009) Effect of ischaemic exercise training of a normal limb on angiogenesis of a pathological ischaemic limb in rabbits. Clin Sci (Lond). 117: 201-208.
23. Lu X, Wu T, Huang P, Lin S, Qiu F, et al. (2008) Effect and mechanism of intermittent myocardial ischemia induced by exercise on coronary collateral formation. Am J Phys Med Rehabil 87: 803-814.
24. Hess DC, Hoda MN, Bhatia K (2013) Remote limb perconditioning [corrected] and postconditioning: will it translate into a promising treatment for acute stroke? Stroke J cerebral circ 44: 1191-1197.
25. Tao L, Wang Y, Gao E, Zhang H, Yuan Y, et al. (2010) Adiponectin: an indispensable molecule in rosiglitazone cardioprotection following myocardial infarction. Cir Res 106: 409-417.
26. Zheng Y, Lu X, Li J, Zhang Q, Reinhardt JD (2014) Impact of remote physiological ischemic training on vascular endothelial growth factor,

endothelial progenitor cells and coronary angiogenesis after myocardial ischemia. Int J Cardiol 177: 894-901.

27. Ni J, Lu H, Lu X, Jiang M, Peng Q, et al. (2015) The evolving concept of physiological ischemia training vs. ischemia preconditioning. J Biomed Res 29: 445-450.

28. Lin S, Chen Y, Li Y, Li J, Lu X (2014) Physical ischaemia induced by isometric exercise facilitated collateral development in the remote ischaemic myocardium of humans. Clin Sci (Lond) 127: 581-588.