Original Article

Repeated Remote Ischemic Conditioning Effect on Ankle-brachial Index in Diabetic Patients - A Randomized Control Trial

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Abstract

Background: Remote ischemic preconditioning (RIPC) is a phenomenon where a short period of ischemia in one organ protects against further ischemia in the other organs. We hypothesized that RIPC occurring in diabetic patients with ankle brachial index (ABI) between 0.70 and 0.90 were included with peripheral arterial disease, would make the better coronary flow resulted in the increasing ABI. **Materials and Methods:** This randomized clinical trial study was done in the Afshar Cardiovascular Hospital in Yazd between 2013 and 2014. Sixty participants were randomly divided into two groups (intervention and control groups). The intervention group was undergoing RIPC, and the control group was tested without RIPC. RIPC was stimulated by giving three cycles of 5 min of ischemia followed by 5 min of reperfusion of both upper arms using a blood pressure cuff inflated to 200 mm Hg (n = 30). This was compared with no RIPC group which consisted of placing a deflated blood pressure cuff on the upper limbs (n = 30). **Results:** The mean of ABI level before intervention in the RIPC and control group group was 0.82 ± 0.055 and 0.83 ± 0.0603 (P = 0.347) respectively, with no significant difference. It was 0.86 ± 0.066 in the RIPC group compared the control 0.83 ± 0.0603 (P = 0.046). So levels of ABI were greater after intervention in the RIPC group. The mean of ABI level increase from 0.82 ± 0.05 to 0.86 ± 0.06 in RIPC group (P = 0.008). So the intervention group showed a significant increase in ABI. **Conclusions:** RIPC through using a simple, noninvasive technique, composing three cycles of 5 min-ischemia of both upper arms, showing a significant increase in ABI level in diabetic patients.

Keywords: *Ankle brachial index, peripheral arterial disease, remote ischemic preconditioning*

Introduction

Peripheral arterial disease (PAD) is found in approximately 60% of patients with coronary artery disease.[1] Coronary heart disease is the leading cause of morbidity and mortality in the worldwide.[2]

PAD, as an ankle brachial index (ABI) of <0.9, increases the long-term risk of cardiovascular and cerebrovascular events, and even death from any cause.[3]

ABI is the ratio of the systolic blood pressure (SBP) measured at the ankle against one measured at the brachial artery; it was described by Winsor[4] in 1950; this index was initially offered for the noninvasive diagnosis of lower-extremity PAD.[5,6] In addition, it was shown that the ABI is an indicator of atherosclerosis at other vascular sites and can be seen as a prognostic marker for cardiovascular events, even in the absence of symptoms.[7,8]

Reperfusion itself can lead to cell damage, which is known as ischemia-reperfusion injury (IRI). Cells in organs other than the heart are very sensitive to the protective effects of IRI of other tissue.[9-11]

Ischemic preconditioning (IPC) is a phenomenon to avoid IRI in different vascular sites. The controlled repeated of short periods of ischemia could also protect tissues against the IRI.

IPC was first described in 1986 by Murry *et al*.[12] as increased cellular resistance to myocardial ischemia when the heart is encountered periods of nonlethal ischemia interspersed with reperfusion. In 1993, Przyklenk *et al*.[13] showed that increased cell resistance to ischemia also occurred in other tissues that were not directly subjected to ischemia. This phenomenon was named remote IPC (RIPC).[14-29] The remote ischemic conditioning (RIC) stimulus could be noninvasively induced

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using a standard blood pressure cuff placed on the upper arm or leg.[30]

The protective effect by IPC has two windows of protection. The first lasts between 4 and 6 h[31] and has been named early preconditioning. The second begins at 24 h lasting up to 72 h after the ischemia and reperfusion stimulus.[32]

Several mechanisms show the phenomenon of RIPC, such as suppression of inflammatory genes, modulation of ATP-sensitive K^+ channels, nuclear factor kappa beta, nitric oxide synthesis, or free radicals pathways.[33]

Based on the evidence of RIPC occurring in the tissues, we predicted that RIPC that occurs in patients with PAD and ABI <0.90, making the better coronary flow that increasing ABI. To test this hypothesis, we performed and compared ABI in patients with and without RIPC.

Materials and Methods

Out of 98 investigated patients, 60 individuals were accepted for this randomized clinical trial study conducted in the Afshar Cardiovascular Hospital in Yazd between 2013 and 2014. About 15 patients did not accept the study protocol and 20 patients did not fulfill the inclusion criteria. The written and oral consent was received from all of the participants. This study was approved by the Ethics Committee of the Shahid Sadoughi University of Medical Sciences.

Diabetic patients with ABI between 0.70 and 0.90 were included. Patients with a history of cerebrovascular accident, or glomerular filtration rate lower than 30 ml/min were excluded. Physical examinations showed normal physiology of the upper limbs in all of the investigated participants.

The participants were randomly divided into two groups (intervention and control groups). The intervention group was undergoing RIPC, and the control group was tested without receiving RIPC. Although those colleagues who both measured the ABI and conducted the statistical analysis were not aware of the engaged groups, the patients were not blinded.

At first ABI was measured with blood pressure cuff, sphygmomanometer, and handheld Doppler devices (Summit Doppler L250 ABI); then calculation was performed that divided the higher of the dorsalis pedis or posterior tibial systolic pressure for each ankle by the higher of the two brachial SBP to get the ABI for each leg; next, RIPC was stimulated by giving three cycles of 5 min of ischemia followed by 5 min of reperfusion of both upper arms using a blood pressure cuff inflated to 200 mm Hg (n = 30). This was compared to no RIPC, which consisted of placing a deflated blood pressure cuff on the upper limbs (n = 30).

The participants were advised to avoid using the following substances, because of their interfering roles during the process of IPC (RIPC), within 2 h of the test: Cilostazol, sildenafil, dipyridamol, glibenclamide, nicorandyl, phenylephrine, angiotensin-converting enzyme inhibitors, angiotensin receptor blocker Π, statins and steroids, caffeine and alcohol.

Statistical analysis

To compare the general characteristics of the two experimental groups, we used the Chi-squared or Fishers' exact test for categorical variables. To compare ABI in each group paired *t*-test and between the two groups, independent *t*-test were used considering the two groups as an independent factor and the ABI as the repeated factor.

Data presented in the form of frequencies, means, and standard deviations. For all of the analyzes, a significance level of $P < 0.05$ was assumed.

Results

Totally 60 patients were analyzed in this study, including 21 males and 39 females ranging from 40 to 74 years old (average 57 years). General patient's characteristics are shown in Table 1. There were no differences between the groups regarding age, gender, hyperlipidemia, hypertension prevalence, and smoking history.

The mean of ABI before and after intervention (RIPC) is shown in Table 2. The mean of ABI before intervention in the RIPC group was 0.82 ± 0.055 in comparison with the control group 0.83 ± 0.0603 (P = 0.347), so no significant differences before intervention were seen between groups.

The mean of ABI after intervention in the RIPC group was 0.86 ± 0.066 in comparison with the control group 0.83 ± 0.0603 (P = 0.046). Hence, comparing

Table 1: General characteristics of participants

Variables	Intervention group (n=30)	Control group (n=30)	P
Age (years)			
Mean±SD	56.57±8.7	55.9±9.39	0.784
Gender (%)			
Female	18 (60)	21 (70)	0.294
Male	12 (40)	9 (30)	
Risk factors (%)			
Hypertension			
Yes	25 (83.3)	23 (76.7)	0.374
No	5 (16.7)	7 (23.3)	
Hyperlipidemia			
Yes	17 (56.7)	16 (53.3)	0.5
No	13 (43.3)	14 (46.7)	
History of smoking			
Yes	7 (23.3)	5 (16.7)	0.374
No	23 (76.7)	25 (83.3)	

Values are expressed as the mean±SD. SD: Standard deviation

Table 2: Effect of RIPC on the ABI in the two experimental groups

Groups	Intervention	Control	*P*
ABI			
Before (mean±SD)	0.82±0.055	0.83±0.0603	0.347
After (mean±SD)	0.86±0.066	0.83±0.0603	0.046
P	0.008	1	

Values are expressed as the mean±SD. SD: Standard deviation, RIPC: Remote ischemic preconditioning, ABI: Ankle brachial index

the control group, the mean of ABI were greater after intervention in the RIPC group.

The mean of ABI level increased from 0.82 ± 0.05 to 0.86 ± 0.06 in RIPC group (P = 0.008). The intervention group showed a significant increase in the ABI in comparison with control group.

Discussion

In this study, we compared the ABI before and after RIPC. Our results showed a significant increase in ABI level in diabetic patients with 0.70≤ ABI ≤0.90 after RIPC.

Przyklenk *et al*. indicated that small cycles of coronary artery occlusion in dogs maintain the myocardial cells from longer periods of ischemia.[13] In addition to the cardiac muscle other organs have been shown to reply to the protective impress of RIPC, including the lungs, kidneys, liver, and skeletal muscle.[34-37]

Hausenloy *et al*. illustrated three 5 min cuff inflations and deflations of a cuff placed on the upper arm to 200 mm Hg, administered before cardiac surgery preoperative myocardial injury (43% less troponin T release) in patients undergoing elective coronary artery bypass grafting surgery.[38]

Hausenloy and Yellon and Tapuria *et al*. reported that the mechanisms underlying the phenomenon of RIC can be noted as three interrelated events: (1) The initial events occurring in the organs in response to the RIC irritant. The application of brief episodes of RIC (Remote Ischemic Conditioning) to the remote organisms believed to produce endogenous factors which can keep them from injury. (2) The transmission of the protective signal may be multi-factorial blood factor(s), neuronal mechanisms, and systemic responses. (3) The events are occurring in the target organ or tissue which present the protective effect.[39,40]

RIPC may be mediated by humoral and neurogenic factors consisting of an early phase that starts about 24 h after RIPC and lasts for nearly 48 h.

Several humoral factors involved in RIPC phenomenon, including opioids, nitric oxide, adenosine, catecholamines, bradykinin, tumor necrosis factor-alpha, free radicals, prostaglandins and angiotensin.

Organs exhibit to ischemia have been shown to induct the protein kinase C intracellular pathways resulting in the nuclear translocation of nuclear factor kappa beta and the activation of nitric oxide synthesis. Current mechanisms involved in target organs and tissues in the early and late process of RIPC stay indeterminate; however, some studies have offered roles for mitochondrial KATP channels and neutrophils.

RIPC was also found to provoke neovascularization in the ischemic myocardium by up-regulation of vascular endothelial growth factor gene expression that limit the infarct size.[41] Not withstanding its promise in animal studies, the clinical benefit of RIPC in human studies remains controversial.

Administration of KATP channel blockers prevents IPC in healthy volunteers, and IPC phenomenon is mimicked by KATP channel opening drugs.[42,43] A number of studies proposed that IRI is dependent on increased oxidative stress,[44,45] making it possible that IPC and RIPC stimulate antioxidant defenses.[46]

Recent studies have discovered the RIPC that occurs in patients with PAD and low ABI[47-49] might lead to the elevation of the circulating levels of some anti-inflammatory, vasodilator, or angiogenesis-inducing contents.

Repeated ischemia-reperfusion occurrence caused by physical training could be irritant for intracellular biochemical changes leading to a more valid use of oxygen by the muscle that ameliorated the endothelial function.[41]

Conclusion

RIPC is a potent innate protective mechanism against ischemic injury. This study demonstrates that RIPC through using a simple, noninvasive technique including three cycles of 5 min of ischemia followed by 5 min of reperfusion of both upper arms showed a significant increase in ABI level in diabetic patients with 0.70≤ ABI ≤0.90. But the mechanism is not yet fully revealed; however, it has indicated a promising point in the clinical trials. No enough trial has been put forward to illustrate the effect of RIPC to decline the incidence of clinically relevant sequel of IRI. However, more powerful studies are necessary to report the case within the next 3–4 years.

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Conflicts of interest

There are no conflicts of interest.

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