RESEARCH ARTICLE

rt-PA with remote ischemic postconditioning for acute ischemic stroke

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Abstract

Objective: To investigate the feasibility and safety of remote ischemic postconditioning (RIPC) in acute ischemic stroke patients after intravenous recombinant tissue plasminogen activator (rt-PA) thrombolysis (IVT). **Methods**: We performed a pilot randomized trial involving acute ischemic stroke patients with IVT. The patients were randomized 1:1 to receive RIPC or standard medical therapy. In the RIPC group, the participants underwent instant RIPC within 2 h of IVT, followed by repeated RIPC therapy for 7 days. The feasibility end point was the completion of RIPC and time from the first RIPC to finishing IVT in the RIPC group. The safety end point included tissue and neurovascular injury resulting from RIPC, changes in vital signs, level of plasma myoglobin, any hemorrhagic transformation, and other adverse events. **Results**: Thirty patients (15 RIPC and 15 Control) were recruited after IVT. The mean age was 65.7 ± 10.2 years, with a National Institutes of Health Stroke Scale (NIHSS) score of 6.5 (4.0–10.0). The completion rate for RIPC was 97.0%. The mean time from first RIPC to completing IVT was 66.0 (25.0–75.0) min in the RIPC group. One case of hemorrhagic transformation was observed in the RIPC group. No significant difference was found in the level of myoglobin between the two groups ($P > 0.05$). **Interpretation**: RIPC is effective and safe for AIS patients after intravenous rt-PA thrombolysis.

Introduction

Acute ischemic stroke (AIS) is one of the leading causes of death and disability worldwide. Since 1995, intravenous recombinant tissue plasminogen activator (rt-PA) therapy (IVT) has been demonstrated to be the most effective treatment for AIS. However, nearly 50% of patients (47.6–61%) who received IVT cannot achieve nondisability (modified Rankin scale [mRS], 0–1) after 90 days.[1–3] Meanwhile, rt-PA may increase cerebral injury due to its fibrinolytic action and cause blood–brain barrier (BBB) disruption, among other effects.[4,5] Moreover, the risk of hemorrhagic transformation is higher than in patients without IVT. The percentage of cases that develop intracranial hemorrhage (ICH) is 9–27%, and 2.1–5% develop symptomatic ICH.[1–3] Therefore, it is necessary to find an effective way to further

improve clinical outcome and reduce the side effects. Combining neuroprotective treatments with reperfusion therapy may be an effective approach to solve this problem.[6]

Remote ischemic conditioning (RIC), a kind of neuroprotection approach, has shown it neuroprotective effect in the treatment of cerebral ischemic stroke these years. It can improve cerebral perfusion, reduce recurrent stroke in patients with symptomatic atherosclerotic intracranial arterial stenosis,[7,8] and decrease ischemic brain injury secondary to carotid artery stenting.[9] Remote ischemic postconditioning (RIPC), which consists of several brief cycles of intermittent ischemia–reperfusion of the arm or leg after intravenous rt-PA, can eliminate the exaggerating effect of rt-PA by reducing reactive oxygen species (ROS) levels, enhancing endothelial function, and improving cerebral blood flow (CBF) after reperfusion.[10–15] Ren et al. reported that RIPC performed in the hind limbs can not only significantly reduce the stroke volume within 3 h, but also ameliorate the outcome of the behavioral test in a rat model. Long-term repeated RIPC therapy can also help improve neurological function.[16] A combination of RIPC and rt-PA can help reduce the infarction volume, which was shown to further improve neurological function in a rat model.[17–19] Thus, it is meaningful to transform these basic experimental results to clinical treatment. In the RECAST-1 trial, RIC was shown to be safe in AIS patients not treated with rt-PA.[20] The prehospital RIC of acute ischemic stroke before rt-PA treatment is safe and may reduce the risk of tissue infarction.[21] However, there is no further explanation for the effects of RIC after rt-PA. Thus, in this study, we aim to demonstrate the safety and feasibility of RIPC in AIS patients who received intravenous rt-PA treatment, as well as to pave the way for further trials on its efficacy.

Materials and Methods

Study design

The RIC-rtPA study (rt-PA Thrombolytic Therapy in Combination with Remote Ischemic Conditioning for Acute Ischemic Stroke) is a single-center, randomized, rater-blinded trial (registered on clinicaltrials.gov with NCT03231384) performed in Xuanwu Hospital, Capital Medical University. This protocol was approved by the Ethics Committee of Xuanwu Hospital, Capital Medical University. All participants or their legally authorized representative provided written informed consent.

Participant selection

Inclusion criteria

The study inclusion criteria were as follows: (1) male or female; (2) age ≥18 years; (3) clinical signs and symptoms consistent with a diagnosis of AIS; (4) onset of stroke symptoms within 4.5 h of initiation of intravenous rt-PA thrombolytic therapy; (5) baseline National Institutes of Health Stroke Scale (NIHSS) score of 1–15 (assessed before intravenous alteplase treatment); (6) mRS ≤1 before onset of stroke symptom; and (7) informed consent obtained.[7,8]

Exclusion criteria

Participants who met any of the following criteria were excluded from the study: (1) contraindication for remote ischemic conditioning, e.g., severe soft tissue injury, fracture, or peripheral vascular disease in the upper limbs; (2) life expectancy <1 year; (3) pregnant or breast-feeding women; (4) unwilling to be followed up or poor compliance for treatment; and (5) patients enrolled or having been enrolled in another clinical trial within 3 months of this clinical trial.[2,3,22]

Randomization

All patients were enrolled consecutively and randomized in a 1:1 ratio to receive either RIPC plus standard stroke unit care or standard stroke unit care only using a computer-generated randomization code. The randomization code was put into an opaque envelope. The on-call physicians would number the participants and open the envelope if the participant met the inclusion criteria and provided informed consent. The treatment plan allocated to each participant was determined by the randomized code. On-call neurologists were not blinded to treatment instructions, but they did not participate in the data analysis or follow-up clinical ratings. The observers who assessed the clinical outcome were blinded to the treatment allocation.

Procedures

We recruited participants with AIS who had been administered intravenous rt-PA (Actilyse® [Recombinant Human Tissue Plasminogen Activator for Injection (rt-PA)]) thrombolytic therapy (0.9 g per kilogram of body weight) within 4.5 h of symptom onset (last known well) according to the guidelines. During IVT, the baseline demographic, clinical, and laboratory information was collected. The eligible participants provided signed informed consent and were randomly assigned into two groups, the control group and RIPC group.

For all participants, a follow-up head Computed tomography (CT) was performed 24 h after intravenous thrombolysis (IVT) or ICH was diagnosed based on clinical deterioration. Doppler ultrasound was scheduled and performed between 24 and 72 h. CT angiography (CTA)

and magnetic resonance imaging (MRI) were performed 24–72 h after IVT. For each participant, baseline characteristics, complications and follow-up outcomes were documented.

In the control group, all participants received standard medical care, including antiplatelet (aspirin 100 mg and/or clopidogrel 75 mg) treatment, if the follow-up CT showed no obvious intracranial hemorrhage (ICH) at 24 h after IVT, and combined with atorvastatin 20 mg/day or rosuvastatin 10 mg/day. In the RIPC group, besides the standard medical care given to the control group, RIPC was performed once within 2 h of intravenous rt-PA and twice daily for the subsequent 6 days consecutively. RIPC was performed on the bilateral upper limbs, but on a single upper limb when the participants were not suitable for the two-armed treatments (e.g., because they were receiving an intravenous infusion, blood pressure monitor use, etc.), at each session. The RIPC was performed using an electric automatically controlled device (patent number ZL201420846209.5, China) that performed five cycles of inflation to a pressure of 200 mmHg and deflation for 5 min alternately on the first day after IVT, and twice a day for 6 consecutive days (13 times × 5 cycles/time). The process was performed with the help of a nurse in the hospital. The device records and documents each RIPC cycle, as well as the heart rate and blood pressure, in real time. The participants could stop the process at any time if they experienced discomfort.

Endpoints assessment

Feasibility end point

(1) The completion of RIPC in the RIPC group; (2) the time from first RIPC to completion of IVT; and (3) the impact of routine medical therapy, requests from the patient or surrogate to cease the RIPC treatment.

Safety monitoring end point

(1) Objective signs of tissue or neurovascular injury resulting from RIPC treatment— inspected by observers blinded to the study protocol, including visual inspection for local edema, erythema, and/or skin lesions, and palpation of distal radial pulses; (2) blood pressure and heart rate were measured immediately before and 15 min after RIPC in the RIPC group daily for 7 days; (3) elevation of plasma myoglobin levels in the two groups; (4) any hemorrhagic transformation within 7 days; and (5) any adverse events within 90 days.

Other end points

1) Neurological function and outcome measured using the NIHSS, modified Rankin Scale score (mRS), and Barthel Index (BI) at baseline (before IVT), 30 ± 7 days,

and 90 ± 7 days after IVT. (2) Stroke recurrence: stroke or transient ischemic attack (TIA) recurrence during the subsequent 90 consecutive days. Stroke recurrence was defined as sudden functional deterioration in neurologic status with a decrease of four or more on the NIHSS, or a new stroke lesion on MRI/DWI located in the territory of the affected intracranial arteries.

Statistical analyses

We compared the data between participants treated with and without RIPC. Per-protocol (PP) analysis was used in this study. For continuous variables, mean ± standard deviation (SD) or median with interquartile range (IQR) is used to summarize data. Two-sided Student's *t*-test or Mann–Whitney *U* test was performed to detect differences between groups. For categorical variables, frequencies and percentages were used to summarize data, and Fisher's Exact Test was used to detect differences between two groups. All data were analyzed using SPSS 21.0 (IBM Corp.©) with a significance level of $P < 0.05$ (two-sided).

Results

Thirty AIS patients were recruited from August 2017 to October 2017 in Xuanwu hospital and 30 participants were analyzed. There were 15 participants (50.0%) in the control group and 15 participants (50.0%) in the interventional (RIPC) group (Fig. 1).

Baseline data

The average age was 65.7 ± 10.2 years old, and 24 participants (80.0%) were male. The baseline NIHSS score was 6.5 (4.0–10.0). Vascular risk factors, etiology of stroke, and operational details are also shown in Table 1, and no significant differences were found between the two groups.

Feasibility

It was expected that each participant would undergo 65 cycles, thus 975 cycles would be performed in total. In the study, 946 cycles were performed, leading to a completion rate of 97.0%; 60% (nine participants) of participants received all 65 cycles. In the RIPC group, the time from first RIPC to completion of IVT was 66.0 (25.0–75.0) min. The RIPC procedure was well tolerated by all participants without the need to cease or affecting routine therapy.

Safety monitoring

All participants (15 participants) in the RIPC group tolerated the whole RIPC procedures. Nine participants
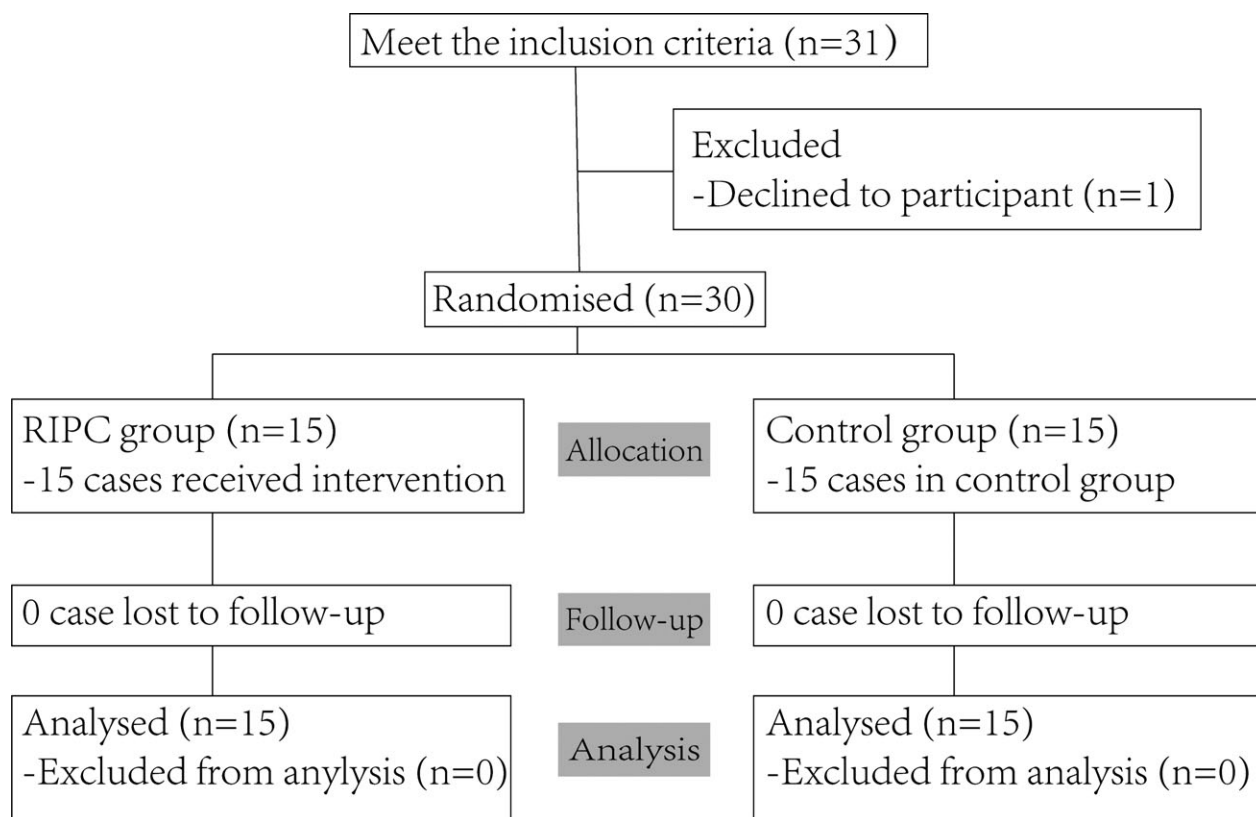
Figure 1. Trial profile. RIPC, remote ischemic postconditioning.

Table 1. Baseline characteristics and therapy of acute ischemic stroke (AIS) patients in the control and RIPC groups.

	Control group (*n* = 15)	RIPC group (*n* = 15)	*P*-value
Age, y (mean ± SD)	65.3 ± 9.4	66.1 ± 11.2	0.737
Male sex, *n* (%)	13 (86.7)	11 (73.3)	0.651
ONT, minutes, median (IQR)	151.0 (116.0-207.0)	128.0 (110.0-212.0)	0.756
Baseline NIHSS, median (IQR)	5 (4–10)	7 (5-10)	0.616
Medical history			
Hypertension, *n* (%)	10(66.7)	11(73.3)	1.0
Diabetes mellitus, *n* (%)	2(13.3)	7(46.7)	0.109
Atrial Fibrillation, *n* (%)	1(6.7)	2(13.3)	1.0
CHD, *n* (%)	4(26.7)	4(26.7)	1.0
Previous stroke, *n* (%)	3(20.0)	4(26.7)	1.0
OCSP			
LACI, *n* (%)	11 (73.3)	11 (73.3)	1.0
TACI, *n* (%)	0 (0)	0 (0)	–
PACI, *n* (%)	2 (13.3)	3 (20.0)	1.0
POCI, *n* (%)	2 (13.3)	1 (6.7)	1.0
Medicine therapy after enrollment			
Aspirin, *n* (%)	7 (46.7)	4 (26.7)	0.450
Clopidogrel, *n* (%)	3 (20.0)	1 (6.7)	0.598
Aspirin + Clopidogrel, *n* (%)	4 (26.7)	8 (53.3)	0.264
Anticoagulation therapy, *n* (%)	1 (6.7)	2 (13.3)	1.0

P < 0.05. ONT, onset to needle time; NIHSS, National Institute of Health stroke scale; CHD, coronary heart disease; LACI, lacunar infarct; TACI, total anterior circulation infarct; PACI, partial anterior circulation infarcts; POCI, posterior circulation infarcts.

(60.0%) developed some pinpoint-like erythema in the superior part of the upper arm without paleness of the skin, edema, or pain or tenderness in the region of the distal radial artery (Fig. 2a,b).

To assess the effect of RIPC on vital signs, blood pressure and heart rate were measured for 7 consecutive days during the study. No statistical differences were found between before and after treatment in systolic pressure,
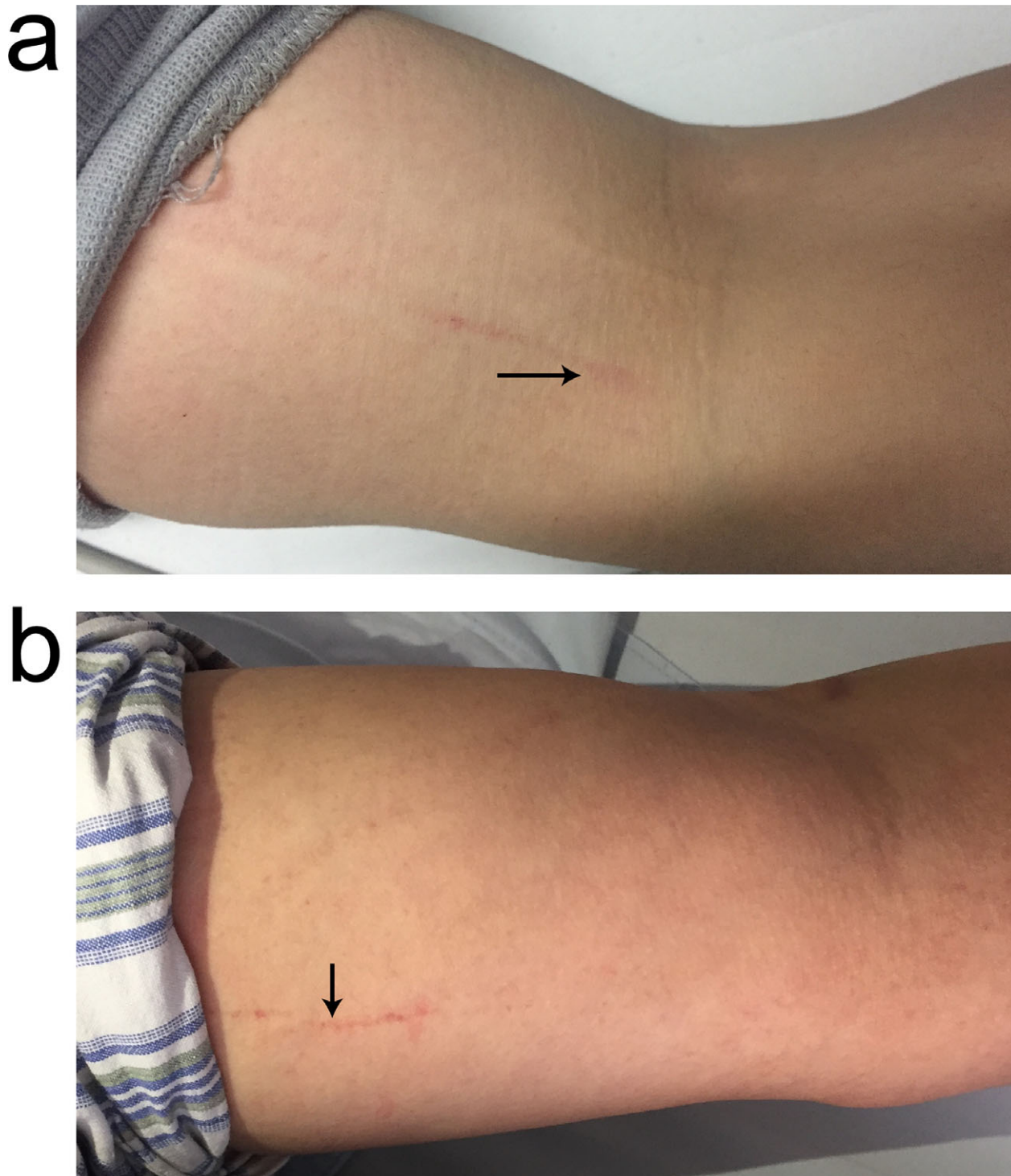

Figure 2. The objective signs of tissue or neurovascular injury. (A, B). the pinpoint-like erythema at the superior part of upper arm.
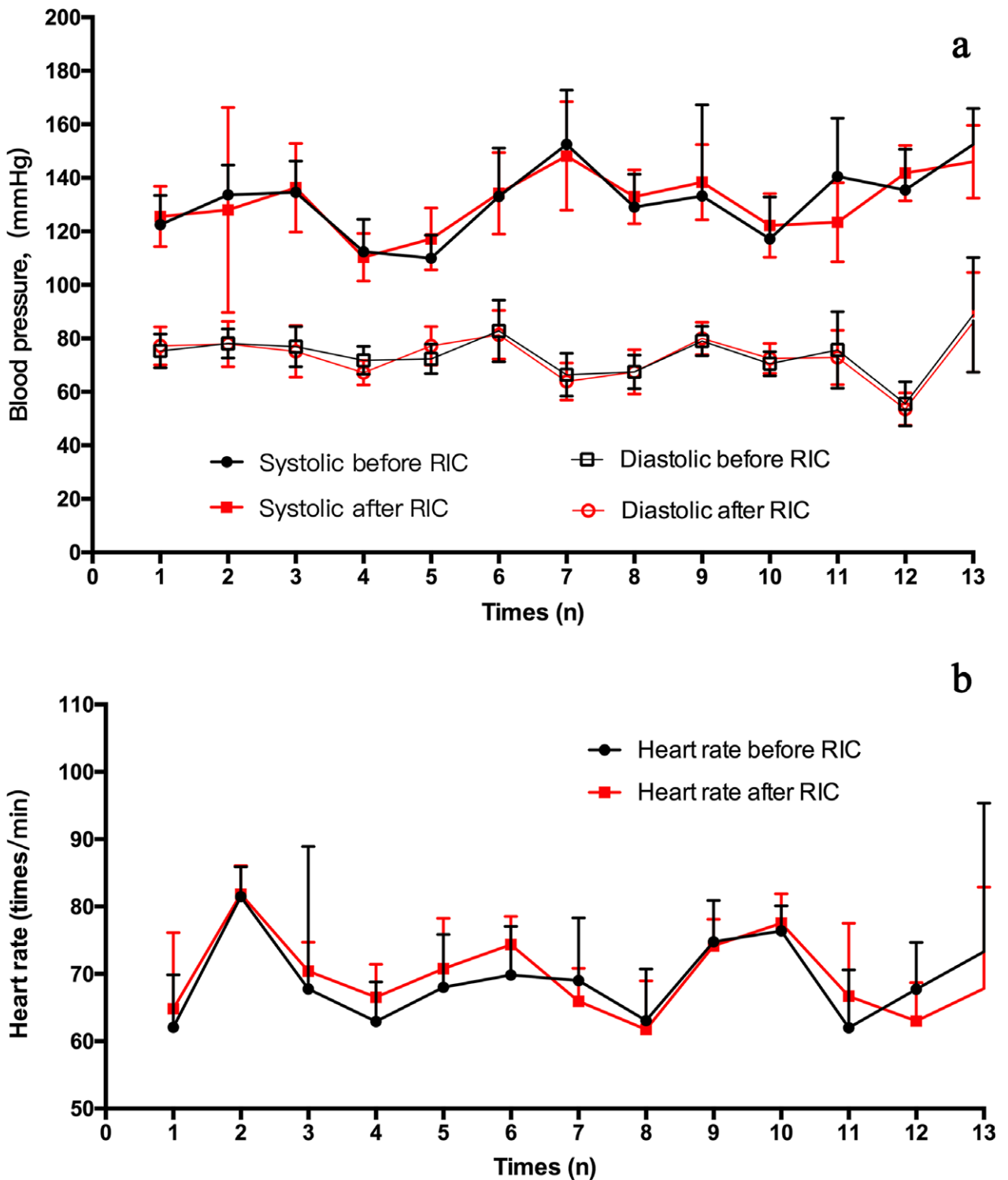
Figure 3. Cardiovascular parameters during 7 days of RIPC treatment (*n* = 15). (A). The black curve represents blood pressure immediately before RIPC, and the red curve represents blood pressure at 15 min after RIPC. (B) The black curve represents the heart rate immediately before RIPC, and the red curve represents the heart rate at 15 min after RIPC.

diastolic pressure, or heart rate ($P = 0.60$, 0.54, 0.56, respectively) (Fig. 3a,b).

The average plasma level of myoglobin was 56.7 (47.7–105.0) μg/L in the control group and 45.6 (37.0–67.9) μg/L in the RIPC group at baseline. On day 7, the level of myoglobin was 34.3 (30.0–43.9) μg/L in the control group, and 35.1 (31.3–42.2) μg/L in the RIPC group. No significant changes were found between baseline and day 7 (-25.1 [-57.1 to -18.1] μg/L vs. -13.3 [-49.3 to 6.2] μg/L, $P = 0.258$).

No participants experienced intracranial hemorrhage within 7 days in the control group. One developed ICH on day 9 and died on day 10 in the RIPC group.

Other end point

No significant difference was observed in the NIHSS, mRS, or BI between the two groups at 90 days ($P = 0.929$, 0.838, 1.0, respectively) (Table 2). However, relative to the participants in the control group, there was a significant decrease in NIHSS score at day 30 in the RIPC group (0 [0–1] vs. 1 [0–2], $P = 0.037$).

Two participants experienced vascular events in the control group: one participant experienced ischemic stroke (day 39) and two TIA (day 7, 11); one of whom experienced a TIA (day 41). At the meantime, one patient met heart failure at day 2. Two participants experienced vascular events in the RIPC group: two participants experienced a TIA (day 2 and 18). No significant difference was observed between the two groups ($P = 0.096$). No other adverse events occurred.

Discussion

Our study indicated that RIPC was tolerated by AIS patients after intravenous rt-PA and seemed to be feasible with a high completion rate and suitable time window according to the study design. Although one patient in the RIPC group developed ICH, there was no significant difference between two groups. Thus, RIPC seems safe.

Although safety and feasibility has been investigated in patients treated with RIC delivered prior to rt-PA, patients treated with RIPC after rt-PA have specific characteristics. Generally, rt-PA is a serine protease that can cleave plasminogen to activate plasmin.[5] In our study, RIC consisted of five cycles of 200 mmHg inflation. This may further increase the risk of hemorrhagic events after IVT in the local area (such as mucocutaneous bleeding) and even ICH. Furthermore, ischemia–reperfusion injury after revascularization is inevitable. Previous studies have indicated that RIC eliminates the effects of rt-PA by reducing ROS levels, reducing excitotoxicity, and protecting the BBB by decreasing the level of MMP-9.[10,13,23] Therefore, it is necessary to elucidate the safety and efficacy of RIPC after rt-PA.

The time window for RIPC treatment was key in our study. In our study, we performed RIPC within 2 h of intravenous rt-PA and continuous RIPC therapy for 7 days. Previous studies have shown that postconditioning attenuates reperfusion injury in a transient focal ischemia–reperfusion model when performed within 3 h of reperfusion.[24–26] This protective effect can last for 48–72 h, which is triggered by ROS, mediated by the modulated inflammatory response and improved endothelial function.[27,28] Furthermore, patients with AIS may experience neurological deterioration hours or days after onset and reach an unexpectedly severe disability status. RIPC can effectively reduce the progressive effects and recurrence of stroke.[8] Thus, long-term daily RIPC was more effective in providing neurological improvement than was a single episode.

In our study, the completion rate of RIPC was 97.0% and the time before starting the first RIPC procedure was

Table 2. Outcome Measures of Patients in the Control and RIPC groups.

	Control group ($n = 15$)	RIPC group ($n = 15$)	P Value
NIHSS			
NIHSS-0 h, median (IQR)	5 (4–10)	7 (5–10)	0.616
NIHSS-30 days, median (IQR)	1 (0–2)	0 (0–1)	0.037
NIHSS-90 days, median (IQR)	0 (0–0)	0 (0–0)	0.929
mRS			
mRS-0 h, median (IQR)	3 (2–4)	4 (2–4)	0.624
mRS-30 days, median (IQR)	1 (1–3)	1 (0–2)	0.486
mRS-90 days, median (IQR)	1 (0–1)	1 (0–1)	0.838
BI			
BI-0 h, median (IQR)	45 (30.0–55.0)	45 (30.0–55.0)	0.935
BI-30 days, median (IQR)	100.0 (65.0–100.0)	100.0 (90.0–100.0)	0.870
BI-90 days, median (IQR)	100.0 (100.0–100.0)	100.0 (100.0–100.0)	1.0

$P < 0.05$. NIHSS, National Institute of Health stroke scale; mRS, modified Rankin scale; BI, Barthel Index.

66.0 min. The result is consistent with our previous study.[29] In the meantime, we found that only 60% (9 participants) of the participants received all 65 cycles. Six participants who have not completed all the treatment, 4 participants required to get discharged earlier than plane (discharged before seven days) because of significant alleviation and 2 participants missed the RIPC treatment because they had to go for an examination (such as CTA, MRI). Although several participants had pinpoint-like erythema in the local area, they could cooperate well to complete the treatment without any pain. Myoglobin is an iron- and oxygen-binding protein found in the muscle tissue and is a sensitive marker for muscle injury.[7,30] No differences were found in the level of myoglobin between the two groups. Therefore, RIPC may be considered safe for patients after IVT.

There are several limitations in this study. First, although the NIHSS score in the RIPC group was lower than that in the control group at 30 days ($P < 0.05$), the sample size was not sufficient to provide the statistical power to detect changes in clinical outcomes and vascular events. Therefore, the differences in neurological improvement may be due to chance or statistical bias. Secondly, the participants in the control group received standard medical therapy only but not sham-RIPC therapy. This may lead to a statistical bias.

In conclusion, these results suggest that RIPC is feasible and safe for AIS patients after intravenous rt-PA thrombolysis. Further investigation is needed to confirm these results and investigate the efficacy of RIC in this patient population.

Acknowledgments

Conflict of Interest

Dr. Xunming Ji is one of the inventors of the electric autocontrol device that has been patented in China (ZL201420846209.5, China). The other authors declare that they have no conflict of interest.

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