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JBR

The Journal of Biomedical Research, 2015, 29(6):445-450

Review Article

The evolving concept of physiological ischemia training *vs.* ischemia preconditioning

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Abstract

Ischemic heart diseases are the leading cause of death with increasing numbers of patients worldwide. Despite advances in revascularization techniques, angiogenic therapies remain highly attractive. Physiological ischemia training, which is first proposed in our laboratory, refers to reversible ischemia training of normal skeletal muscles by using a tourniquet or isometric contraction to cause physiologic ischemia for about 4 weeks for the sake of triggering molecular and cellular mechanisms to promote angiogenesis and formation of collateral vessels and protect remote ischemia areas. Physiological ischemia training therapy augments angiogenesis in the ischemic myocardium by inducing differential expression of proteins involved in energy metabolism, cell migration, protein folding, and generation. It upregulates the expressions of vascular endothelial growth factor, and induces angiogenesis, protects the myocardium when infarction occurs by increasing circulating endothelial progenitor cells and enhancing their migration, which is in accordance with physical training in heart disease rehabilitation. These findings may lead to a new approach of therapeutic angiogenesis for patients with ischemic heart diseases. On the basis of the promising results in animal studies, studies were also conducted in patients with coronary artery disease without any adverse effect *in vivo*, indicating that physiological ischemia training therapy is a safe, effective and non-invasive angiogenic approach for cardiovascular rehabilitation. Preconditioning is considered to be the most protective intervention against myocardial ischemia-reperfusion injury to date. Physiological ischemia training is different from preconditioning. This review summarizes the preclinical and clinical data of physiological ischemia training and its difference from preconditioning.

Keywords: physiological ischemia training, ischemia preconditioning, angiogenesis, vascular endothelial growth factor

Introduction

Ischemic cardiovascular diseases including coronary artery disease (CAD) are the leading cause of death worldwide. Despite substantial advances in revascularization techniques such as surgical revascularization or percutaneous catheter intervention (PCI), angiogenic therapies remain the mainstay for the treatment of

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CLC number: R541.4, Document code: A

These authors reported no conflict of interests.

Received 23 October 2014, Revised 24 January 2015, Accepted 22 May 2015, Epub 10 September 2015

doi: 10.7555/JBR.29.20140142

ischemic diseases[1]. Myocardial ischemia is important for the development of collateral circulation[2]. Intensive exercise training may facilitate collateral formation in CAD patients. However, the risk of high-intensity exercise in these patients is a major clinical concern. Therefore, it is necessary to explore approaches that can safely induce angiogenesis[1].

Physiological ischemia training (PIT) is a reversible ischemia training of normal skeletal muscles induced by tourniquet or isometric contraction to cause physiologic ischemia for about 1 month for the sake of developing angiogenesis and collateral vessels to protect the ischemic area. PIT is usually applied after the occurrence of an ischemic vascular disease. It was first proposed in our laboratory as a novel and safe technique for cardiac rehabilitation[3-14].

Ischemic preconditioning (IPC) refers to the deliberate induction of a series of transient ischemia and reperfusion before the lethal 'index' ischemic event occurs. At present, there is a clear consensus that IPC is cardioprotective across all investigated animal species and is regarded as the most protective intervention against myocardial ischemia-reperfusion injury today[15-16]. Although IPC has been widely accepted, PIT remains a new concept and is likely to be confused with IPC.

This article summarizes recent works of PIT in animals and humans, and discusses the potential mechanism underlying PIT therapy that promotes collateral formation and the advantages of PIT. We also discuss the difference between PIT and IPC.

PIT therapy for myocardial infarction

Clinical studies have reported that patients who have had a myocardial infarct may have smaller infarcts[17] and a lower mortality rate if they had angina pectoris before[18]. It was also demonstrated that well-developed collateral circulation may protect the residual viable myocardium and decrease the infarct size in patients with myocardial infarction[19-20].

Lu *et al.* performed an animal experiment with pigs *in vivo* using a porcine model of chronic myocardial ischemia by implanting a balloon constrictor in the first obtuse marginal coronary artery[21]. It was shown that high-intensity exercise could improve the formation of coronary collateral circulation in pigs with myocardial ischemia[21]. Unfortunately, high-intensity exercise may be a significant risk for cardiovascular patients and has been limited for years due to the risk of triggering cardiovascular events[22,24].

Great progress has been made in PCI and the surgical revascularization field. However, there are still some patients with severe CAD who cannot sustain PCI or surgical revascularization and they remain poor due to the lack of effective treatments. Therefore, safer approaches for inducing angiogenesis should be explored. PIT was first proposed in our laboratory. Rabbit models of controllable myocardial ischemia are established by using an implanted water balloon constrictor. Controllable coronary artery stenosis and reperfusion are induced by inflating and deflating the balloon by injecting and sucking out sterile water using a syringe, a process that mimics the pathologic status of stable myocardial ischemia[3-4,6]. We treated these models with PIT for 4 weeks. Stimulation was applied using a 1-m second square wave pulse applied at a frequency of 40 Hz, which was sufficient to cause the hind limb to produce 40% of its maximal force without pain. The stimulation protocol was repeated for 4 minutes in each session at a 2-day interval for 4 weeks. The experiment has demonstrated that PIT induces the formation of collateral circulation[3,6,10]. On the basis of these experiments, the 30 remaining rabbits were randomly assigned into 3 groups: the pure ischemia group that underwent a myocardial ischemia only to imitate stable ischemia; the exercise training group that received PIT along with the same ischemic stimulation as the PIT group; the sham-operated group that remained inactive. There were significant differences in infarct size among the groups ($P<0.01$) after 4 weeks of training. The findings suggest that PIT for 4 weeks can effectively decrease the myocardial infarct size, which is significantly correlated with heart function, myocardial blood flow and capillary density (CD). This is the first report that demonstrated the function of PIT in inducing collateral circulation formation and protecting the myocardium when an infarction occurs.

PIT therapy for peripheral arterial disease

Vascular occlusion-induced peripheral arterial disease is a common disorder. With the rapid increase of the elderly population, the number of patients with peripheral arterial disease continues to increase. To date, critical limb ischemia in peripheral arterial disease has become a great challenge in medical therapy.

A number of studies have reported that angiogenesis is an effective method to improve collateral-dependent blood flow to distal tissues at risk of ischemia, and demonstrated that exercise can induce angiogenesis in the peripheral circulation[25]. Most studies focused their attention of electrical stimulation or aerobic exercise primarily on the local ischemic muscle. In fact, upregulation of angiogenic factors should not be limited to a local site. Remote expression of angiogenic factors induced by exercise and ischemia has been reported[26-27].

Liu *et al.* reported the up-regulation of vascular endothelial growth factor (VEGF) in serum and skeletal muscles after myocardial ischemia[28].

As local exercise increases cytokines and leucocytes in the circulation or non-exercised tissues, it may promote angiogenesis in the distal pathological ischemic site *via* a remote effect of the angiogenic factors. Therefore, a study was designed to test the hypothesis that local exercise of a normal limb could promote angiogenesis in the pathologically ischemic limb. Animal experiments were performed in a rabbit model by chronically ligating the femoral artery of the left hindlimb, and implanting an electrode onto the sciatic nerve of the right hindlimb[10]. The animals were then randomly assigned to 4 groups, including the Lig-N group without receiving electrical stimulation, the Lig-High group receiving high-intensity electrical stimulation on the right hindlimb, the Lig-Low group receiving low-intensity electrical stimulation on the right hindlimb), and the Double-Lig-High group receiving both ligation of the left femoral artery and high-intensity electrical stimulation on the right hindlimb. In the Lig-High and Double-Lig-High groups, the high-intensity electrical stimulation was applied at a frequency of 40 Hz for a 1-m second duration, and the maximal force reached a plateau of 13-16 N at a current of 2.5 mA. In the Lig-Low group, low-intensity electrical stimulation was defined as 0.3 mA (40 Hz for 1 m second) to cause minimal macroscopic muscle contraction. The electrical stimulation procedure included an episode of 5-minute stimulation and a 5-minute rest, 8 times daily for 4 weeks. Upon gross examination with angiography, the number of collaterals was significantly higher in the Lig-High group compared with the three groups ($P<0.01$). Blood flow, capillary density and VEGF mRNA levels in the gastrocnemius muscle in the Lig-High group were also noticeably higher than the other three groups ($P<0.01$). The value of VEGF protein levels in the Lig-High group was significantly higher than that of the other three groups ($P<0.01$). The findings demonstrated that PIT in normal limbs with high intensity electrical stimulation may significantly increase collateral blood flow and capillary supply in the pathological ischemia site, indicating that physiological ischemic exercise training in the normal limb for 4 weeks facilitated angiogenesis and up-regulated VEGF expression in the pathological ischemic limb[9-10].

Mechanisms underlying PIT therapy

Molecule mechanisms

Although the underlying mechanism responsible for the effect of PIT remains not clearly understood, some evidence may provide meaningful insights. Firstly, ischemic exercise of the normal skeletal muscles elevates circulating VEGF binding to the site with pathological ischemia. Remote expression of VEGF has been demonstrated in the skeletal muscles induced by 4-week stenosis of the coronary artery[28]. Secondly, apart from VEGF synthesis in the muscle, circulating T-cells[29] and monocytes[30] provide another source of VEGF. Our study demonstrated that PIT augmented angiogenesis in the ischemic myocardium through differential expression of proteins involved in energy metabolism, cell migration, protein folding, and generation[31]. Logically, remote expression of angiogenic factors implies potential remote angiogenesis.

Coronary collaterals may develop in response to intermittent brief myocardial ischemia in humans, and that collaterals may be preserved and could immediately function when a coronary artery is acutely occluded. This notion has been supported by some researchers who found that coronary collateral circulation could extend the "golden time" before coronary reperfusion when infarction occurred. Well-developed collaterals were found to decrease the infarct size of patients who had experienced infarction[32-33]. Only useful, efficient, or functional collateral flow could prevent myocardial ischemia. Our study supports these findings.

Treadmill test showed that exercise training increased VEGF levels by 310% in patients with peripheral arterial disease, where the presence of ischemia was documented as an increase in venous lactate levels and complaint of ischemic leg pain. With an increase in exercise intensity, lactate accumulated in the muscle and the concentration of lactate elevated significantly. At baseline and immediately after exercise, venous lactate was measured to confirm ischemia and hypoxia during high-intensity electrical stimulation exercise training. Rabbits in the low-intensity group were not subjected to ischemic stimulation. The absence of ischemia was shown by no change in venous lactate levels. The levels of *VEGF* mRNA and protein in the left gastrocnemius muscle were all up-regulated in the high-intensity group compared with those in the low-intensity group. This is due to the induction of ischemia by high-intensity exercise in the Lig-High group[10]. We used high frequency (40 Hz) to produce intense muscle contractions. High-intensity static muscle contraction is important to perform resistive exercises[10].

The higher intensity of resistance will account for the larger proportion of static muscle contractions in resistive exercises. According to the previous results[9-10], high-intensity resistance exercise promoted angiogenesis in remote ischemic tissues. However, high-intensity exercise has been limited as a treatment for patients

with cardiovascular disease because of the cardiovascular risk[7]. This finding may lead to a potential new approach of applying PIT of skeletal muscles in the treatment of patients with ischemic diseases.

Cellular mechanisms

The notion that PIT could increase systemic endothelial progenitor cells (EPCs) has been supported by the literature. A 12-week period of regular physical exercise resulted in a 2.9-fold increase in circulating EPCs, and therefore promoted cardiovascular health[34]. However, another study[5] showed that PIT could also increase circulating EPCs, which is in accordance with physical training in heart disease rehabilitation.

It was also found that exercise induced a highly significant enhancement of migratory capacity in all groups[34]. Wan C investigated the relationship between PIT and the function of EPCs. PIT, like circulating EPCs, also increases both the quantity of migrating EPCs and the migratory activity of EPCs, which is consistent with the situation in physical training. In addition, PIT improves capillary density in the ischemic cardiac area, which is in accordance with the circulating EPCs and EPC migration activity, and hence might be applied as a promising new approach in cardiac rehabilitation. The statistics showed that circulating EPCs were positively correlated with capillary density in the ischemic cardiac area. This may be due to homing of EPCs. When EPCs homed to the injured tissue, they could promote angiogenesis and then improve local microenvironment. Meanwhile, the improved microenvironment in turn could promote homing and functioning of EPCs. Our study showed that the homing of EPCs was involved in the effect of PIT on angiogenesis in the remote ischemic cardiac area, during which the quantity and activity of EPCs were shown to be the key factors.

Clinical studies

A recent study was designed by Lin S *et al.*[12] has confirmed that PIT in patients with CAD could increase coronary collateral flow during acute vessel occlusion, which was significantly different from control occlusion. A randomized controlled study was performed to determine whether isometric exercise could increase collateral flow in the remote ischemic myocardium in an acute coronary occlusion model in 65 patients with one-vessel CAD. The patients were randomly assigned to either the isometric exercise group or non-exercise group. Patients in the exercise group performed isometric handgrip exercises (50% maximal voluntary contraction) during 1-minute coronary balloon occlusion, while patients in the non-exercise group remained sedentary. Collateral flow index, heart rate, systolic blood pressure and diastolic blood pressure were determined prior to and 1 minute after coronary occlusion. In the exercise group, the collateral flow index after and before coronary occlusion was significantly higher than that of the non-exercise group ($P<0.01$), and so were heart rate, systolic and diastolic blood pressure ($P<0.01$).

Evolving concept of preconditioning

Currently, there is a clear consensus that IPC is cardioprotective across all animal species investigated and is the most protective intervention against myocardial ischemia-reperfusion injury[15]. Remote ischemic conditioning was first discovered to offer protection within one organ[35], gaining interest as a potential method to induce resistance against ischemia reperfusion injury in a variety of clinical settings. It is an approach of conditioning in which not the target organ, such as the heart, but instead a more accessible tissue is submitted to a conditioning stimulus. Remote preconditioning has also been currently found to offer protection against ischemia reperfusion injury when a different organ, or even the skeletal muscle tissue is used for conditioning[36]. However, it is a matter of fact that remote ischemic conditioning reduces the incidence of periprocedural myocardial infarction and the release of troponin. There is no evidence that remote ischemic conditioning reduces ischemic event-related mortality, nor does it reduce major adverse cardiovascular events.

The concept of PIT is different with preconditioning, which renders the organ resistant to a more prolonged episode of ischemia. Remote ischemic preconditioning applies the precedent ischemic stimulus to a distant site from the organ or tissue that is afterward exposed to ischemia injury. PIT needs to train for several weeks while adaptation of IPC or remote ischemic preconditioning only needs several times. The underlying mechanisms of PIT and remote ischemic preconditioning are very complex and not yet fully defined. It has been hypothesized that remote ischemic preconditioning predominantly involves systemic multifactorial anti-inflammatory, neuronal and humoral signaling pathways, which are likely to interact with each other[37]. Remote ischemic preconditioning has been described to reduce ischemia-reperfusion injury in various animal models. Remote effect of PIT may facilitate coronary collateral formation of the myocardium by repeated episodes of short-term skeletal muscle ischemia after the occurrence of an ischemic event. PIT therapy augments angiogenesis in the ischemic myocardium by inducing

differential expression of proteins involved in energy metabolism, cell migration, protein folding, and generation[14,31]. It upregulates the expressions of VEGF and VEGF mRNA, induces angiogenesis, protects the myocardium when infarction occurs by increasing circulating EPCs and enhancing migration as well as migratory activity of EPCs[3,5,8,10]. Further studies are needed to understand the different mechanisms.

The cardioprotective effect of short-term skeletal muscle ischemia has been previously evaluated in experimental and clinical studies and the beneficial effect on the ventricular myocardium is not specific for a particular species[38]. Physiologic ischemia is reversible noninvasive ischemia of normal skeletal muscles caused by tourniquet or isometric contraction, and induces the development of collateral circulation in the myocardium. The beneficial effect of repeated ischemic training arises from collateral formation rather than from tissue adaptation.

Conclusion

Our research has demonstrated that physiologic ischemic training induces collateral circulation formation and protects the myocardium from complete occlusion of a coronary artery and peripheral arterial disease. These findings may serve as a theoretic basis for further research and a new way of thinking for rehabilitation of ischemic disease.

Acknowledgement

This work was supported by grants from Nantong City (No. BK2014040 to Jun Ni and No. HS2012057 to Hongjian Lu), and from Chinese National Science Fund of China (No. 81070181 to Xiao Lu).

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