

Original Article

Limb remote ischemic preconditioning for ischemic cerebrovascular disease

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Abstract: Objective: The aim of this study was to investigate the efficacy of limb remote ischemic preconditioning (RIPC) in patients with ischemic cerebrovascular disease (ICD). Methods: One hundred patients with confirmed ICD, admitted to the Department of Neurology in Qianfoshan Hospital Affiliated to Shandong University, between January 2015 and December 2016, were enrolled in this study. Patients were randomly divided into an intervention group or control group, with 50 patients in each group. Patients in the control group were treated with standard care while those in the intervention group received limb RIPC, in addition to standard care. The intervention period for all patients was 6 months. At the end of intervention, improvement in neurologic function and rates of newly-developed cerebral infarction were compared between the two groups. Mean blood velocities in the middle cerebral artery, anterior cerebral artery, posterior cerebral artery, vertebral artery, and basilar arteries of patients were measured by transcranial Doppler ultrasonography. Results were compared between the two groups. Barthel activities of daily living (ADL) index scores and 36-item short form health survey (SF-36) scores of patients, as well as serum matrix metalloproteinase-9 (MMP-9) and brain-derived neurotrophic factor (BDNF), were compared between the two groups. Results: Rates of newly-developed cerebral infarction (6% vs 20%, $\chi^2=4.540$, $P=0.033$) were strikingly lower but the improvement rate in neurologic function (84% vs 62%, $\chi^2=6.139$, $P=0.013$) was remarkably higher in the intervention group than control group. Mean blood velocities in the middle cerebral artery, anterior cerebral artery, posterior cerebral artery, vertebral artery, and basilar artery ($P < 0.05$) as well as Barthel ADL index scores (70.2 ± 6.3 vs 61.7 ± 5.8 ; $P=0.038$) and SF-36 scores (67.2 ± 6.1 vs 58.4 ± 5.7 ; $P=0.013$) were all substantially higher in the intervention group than control group. Moreover, much lower serum MMP-9 (215.8 ± 41.7 $\mu\text{g/L}$ vs 277.2 ± 45.8 $\mu\text{g/L}$) levels and markedly higher BDNF levels (34.2 ± 7.2 $\mu\text{g/L}$ vs 20.5 ± 6.9 $\mu\text{g/L}$) were noted in the intervention group (both $P < 0.001$). Conclusion: Limb RIPC training is associated with a reduction in newly-developed cerebral infarction, improvements in neurologic function, blood velocities in the cerebral arteries, quality of life (QoL), serum MMP-9, and BDNF expression in ICD patients. Therefore, it is beneficial for patients with ICD.

Keywords: Limb remote ischemic preconditioning, ischemic cerebrovascular disease, curative effect

Introduction

Ischemic cerebrovascular disease (ICD) is a disorder in which atherosclerosis and other factors lead to insufficient blood supply to the brain and irreversible anoxic changes in nerve cells. With high mortality and disability, this disease seriously threatens health and quality of life (QoL) of many [1-3]. Nevertheless, in treatment of ICD, effective ways to protect the nerve cells have not yet been found. In recent years, animal experiments have shown that remote ischemic preconditioning (RIPC) training reduces the volume of cerebral infarction and has a

protective effect on brain tissues [4, 5]. RIPC is the phenomenon whereby repeated ischemia and anoxia of local organs enhance the tolerance of organs to ischemia and hypoxia, as well as the tolerance of remote target organs to ischemia and hypoxia [6, 7]. According to the results of one study, RIPC plays a protective role in brain cells of rats with ICD [8]. However, few reports have been concerned with the clinical application of RIPC. Therefore, in this current study, bilateral upper-limb RIPC training was applied in intervention of patients with ICD to investigate the effects of RIPC on newly-developed cerebral infarction, improvements in

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neurologic function, blood flow in the brain, QoL, and serum factors of patients. This study aimed to add new insight into the treatment of ICD.

Materials and methods

Patients

This study obtained approval from the Medical Ethics Committee of Qianfoshan Hospital Affiliated to Shandong University. All patients provided written informed consent. A total of 100 patients, admitted to Qianfoshan Hospital Affiliated to Shandong University, from January 2015 to December 2016, confirmed as having ICD (cerebral infarction or transient cerebral ischemic attack) by clinical symptoms and cranial CT or MRI examinations, were recruited for this study. Patients older than 18 years of age were enrolled if they had a history of cerebral infarction or transient cerebral ischemic attack; if they had presence of cerebral infarct 1 year prior; if they had multiple cerebral artery or carotid artery stenosis with narrowing greater than 50% at one site, as demonstrated by imaging examinations; if they refused to undergo carotid endarterectomy or stent implantation; and, if they took routine medication to control potential risk factors (blood glucose < 7 mmol/L, blood lipid (low-density lipoprotein < 2.6 mmol/L) or blood pressure < 140/90 mmHg) within the normal range. Patients cooperated voluntarily, they stopped smoking and drinking. Patients were ineligible if they had malignancy, severe hepatorenal dysfunction, and mental disorders or if they had undergone surgery or had a severe infection within 3 months.

Enrolled patients were randomly divided into an intervention group (n=50) and control group (n=50), in terms of a random number table. Patients in the control group were given standard care (routine medication) whereas those in the intervention group underwent limb RIPD, in addition to standard care. Specific procedures were as follows: upper-limb blood pressure of each patient was pressurized to 180 to 200 mmHg by sphygmomanometers for 5 minutes, followed by a 5 minute deflation, forming a cycle. Each patient received 5-cycle training, once a day, for 6 months. During the 6-month intervention, all patients were reviewed by cranial CT or MRI once a month. Patients were

reviewed at any time if they presented with suspected symptoms and signs of cerebral infarction. They were not instructed to do follow up training when newly-developed cerebral infarctions were detected by imaging examinations during the intervention period.

Outcome measures

Rates of cerebral infarction and improvement rates in neurologic function of patients were compared between the two groups. Over the intervention period, cranial MRI was performed to check newly-developed cerebral infarction lesions once a month. At the end of intervention, new injury severity score (NISS) was used to evaluate patient recovery of neurologic function. It, primarily, included awareness, gaze, vision, facial paralysis, upper-limb motor and lower-limb motor, ataxia, dysarthria, feeling, language, dysarthria, and neglect. An improvement in neurologic function was defined as the NISS score decreasing by more than 18%. No improvement was defined as the NISS score decreasing by less than 18%, or increasing. Patients with transient cerebral ischemic attacks were considered as improved if they had no cerebral ischemia events. They were considered as not improved if they had cerebral ischemia events.

Mean blood velocities in the middle cerebral, anterior cerebral, posterior cerebral, vertebral, and basilar arteries of patients were measured using transcranial Doppler ultrasonography, immediately after intervention, and compared between the two groups.

Barthel activities of daily living (ADL) index scores and QoL were compared between the two groups. Immediately after intervention, the neurologic deficits of patients were assessed with use of the Barthel ADL Index. QoL of patients was evaluated according to the 36-item short form health survey (SF-36) scores (Scores ranging from 0 to 100 with higher scores indicating higher QoL), which mainly covered physical and psychosocial health. Physical health included physical pain, physical activity, work efficiency, and overall health status while psychosocial health covered sociability, mental status, vitality, and emotional status.

Serum matrix metalloproteinase-9 (MMP-9) and brain-derived neurotrophic factor (BDNF)

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Table 1. Baseline characteristics of patients

Variables	Case	M/F (n)	Age (year)	ICD (n)		NIHSS score	DM (n)	HT (n)	HL (n)
				CI	TIA				
IG	50	23/27	52.3±4.7	19	31	3.6±1.6	10	30	13
CG	50	26/24	54.1±5.3	21	29	3.8±1.8	9	28	16
t/χ ²		0.160	1.797	0.042	0.587	0.065	0.164	0.437	
P		0.689	0.076	0.838	0.558	0.799	0.685	0.509	

Note: Male/Female denotes M/F; ICD, ischemic cerebrovascular disease; NIHSS, National Institutes of Health Stroke Scale; CI, cerebral infarction; TIA, transient cerebral ischemic attack; DM, diabetes mellitus; HT, hypertension; HL, hyperlipidemia; IG, intervention group; CG, control group.

expression of patients were compared between the two groups. Six months after intervention, venous blood (3 mL) was drawn from the elbow vein of each patient and placed into an anticoagulant tube, followed by serum centrifugation at 3000 r/min for 15 minutes. Serum MMP-9 and the BDNF levels of patients were detected by ELISA, following manufacturer instructions (Promega, USA).

Statistical analysis

Statistical data analyses were conducted with application of SPSS statistical software, version 21. Measurement data are presented as mean ± standard deviation, with independent sample t-tests for intergroup comparisons and paired t-tests for intragroup comparisons, before and after treatment. Count data are described as rates, with Chi-square tests for inter-group comparisons. $P < 0.05$ was deemed as a statistically significant difference.

Results

Baseline characteristics of patients

The patients were largely well-balanced in risk factors (sex ratio, age, scores for the National Institutes of Health Stroke Scale (NIHSS), hypertension, hyperlipidemia, and other factors) (all $P > 0.05$). Hence, they were comparable, as indicated in **Table 1**.

Newly-developed cerebral infarction and improvement in neurologic function

At 6-months after intervention, rates of newly-developed cerebral infarction were remarkably lower in the intervention group (6% vs 20%, $\chi^2=4.540$, $P=0.033$), whereas the improvement rate in neurologic function was substan-

tially higher in the intervention group than control group (84% vs 62%, $\chi^2=6.139$, $P=0.013$), as illustrated in **Figure 1**.

Blood velocities in cerebral arteries

Before intervention, mean blood velocities in the middle cerebral, anterior cerebral, posterior cerebral, vertebral, and basilar arteries of patients differed insignificantly between the two groups (all $P > 0.05$).

Six months after intervention, mean blood velocities in the abovementioned cerebral arteries in the control group were insignificantly different than before intervention (all $P > 0.05$). However, corresponding velocities in the intervention group were strikingly higher than before intervention (all $P < 0.05$) and those in the control group (all $P < 0.05$; **Tables 2 and 3**).

Barthel ADL index scores and SF-36 scores

Before intervention, no significant differences in Barthel ADL index scores and SF-36 scores were observed between the two groups (both $P > 0.05$). Six months after intervention, Barthel ADL index scores (61.7±5.8) of the control group differed insignificantly from that before intervention ($P > 0.05$); corresponding scores (70.2±6.3) of the intervention group were considerably higher than before intervention ($P < 0.05$). A great disparity in Barthel ADL index scores after intervention was noted between the two groups ($P=0.038$). SF-36 scores (67.2±6.1) in the intervention group after intervention was remarkably higher than that (54.9±5.1) before intervention and that (58.4±5.7) of the control group after intervention ($P=0.013$). Conversely, SF-36 scores, before and after intervention, differed insignificantly among patients in the control group ($P > 0.05$; **Table 4**).

Expression of serum MMP-9 and serum BDNF

Before intervention, there were no significant differences in serum MMP-9 (286.4±50.2 µg/L vs 291.5±52.4 µg/L, $t=0.497$, $P=0.620$) and BDNF levels (18.4±5.9 µg/L vs 17.6±5.3 µg/L;

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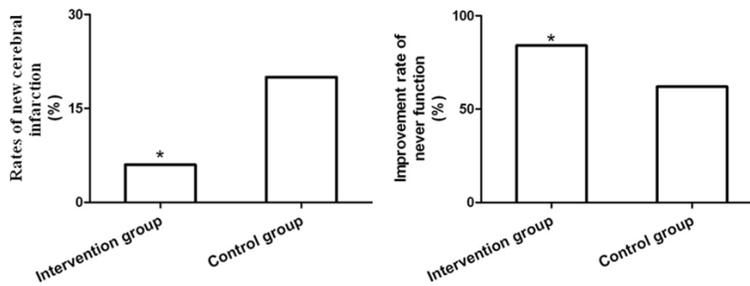


Figure 1. Comparison of rates of newly-developed cerebral infarction and improvement rates in neurologic function between the two groups. Compared with the control group, * $P < 0.05$.

Table 2. Blood velocities in the cerebral arteries before intervention (cm/s)

Variables	Case	MCA	ACA	PCA	VA	BA
IG	50	40.2±9.1	31.5±9.4	30.6±7.4	31.1±7.9	34.9±6.7
CG	50	39.5±8.8	31.8±8.5	31.3±7.2	30.8±7.6	34.2±6.3
t		0.096	0.041	0.117	0.047	0.132
P		0.928	0.969	0.912	0.964	0.901

Note: MCA denotes middle cerebral artery; ACA, anterior cerebral artery; PCA, posterior cerebral artery; VA, vertebral artery; BA, basilar artery; IG, intervention group; CG, control group.

Table 3. Blood velocities in the cerebral arteries after intervention (cm/s)

Variables	Case	MCA	ACA	PCA	VA	BA
IG	50	50.1±8.7	39.2±9.1	37.4±7.8	36.9±7.2	40.4±7.5
CG	50	40.7±8.3	34.8±8.9	32.5±6.9	31.7±6.5	35.3±7.1
t		18.124	2.057	2.080	4.201	3.129
P		< 0.001	0.041	0.039	0.004	0.011

Note: MCA denotes middle cerebral artery; ACA, anterior cerebral artery; PCA, posterior cerebral artery; VA, vertebral artery; BA, basilar artery; IG, intervention group; CG, control group.

$t=0.175$, $P=0.870$) between the intervention group and control group (both $P > 0.05$). Among patients in the control group, serum MMP-9 ($291.5 \pm 52.4 \mu\text{g/L}$ vs $277.2 \pm 45.8 \mu\text{g/L}$, $t=0.356$, $P=0.740$) and BDNF levels ($17.6 \pm 5.3 \mu\text{g/L}$ vs $20.5 \pm 6.9 \mu\text{g/L}$, $t=0.577$, $P=0.595$) before intervention were insignificantly different from those after intervention (both $P > 0.05$); conversely, among patients in the intervention group, serum MMP-9 ($286.4 \pm 50.2 \mu\text{g/L}$ vs $215.8 \pm 41.7 \mu\text{g/L}$, $t=0.2964$, $P=0.014$) and BDNF levels ($18.4 \pm 5.9 \mu\text{g/L}$ vs $34.2 \pm 7.2 \mu\text{g/L}$, $t=4.648$, $P=0.001$) before intervention were greatly different from those after intervention (both $P < 0.05$).

Six months after intervention, results of ELISA revealed a considerable decrease in serum

MMP-9 levels and a striking rise in BDNF levels was noted among patients in the intervention group compared with those in the control group ($P < 0.001$, **Figure 2**).

Discussion

ICD is a common cardiovascular and cerebrovascular disease. With rising morbidity and a trend of younger patients, clinically, ICD has become an urgent medical concern. In 1986, Murry et al. reported that one or more episodes of ischemia reperfusion results in an increase in the tolerance of myocardial cells to subsequent severe ischemia reperfusion injuries [9]. Ischemic preconditioning (IPC) refers to a phenomenon whereby repeated brief episodes of IPC reduce or alleviate subsequent severe ischemia reperfusion injury in tissues. Previous studies have proven IPC as a novel therapeutic technique with favorable curative effects in treatment of cardiovascular disease and spinal cord-associated disease [10-12]. However, IPC directly acts on target organs and cerebral cells are sensitive to ischemia, so

irreversible injury might occur after ischemia [13, 14]. Therefore, *in situ* IPC cannot be applied in the management of ICD.

In recent years, the protective effects of RIPC on cerebral tissue have been drawing increasing attention from relevant scholars. Previous literature has indicated that limb IPC is associated with reduction in the apoptosis of hippocampal neurons induced by cerebral ischemia-reperfusion injury and in delayed death of neuronal cells after ischemia in rats [15]. According to another study, IPC training of the iliac artery in rats significantly reduced the volume of cerebral infarcts, in the rat model, of middle cerebral artery occlusion [16]. Some scholars have argued that mechanisms for the protective effects of RIPC on cerebral tissue

Table 4. Barthel ADL index scores and SF-36 scores of patients

Variables	Case	Barthe ADL index score		t	P	SF-36 score		t	P
		BI	AI			BI	AT		
IG	50	58.4±4.9	70.2±6.3	3.070	0.018	54.9±5.1	67.2±6.1	3.247	0.014
CG	50	58.7±5.2	61.7±5.8	0.667	0.541	55.2±5.4	58.4±5.7	0.706	0.519
t		0.073	4.982			0.070	6.124		
P		0.946	0.038			0.948	0.013		

Note: BI denotes before intervention; AI, after intervention; Barthe ADL index, Barthel activities of daily living (ADL) index; IG, intervention group; CG, control group.

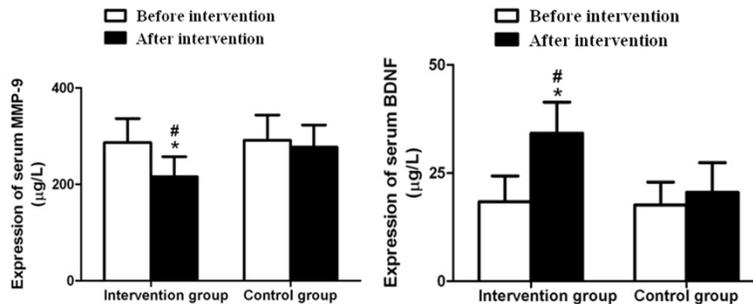


Figure 2. Expression of serum MMP-9 and serum BDNF of patients before and after intervention. Compared with that in the same group before intervention, *P < 0.05; Compared with that in the control group, #P < 0.05.

might be implicated in regulation of immunity and humoral and nervous functions. The specific mechanism, however, remains unclear [17, 18]. Studies on RIPC have been mostly focused on experimental animals. With the advantages of a simple operation, non-invasion, and safety, RIPC has become a hotspot in clinical practice [19].

In this current study, patients with cerebral infarction and transient cerebral ischemic attacks were selected as subjects. It was found that RIPC training resulted in much lower rates of newly-developed cerebral infarction and significantly improved neurologic function, suggesting that RIPC has a protective effect on cerebral tissues in patients with ICD. Moreover, this study detected the effects of RIPC on blood velocities of cerebral arteries with the use of transcranial Doppler ultrasonography, finding greater improvement in mean blood velocities in the middle cerebral, anterior cerebral, posterior cerebral, vertebral, and basilar arteries of patients in the intervention group compared to the control group. Imaging examinations demonstrated that cerebral protection effects of RIPC might be correlated with an increase in cerebral blood flow perfusion, consistent with

findings reported by Etz et al. [20].

Abnormalities in expression of serum factors have been observed in ICD patients in the pathological process [21]. MMP-9, a crucial member of the MMPs family, degrades the extracellular matrix. Elevated MMP-9 levels, after ischemia-reperfusion injury, lead to accelerated damages to nerve cells [22]. BDNF is a neurotrophic substance protective to the nervous system, as it is effective in repair of injured nerve cells [23].

The results of this current study indicated that serum MMP-9 levels in the intervention group were remarkably lower than the control group, while BDNF levels were substantially higher. These results imply that the neuroprotection of RIPC might be correlated with enhancement in the protection function by BDNF and decrease in injuries by MMP-9. Additionally, the current study demonstrated that RIPC significantly improved QoL of patients, compared to the control group, similar to results reported in previous studies [24].

In conclusion, limb RIPC training has a favorable effect on ICD patients, significantly reducing rates of newly-developed cerebral infarction, improving neurologic function, enhancing life and treatment, increasing the blood supply in cerebral arteries, and changing the expression of MMP-9 and BDNF. Limb RIPC training provides a new method for prevention and treatment of ICD. Nevertheless, in future studies, larger sample sizes and longer follow up periods are necessary to clarify the possible humoral or neural mechanisms for cerebral protection of limb RIPC.

Disclosure of conflict of interest

None.

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