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Chronic remote ischemic conditioning in patients with symptomatic intracranial arterial stenosis (RICA): a multi-center, randomized, double-blind, sham-controlled, device trial

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Abstract:	Background Intracranial atherosclerotic stenosis (ICAS) is one of the most common causes of stroke worldwide and is associated with a high risk of recurrent stroke on currently recommended treatments. We aimed to evaluate the effect of chronic remote ischemic conditioning (RIC) on preventing ischemic events in patients with symptomatic ICAS. Methods This multicenter, randomized, double-blind, sham-controlled trial was conducted at 84 sites in China. Patients aged 40-80 years with ischemic stroke or transient ischemic attack (TIA) attributable to angiographically verified 50-99% stenosis of a major intracranial artery were randomly assigned (1:1), via an interactive web-based system by computer-generated randomization code, to either RIC or sham RIC once daily for 12 months and voluntarily thereafter. All investigators and patients were masked to treatment allocation. The primary outcome was the occurrence of an ischemic stroke. Analyses were done on the intention-to-treat population and the per-protocol population who were at least 50% compliant with RIC during the first 12 months of follow-up. This study is registered with ClinicalTrials.gov, number NCT02534545. Findings Between Oct 28, 2015, and Feb 28, 2019, 3033 patients were enrolled and randomly assigned to receive RIC (n=1517) or sham RIC (n=1516). Median follow-up was 3.5 years (IQR 2.7-4.4). In the intention-to-treat analysis, the primary outcome occurred in 257 (16.9%) patients in RIC group compared with 288 (19.0%) patients in sham RIC group, with no significant difference between the two groups (hazard ratio [HR] 0.87, 95% CI 0.74-1.03; p=0.12). In the per-protocol analysis, there was a significant reduction in primary outcome with RIC compared to sham RIC (103 [14.7%] of 703 patients vs. 132 [18.7%] of 706 patients; HR 0.76, 95% CI 0.59-0.99, p=0.038). No RIC-related serious adverse events were observed. Interpretation In the entire cohort, RIC did not lower the risk of ischemic stroke in patients with symptomatic ICAS. However, in patients who were at least 50% compliant with the treatment, RIC safely reduced the occurrence of ischemic stroke.

Title Page

Title: Chronic remote ischemic conditioning in patients with symptomatic intracranial arterial stenosis (RICA): a multi-center, randomized, double-blind, sham-controlled, device trial

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Summary

Background Intracranial atherosclerotic stenosis (ICAS) is one of the most common causes of stroke worldwide and is associated with a high risk of recurrent stroke on currently recommended treatments. We aimed to evaluate the effect of chronic remote ischemic conditioning (RIC) on preventing ischemic events in patients with symptomatic ICAS.

Methods This multicenter, randomized, double-blind, sham-controlled trial was conducted at 84 sites in China. Patients aged 40-80 years with ischemic stroke or transient ischemic attack (TIA) attributable to angiographically verified 50-99% stenosis of a major intracranial artery were randomly assigned (1:1), via an interactive web-based system by computer-generated randomization code, to either RIC or sham RIC once daily for 12 months and voluntarily thereafter. All investigators and patients were masked to treatment allocation. The primary outcome was the occurrence of an ischemic stroke. Analyses were done on the intention-to-treat population and the per-protocol population who were at least 50% compliant with RIC during the first 12 months of follow-up. This study is registered with ClinicalTrials.gov, number NCT02534545.

Findings Between Oct 28, 2015, and Feb 28, 2019, 3033 patients were enrolled and randomly assigned to receive RIC (n=1517) or sham RIC (n=1516). Median follow-up was 3.5 years (IQR 2.7-4.4). In the intention-to-treat analysis, the primary outcome occurred in 257 (16.9%) patients in RIC group compared with 288 (19.0%) patients in sham RIC group, with no significant difference between the two groups (hazard ratio [HR] 0.87, 95% CI 0.74-1.03; p=0.12). In the per-protocol analysis, there was a significant reduction in primary outcome with RIC compared to sham RIC (103 [14.7%] of 703 patients *vs.* 132 [18.7%] of 706 patients; HR 0.76, 95% CI 0.59-0.99, p=0.038). No RIC-related serious adverse events were observed.

Interpretation In the entire cohort, RIC did not lower the risk of ischemic stroke in patients with symptomatic ICAS. However, in patients who were at least 50% compliant with the treatment, RIC safely reduced the occurrence of ischemic stroke.

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Introduction

Stroke is the second leading cause of death and the third leading cause of death and disability combined globally,[1] and the disease burden is especially great in China.[2] Intracranial atherosclerotic stenosis (ICAS) is one of the most common causes of stroke worldwide and is associated with a substantial risk of recurrent stroke.[3] Effective treatments for ICAS are limited. Extracranial to intracranial bypass surgery and arterial stenting have both been associated with worse outcomes compared with aggressive medical treatment.[4-6] Even with aggressive medical treatment consisting of dual antiplatelet treatment and intensive management of vascular risk factor, patients are still at high risk of recurrent stroke.[7] Therefore, additional treatment is needed to reduce the recurrence of stroke in ICAS.

Remote ischemic conditioning (RIC) is a promising therapy that has been recommended for further investigation in subjects with ICAS.[8] RIC is an intervention producing repetitive transient ischemia of limbs by inflating blood pressure cuffs with the intention of protecting remote organs like the brain or heart from subsequent ischemic injury.[9,10] A small single-center randomized trial including 68 Chinese patients aged <80 years with ICAS who had an ischemic stroke or transient ischemic attack (TIA) within the previous 30 days showed that five cycles of RIC of both upper limbs for 300 days reduced the risk of recurrent stroke in the per-protocol analysis.[11] Another single-center clinical trial with 58 patients showed that RIC intervention for 180 days reduced stroke recurrence and ameliorated plasma biomarkers of inflammation in patients aged 80-95 years with symptomatic ICAS.[12] These two trials were single-center studies with small sample sizes that did not permit a definite conclusion regarding the effect of RIC on ICAS.

In the current multi-center, large-scale trial, we aimed to evaluate the efficacy of chronic RIC for preventing ischemic events in a broader range of patients with ischemic stroke or TIA attributable to stenosis of a major intracranial artery.

Methods

Study design and participants

RICA was an investigator-initiated, multi-center, randomized, double-blind, sham-controlled, parallel-group trial conducted at 84 clinical centers in China (appendix p 2-8). All sites participating in this study were comprehensive stroke centers certified by the China Stroke Prevention Project Committee affiliated with the National Health Commission of China (www.cnstroke.com). The trial protocol and the statistical analysis plan are provided in the appendix. Briefly, patients were eligible if aged 40-80 years, presented with ischemic stroke that occurred within 30 days or TIA within 15 days before randomization, and the stroke or TIA was attributable to 50 to 99 percent stenosis of a major intracranial artery (carotid, middle cerebral, vertebral, or basilar) verified by computed tomography angiography or magnetic

resonance angiography.[13] The site investigators were responsible for attributing the qualifying TIA or ischemic stroke to the stenotic artery as done in previous symptomatic intracranial stenosis trials.[5,7] Key exclusion criteria included cerebral venous thrombosis/stenosis, any cardiac source of embolism, extracranial carotid artery stenosis ≥50%, subclavian arterial stenosis ≥50% or subclavian steal syndrome, intracranial neoplasm, cerebral aneurysm or arteriovenous malformation, and a contraindication to remote ischemic conditioning therapy including severe soft tissue injury, fracture, or peripheral vascular disease in the upper limbs. The complete list of inclusion and exclusion criteria is provided in the appendix (p 10-11).

The study complied with the provisions of the Declaration of Helsinki and was approved by the ethics committee at each participating center. All patients or their legally authorized representatives provided written informed consent before enrollment. An independent data monitoring committee oversaw the study data.

Randomization and masking

Eligible patients were randomly assigned (1:1) to either the RIC group or the sham RIC group via an interactive web-based response system at Air Force Medical University (Shaanxi, China). Randomization was done with constant block size of four allocated to each center, stratified by qualifying event (ischemic stroke/TIA), to ensure balance between treatment groups. The randomization sequence was computer generated by an independent statistician who was not involved in the trial. After a patient's eligibility was determined, the investigator accessed the randomization interface to complete a randomization form, and then the web interface displayed a randomization code. The randomization code was used to link to the device (i.e., RIC device or sham RIC device) that was dispensed to the patient. For blinding purposes, RIC devices and sham RIC devices had identical appearance, and the patients could feel pressure on their arms when they used the devices whether the inflation pressure was 200mmHg or 60mmHg. All patients, investigators, and central study staff involved in the trial were masked to treatment assignment. After the end of the study, patients were contacted again to ask their knowledge of group allocation by answering the question: did you know which intervention you had received? If the answer was yes, the following question was asked: which intervention did you think you had received?

Procedures

Study patients were randomized to the RIC group or the sham RIC group. In both groups, the blood pressure cuffs of the automated device (appendix, p 12) were placed on a patient's bilateral upper arms to deliver the intervention which comprised of five programmed cycles of five-minute inflation of the cuffs followed by five-minute deflation.[11] Thus, one treatment cycle lasted for 45 minutes. In the RIC group the inflation pressure was 200mmHg whereas it was

60mmHg in the sham RIC group. The protocol required that both interventions were performed by patients once per day for the first 12 months after randomization because the risk of stroke from ICAS is highest during this period and then plateaus.[3,5,7] After each performance of the intervention, the signal was uploaded automatically by the RIC device to the central database. The calculation method for the compliance rate in the first 12 months is presented in the appendix (p 13). Use of the study interventions after 12 months was voluntary. Patients received other stroke preventive therapy as considered appropriate by the investigator at each participating site, including antiplatelet therapy, blood pressure management, lipid management, blood glucose management, following Chinese guidelines for secondary prevention of ischemic stroke and transient ischemic attack.[14]

Clinic visits were scheduled at months 1, 3, 6,12, and the end of the study, and these visits were supplemented by monthly follow-up telephone calls to the patient until the end of the study (appendix p 14). At each follow-up visit, any outcome events and adverse events were evaluated and recorded and counselling by the study team was conducted to improve compliance with use of the study device.

Outcomes

The primary outcome was the time to first occurrence of ischemic stroke. The pre-specified secondary outcomes included a composite of the time to first occurrence of any stroke (ischemic or hemorrhagic), TIA, or myocardial infarction; each component of the composite outcome; and the time to occurrence of all-cause death. Criteria for defining these outcome events are listed in the appendix (p 15). Patients were assessed until their last study visit, withdrawal from the study, lost to follow-up, or death. When an investigator suspected the occurrence of any outcome event or a patient reported a possible outcome event, the investigator evaluated the patient in person. If a stroke was suspected, the patient underwent neuroimaging (CT or MRI). All available source documentation including clinical records, results of neuroimaging, hospital discharge summary, and death certificate (if applicable) were provided to the event adjudication committee to determine whether the outcome event met the criteria for an endpoint. The final decision was made by the event adjudication committee, who were masked to treatment allocation. Prespecified adverse events of special interest during RIC or sham RIC based on our experience from previous single center studies included skin petechiae caused by cuff inflation, dizziness, and nausea.

Statistical analysis

We determined that the occurrence of 597 primary outcome events would provide the trial with 90% statistical power to detect a 22% lower relative risk in the RIC group than in the sham RIC group. We estimated that the target number of events would be obtained by enrolling

approximately 3000 patients over a period of 36 months with a minimum follow-up of 12 months, on the basis of an estimated rate of the primary outcome of 14% per year in the sham RIC group,[7] and accounting for 10% loss to follow-up and 5% noncompliance. Owing to the lower-than-expected primary outcome event rate and slower-than-expected patient recruitment, the study period was extended in order to achieve as close to the target number of events. Follow-up ended on Feb 28, 2021 and lasted approximately 64 months during which 545 primary outcome events occurred. This provided approximately 87% power for the primary outcome. Initially, one interim analysis of efficacy was planned with a stopping boundary corresponding to a two-sided alpha level of 0.003, and the final analysis was tested at a two-sided alpha level of 0.047 with the overall type I error preserved at 0.05. About a year after the start of the study, the planned interim analysis was cancelled in order to set a larger significance level (0.05 for two-sided alpha level) in the final analysis.

Efficacy analyses were done first on the intention-to-treat population that included all patients randomly assigned to treatment groups regardless of study treatment compliance, then on a per-protocol population that was prespecified as patients who were at least 50% compliant with the study intervention during the first 12 months of follow-up. Cumulative event rates were estimated with the Kaplan-Meier method and compared with stratified log-rank tests according to qualifying event (ischemic stroke/TIA). Hazard ratios (HRs) with 95% CIs were estimated with the Cox proportional-hazards model with stratification.[15] Event-free patients were censored at the time of study termination, withdrawal of consent, or lost to follow-up whichever occurred first. The proportional hazard assumptions were graphically inspected in the log-cumulative hazard plot and were also confirmed with the Schoenfeld residual test, which did not detect any significant violation. For the intention-to-treat analysis, an unadjusted Cox model was constructed with treatment group as the only explanatory variable. For the per-protocol analysis, an unadjusted Cox model and a second model adjusted for age, sex, body-mass index, time from qualifying event to randomization, previous ischemic stroke, previous TIA, previous myocardial infarction, systolic blood pressure, low-density lipoprotein cholesterol, fasting blood glucose, smoking status, symptomatic qualifying artery, and stenotic degree of qualifying artery were constructed to calculate the HRs. The effect of the intervention on the primary and secondary composite efficacy outcomes was tested in prespecified subgroups based on baseline characteristics (age, sex, qualifying event, previous ischemic stroke, previous TIA, previous myocardial infarction, hypertension, hyperlipidemia, diabetes mellitus, smoking status, and stenotic degree of qualifying artery) by including the subgroup and interaction term in the Cox model. Safety analyses were done on all patients with at least one exposure to study intervention. The proportions of patients with adverse events in both treatment groups were compared using Fisher's Exact test. The effectiveness of blinding (post hoc analysis) was assessed by the distribution of patients who thought they received RIC, thought they received

sham RIC, or did not know, using χ^2 test and Bang's blinding index.[16]

All tests were done at a two-sided 0.05 significance level. No adjustments were made for multiple comparisons. Statistical tests were done using R programming language version 4.0.3. This trial is registered with ClinicalTrials.gov, number NCT02534545.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. XJ, CH, JL, and YL had full access to all the data in the study, and XJ had final responsibility for the decision to submit for publication.

Results

Between Oct 28, 2015, and Feb 28, 2019, 5721 patients were screened at 84 sites in China. Of these, 3033 patients were randomly assigned to RIC group (n=1517) or sham RIC group (n=1516). 2688 patients were excluded, of whom the majority (n=1962 [73.0%]) did not meet eligibility criteria (figure 1). The median follow-up was 3.5 years (IQR 2.7-4.4), and 2919 (96.2%) patients completed follow-up (79 patients were lost to follow-up and 35 withdrew consent) (figure 1). In the study cohort, 46.5% of patients were at least 50% compliant with the study intervention in the first 12 months and the compliance was similar between the two groups (appendix figure S1).

Baseline demographic and clinical characteristics of the patients were balanced between the intervention groups (table 1) in the intention-to-treat population. The mean age was 61.1 years (SD 9.1), and 1079 patients (35.6%) were female. The most prevalent risk factors were hypertension (83.2%), hyperlipidemia (66.9%), and current or previous smoking (62.4%). The median time from the onset of the qualifying event to randomization was 9 days (IQR 6-11) for TIA and 12 days (IQR 8-16) for ischemic stroke. The qualifying event was an ischemic stroke in 2446 patients (80.6%). The baseline characteristics of the per-protocol population are shown in the appendix (table S1).

In the intention-to-treat analyses, the primary outcome occurred in 257 (16.9%) of 1517 patients in the RIC group and 288 (19.0%) of 1516 patients in the sham RIC group, with no significant difference between the two groups (HR 0.87, 95% CI 0.74-1.03, p=0.12; table 2, figure 2A). The incidence of the secondary composite outcome was significantly lower in patients assigned to RIC intervention than in those assigned to sham RIC intervention (318 [21.0%] *vs.* 376 [24.8%]; HR 0.82, 95% CI 0.71-0.95, p=0.0089; table 2, figure 2B), but there was no significant difference between the two groups in the individual components of the secondary composite outcome. All-cause death did not differ significantly between RIC and sham RIC group (79

[5.2%] *vs.* 84 [5.5%]; HR 0.93, 95% CI 0.68-1.27, p=0.65; table 2). In the per-protocol analyses, there was a significant reduction of the primary outcome with RIC intervention compared to sham RIC intervention (103 [14.7%] of 703 patients *vs.* 132 [18.7%] of 706 patients; HR 0.76, 95% CI 0.59-0.99, p=0.038; adjusted HR 0.76, 95% CI 0.59-0.98, p=0.037; table 2, and appendix table S2 and figure S2A). The statistical power was estimated based on the actual hazard ratio and sample size in the per-protocol analysis, which provided power of 72% for the primary outcome.

The treatment effects of RIC compared with sham RIC on the primary outcome and the secondary composite outcome were consistent across all prespecified subgroups in both the intention-to-treat population and per-protocol population, with no significant interaction ($P_{interaction}$>0.10 for all comparisons; figure 3, and appendix figure S3, figure S4, and figure S5).

More patients in the RIC group than in the sham RIC group had skin petechiae (104 [6.9%] of 1515 patients *vs.* 1 [0.1%] of 1514 patients; P<0.0001; table 3), and dizziness or nausea (19 [1.3%] *vs.* 2 [0.1%]; P=0.0002; table 3) at the time of the intervention; there were no long-term consequences in either group. The proportion of patients with serious adverse events was similar in both intervention groups (327 [21.6%] in RIC group *vs.* 312 [20.6%] in sham RIC group; appendix table S3). There were no serious adverse events reported related to use of the device.

A post-hoc analysis was performed to evaluate the effectiveness of blinding in 2524 patients (1280 in the RIC group and 1244 in the sham RIC group). Of the patients in the RIC group, 259 (20.2%) believed they received active RIC and 196 (15.3%) believed they received sham RIC. Of the patients in the sham RIC group, 240 (19.3%) believed they received active RIC and 231 (18.6%) believed they received sham RIC. The remaining patients in both groups responded that they did not know. There was no significant difference in the distribution of the response between the two groups (P=0.092; appendix table S4). The Bang's blinding index demonstrated good effectiveness of blinding as well (RIC group score 0.049, 95% CI 0.017-0.082; sham RIC group score -0.007, 95% CI -0.041-0.027; appendix table S4).

Discussion

In this trial of 3033 patients with ischemic stroke or TIA attributable to stenosis of a major intracranial artery, chronic RIC did not significantly reduce ischemic stroke in the intention-to-treat population. However, a prespecified per-protocol analysis was done in patients performing the intervention on at least 50% of the potential treatment days in the first 12 months, and the result showed that chronic RIC significantly reduced the occurrence of ischemic stroke (p=0.038). Given the borderline statistical significance in the per-protocol analysis, we

estimated the statistical power based on the actual hazard ratio and sample size in the per-protocol analysis. This showed power of 72% for the primary outcome which provides support that the positive result in the per-protocol analysis is likely reliable. Additionally, this result is consistent with our previous single-center clinical trial that repetitive RIC may be an effective way to reduce recurrent stroke in patients with symptomatic ICAS if patients are compliant with the therapy.[11] RIC also significantly reduced the secondary composite outcome of stroke (ischemic or hemorrhagic), TIA, or myocardial infarction in both the intention-to-treat and per-protocol populations.

There are three main hypothesized mechanisms of stroke related to ICAS: hemodynamic compromise, artery-to-artery embolism, and branch occlusive disease.[3] There are several means by which RIC may lower the risk of stroke in ICAS subjects. In preclinical studies of chronic cerebral hypoperfusion, RIC once a day for 28 days or 4 months significantly improved cerebral blood flow and increased angiogenesis, arteriogenesis, capillary density, capillary diameter, and collateral formation.[17,18] In a previous clinical trial in ICAS, RIC for 300 days increased cerebral perfusion measured by SPECT imaging and improved collateral flow measured by transcranial Doppler sonography.[11] In patients with cerebral small vessel disease, RIC for one year improved cerebral perfusion and alleviated the resistance of distal vessels.[19,20] Based on these reports, long-term RIC could possibly improve cerebral blood flow in ICAS subjects with hemodynamic compromise via collateral remodeling, thereby lowering their risk of stroke.

In addition to improving hemodynamic compromise, chronic RIC might reduce the occurrence of artery-to-artery embolism. In patients with severe carotid artery stenosis, RIC for 2 weeks before carotid artery stenting lowered the incidence of new embolic infarctions.[21] Cerebrovascular embolic events were closely related to the instability of atherosclerotic plaque structure, which was shown to correlate with upregulation of several plasma markers such as high sensitivity C reactive protein (hs-CRP) and interleukin-6 (IL-6).[22] Previous clinical trials showed RIC for several months decreased plasma hs-CRP and IL-6,[12,23] suggesting RIC could possibly stabilize the structure of atherosclerotic plaque to reduce the occurrence of artery-to-artery embolism. Chronic RIC might also decrease the progression of branch occlusive disease. In a preclinical experiment, RIC for 12 months prevented atherosclerosis progression by reducing the percentage of plaque area in the aorta of hypercholesterolemic rabbits.[24] In patients with cerebral small vessel disease, RIC for 12 months significantly lowered plasma triglycerides, total cholesterol, and low-density lipoprotein,[20] which were related to the progression of atherosclerosis.[25] These results provided evidence that RIC might slow the progression of atherosclerosis to reduce the occurrence of ischemic stroke.

Although previous multi-center trials incorporating one-time use of RIC for neuroprotection or cardioprotection after acute stroke or myocardial infarction, or graft protection after renal transplant did not show a benefit of RIC,[26-30] this may be because the protection afforded by one-time RIC is limited. The mechanisms by which RIC might prevent stroke in patients with ICAS described above (i.e., improved collaterals, stabilization of plaque, slowing the progression of atherosclerosis) likely requires repeated RIC over a longer period. This is supported by the neutral results of our intention-to-treat analysis but positive results in per-protocol analysis, i.e., poor compliance was not associated with a reduction in ischemic stroke whereas good compliance was. The suggested effectiveness of repeated RIC observed in our trial may provide a rationale for cardio-protection trials in subjects with coronary disease and renal graft protection trials in subjects following renal transplant using repeated RIC over a longer period rather than one-time use of RIC.

This trial has many strengths including the large sample size, long follow-up, low drop-out rate, standardized device, automated upload of adherence data, and effective blinding. Nevertheless, there were some important limitations. First, compliance with RIC was much lower than expected largely because the device was considered inconvenient to use by many patients who had to sit quietly for 45 minutes with both upper arms confined by the device and who had to transport the device if patients were away from home. Further studies are needed to determine whether less frequent and shorter cycles of RIC are effective for the treatment of ICAS. Additionally, ongoing improvements in the design of the device to make it more practical to use (e.g., making a wearable device - see appendix p 12) will help improve portability and compliance. When better compliance is achieved, further study should be conducted to provide more definitive evidence to support RIC treatment for ICAS. Second, we did not collect data on control of some risk factors (e.g., lipid and hemoglobin A1c levels) during follow-up so we cannot be certain that the differences in outcomes between the two arms was solely due to RIC and not to differences in other secondary prevention treatment in the trial. However, given the large sample size in this randomized trial with effective blinding it highly unlikely that there would have been significant differences in the other secondary prevention treatments in the two arms of the trial. Third, fewer women were enrolled in this study than men in large part because stroke is much more prevalent in men,[31] especially in ICAS related stroke.[7] The low proportion of women in our trial may also represent a bias against women in recruitment, and we will recruit more women in our future stroke trials. Importantly, there was no interaction between sex and outcome in this trial. Finally, there might be concerns about the generalizability of the trial results because all patients were Chinese. Confirmation in other populations is required.

In conclusion, although chronic RIC treatment did not lower the risk of ischemic stroke in patients with symptomatic ICAS in the entire cohort, it reduced the occurrence of ischemic

stroke in patients who were compliant with the therapy. Also, RIC lowered the risk of combined cerebro- and cardio-vascular events in all patients and was associated with only minor adverse events. Further studies are needed to confirm these findings in non-Chinese populations.

Research in context

Evidence before this study

Intracranial atherosclerotic stenosis (ICAS) is one of the most common causes of stroke worldwide and is associated with a high risk of recurrent stroke on currently recommended treatments. Remote ischemic conditioning (RIC) is a promising therapy for secondary stroke prevention that warrants further investigation in large randomized trials. We searched PubMed, using the search terms “(intracranial arterial stenosis or intracranial atherosclerotic stenosis)” and “(conditioning or preconditioning or perconditioning or postconditioning)”, for studies published up to February 12, 2022, without language restrictions. 18 articles were retrieved, and 2 clinical studies were identified suggesting that RIC may safely reduce recurrent stroke in patients with symptomatic ICAS. However, the sample sizes in these two single-center trials were too small to draw any definite conclusions regarding the efficacy of RIC for preventing stroke in patients with ICAS.

Added value of this study

This RICA study is, to the best of our knowledge, the first multi-center, large-scale, randomized controlled trial to investigate the effect of RIC on clinical outcomes in patients with a recent cerebral ischemic event attributable to ICAS. The rigorous nature of this trial (large sample size, long follow-up, low drop-out rate, standardized device, automated upload of adherence data, and effective blinding) provides unique data on the potential role of RIC for secondary prevention of stroke in patients with symptomatic ICAS but also identifies challenges in patient compliance with the treatment that must be overcome to make RIC a practical and effective treatment for this disease.

Implications of all the available evidence

The results of this trial suggest that chronic RIC might be an efficacious therapy for patients with symptomatic ICAS in compliant patients. If confirmed in other trials of non-Chinese patients, together these findings will provide high level evidence to support using RIC for stroke prevention in patients with ICAS. Additionally, since the pathophysiology and treatment of ICAS are similar to coronary artery disease and atherosclerosis in other vascular beds (peripheral vascular, renal), the results of this trial also provide a strong rationale for clinical trials in patients with those diseases in which patients are treated with repeated RIC over a longer period.

Table 1: Baseline characteristics in the intention-to-treat population

	RIC group (n=1517)	Sham RIC group (n=1516)
Age, years	61.1 (9.1)	61.0 (9.1)
Sex		
Female	536 (35.3%)	543 (35.8%)
Male	981 (64.7%)	973 (64.2%)
Body-mass index, kg/m²	25.0 (3.1)	25.1 (3.1)
Qualifying event		
TIA	296 (19.5%)	291 (19.2%)
Ischemic stroke	1221 (80.5%)	1225 (80.8%)
Time from qualifying event to randomization, days		
TIA	9 (6-11)	9 (6-11)
Ischemic stroke	12 (8-16)	12 (8-16)
Neurologic score		
ABCD² for TIA*	4 (4-5)	4 (4-5)
mRS for ischemic stroke†	1 (1-2)	1 (1-2)
Comorbidities (Medical history and risk factors)		
Previous ischemic stroke	369 (24.3%)	360 (23.7%)
Previous TIA	172 (11.3%)	164 (10.8%)
Previous myocardial infarction	129 (8.5%)	127 (8.4%)
Hypertension	1254 (82.7%)	1268 (83.6%)
Hyperlipidemia	1000 (65.9%)	1030 (67.9%)
Diabetes mellitus	545 (35.9%)	553 (36.5%)
Current or previous smoking	954 (62.9%)	939 (61.9%)
Symptomatic qualifying artery		
Internal carotid	210 (13.8%)	207 (13.7%)
Middle cerebral	768 (50.6%)	774 (51.1%)
Basilar	266 (17.5%)	281 (18.5%)
Vertebral	205 (13.5%)	191 (12.6%)
Multiple arteries‡	68 (4.5%)	63 (4.2%)
Stenosis of qualifying artery ≥70%§	597 (39.4%)	605 (39.9%)
Fasting blood glucose, mmol/l	6.9 (2.8)	6.8 (2.8)
Blood pressure		
Systolic, mmHg	141.4 (16.7)	142.0 (16.4)
Diastolic, mmHg	84.8 (11.6)	85.2 (11.0)
Lipids		
LDL cholesterol, mg/dl	113.3 (32.9)	113.4 (32.5)
HDL cholesterol, mg/dl	43.5 (11.3)	43.7 (11.2)
Total cholesterol, mg/dl	184.1 (37.8)	183.8 (38.6)

Data are n (%), mean (SD), or median (IQR). RIC=remote ischemic conditioning. TIA=transient ischemic attack. mRS=modified Rankin scale. LDL=low-density lipoprotein. HDL=high-density lipoprotein. * Scores on the ABCD² scale range from 0 to 7, with higher scores indicating a

greater risk of stroke. † Scores on modified Rankin scale (mRS) range from 0 to 6, with higher scores indicating a greater stroke severity. ‡ The affected arteries were a combination of the internal carotid and middle cerebral arteries, the vertebral and basilar arteries, or the left and right vertebral arteries. § Stenosis was quantified on the basis of a reading of the angiogram by the site investigators.

Table 2: Efficacy outcomes in the intention-to-treat and per-protocol populations

	Intention-to-treat population				Per-protocol population			
	RIC group (n=1517)	Sham RIC group (n=1516)	HR (95% CI)	p value	RIC group (n=703)	Sham RIC group (n=706)	HR (95% CI)	p value
Primary outcome								
Ischemic stroke	257 (16.9%)	288 (19.0%)	0.87 (0.74-1.03)	0.12	103 (14.7%)	132 (18.7%)	0.76 (0.59-0.99)	0.038
Non-fatal	247 (16.3%)	277 (18.3%)			99 (14.1%)	127 (18.0%)		
Fatal	10 (0.7%)	11 (0.7%)			4 (0.6%)	5 (0.7%)		
Secondary outcomes								
Composite of stroke (ischemic or hemorrhagic), TIA, or myocardial infarction	318 (21.0%)	376 (24.8%)	0.82 (0.71-0.95)	0.0089	124 (17.6%)	170 (24.1%)	0.70 (0.56-0.88)	0.0026
Stroke (ischemic or hemorrhagic)	262 (17.3%)	294 (19.4%)	0.87 (0.74-1.03)	0.11	105 (14.9%)	134 (19.0%)	0.77 (0.59-0.99)	0.040
Non-fatal	251 (16.5%)	281 (18.5%)			101 (14.4%)	128 (18.1%)		
Fatal	11 (0.7%)	13 (0.9%)			4 (0.6%)	6 (0.8%)		
TIA	41 (2.7%)	53 (3.5%)	0.77 (0.51-1.16)	0.20	14 (2.0%)	21 (3.0%)	0.67 (0.34-1.31)	0.24
Myocardial infarction	43 (2.8%)	53 (3.5%)	0.80 (0.54-1.20)	0.28	17 (2.4%)	23 (3.3%)	0.73 (0.39-1.37)	0.33
Non-fatal	39 (2.6%)	47 (3.1%)			15 (2.1%)	21 (3.0%)		
Fatal	4 (0.3%)	6 (0.4%)			2 (0.3%)	2 (0.3%)		
All-cause death	79 (5.2%)	84 (5.5%)	0.93 (0.68-1.27)	0.65	33 (4.7%)	36 (5.1%)	0.92 (0.57-1.47)	0.72

Data are number of first events (%). RIC=remote ischemic conditioning.HR=hazard ratio. TIA=transient ischemic attack.

Table 3: Adverse events of special interest during RIC or sham RIC intervention

	RIC group (n=1515)	Sham RIC group (n=1514)	P value
Total	123 (8.1%)	3 (0.2%)	< 0.0001
Skin petechiae caused by cuff inflation	104 (6.9%)	1 (0.1%)	< 0.0001
Dizziness, or nausea	19 (1.3%)	2 (0.1%)	0.0002

Data are number (%) of patients, in those with at least one exposure to study intervention. RIC=remote ischemic conditioning.

Figure legends

Figure 1: Trial profile

RIC=remote ischemic conditioning. ITT=intention-to-treat.

Figure 2: Kaplan-Meier event curve for the primary outcome and the secondary composite outcome in the intention-to-treat population

(A) Primary outcome (ischemic stroke). (B) Secondary composite outcome (stroke, transient ischemic attack, or myocardial infarction). RIC=remote ischemic conditioning. HR=hazard ratio.

Figure 3: Prespecified subgroup analyses of the primary outcome in the intention-to-treat population

$p_{interaction}$ represents the likelihood of interaction between the subgroup variable and the intervention strategy. RIC=remote ischemic conditioning. TIA=transient ischemic attack. HR=hazard ratio.

Contributors

XJ, MIC, DCH, DSL, CH, JL, and RM conceived and designed the study. HS, YW, JHuang, ZL, XH, JY, JZ, PC, XZ, PH, HP, WC, HC, GL, DT, WY, and ZG participated in data collection. CH and YL did the statistical analyses. XJ, CH, JL, HS, WZ, SL, JHao, RM, YD, MIC, and HC interpreted the data. CH and JL wrote the first draft. XJ, JHao, HS, MIC, MF, DCH, DSL, and DJH revised the manuscript. All authors approved the final version of the manuscript.

Declaration of interests

CH has received speaker fees from Novo Nordisk and Pfizer. HS has received speaker fees from AstraZeneca, Pzier, Amgen, Boehringer Ingelheim, Novartis, and Sanofi. WZ has received speaker fees from Tasly Pharma. RM has received grants from Ministry of Science and Technology of the People's Republic of China. MIC has received grants from NIH (CAPTIVA, U01 NS117450-01A1). MIC has received honoraria for lectures on Intracranial Stenosis at Taiwan Neurological Conference and grand rounds at other universities. DSL has served as CEC member. DJH has received consultant fees from Faraday Pharmaceuticals Inc. and Boehringer Ingelheim International GmbH; honoraria from Servier; and research funding from Astra Zeneca and Merck Sharp & Dohme Corp. XZ has received speaker fees from AstraZeneca and Bayer. GL has received speaker fees from Techpool, CSPC, and Boehringer Ingelheim. WY has participated in speakers bureaus and received speaker fees from Bayer, Sanofi, Novartis, Xian Janssen and Amgen. XJ has received grants from Beijing Municipal Education Commission and Beijing Municipal Finance Bureau. XJ has received speaker fees from AstraZeneca, Medtronic, and Sanofi. The remaining authors declare no competing interests.

Data sharing

RICA study is an investigator-initiated trial. Internal investigators who actively participated in the study and provide a methodologically sound study proposal will be granted priority access to the study data for a period of 48 months. After 48 months, individual, deidentified participant data underlying the findings described in this manuscript will be available by request to any external investigator following approval of a proposal by the steering committee. Study proposals can be filed at rica_group@ccmu.edu.cn.

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Figure 1

Figure 2

Figure 3

Title Page

Title: Chronic remote ischemic conditioning in patients with symptomatic intracranial arterial stenosis (RICA): a multi-center, randomized, double-blind, sham-controlled, device trial

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Summary

Background Intracranial atherosclerotic stenosis (ICAS) is one of the most common causes of stroke worldwide and is associated with a high risk of recurrent stroke on currently recommended treatments. We aimed to evaluate the effect of chronic remote ischemic conditioning (RIC) on preventing ischemic events in patients with symptomatic ICAS.

Methods This multicenter, randomized, double-blind, sham-controlled trial was conducted at 84 sites in China. Patients aged 40-80 years with ischemic stroke or transient ischemic attack (TIA) attributable to angiographically verified 50-99% stenosis of a major intracranial artery were randomly assigned (1:1), via an interactive web-based system by computer-generated randomization code, to either RIC or sham RIC once daily for 12 months and voluntarily thereafter. All investigators and patients were masked to treatment allocation. The primary outcome was the occurrence of an ischemic stroke. Analyses were done on the intention-to-treat population and the per-protocol population who were at least 50% compliant with RIC during the first 12 months of follow-up. This study is registered with ClinicalTrials.gov, number NCT02534545.

Findings Between Oct 28, 2015, and Feb 28, 2019, 3033 patients were enrolled and randomly assigned to receive RIC (n=1517) or sham RIC (n=1516). Median follow-up was 3.5 years (IQR 2.7-4.4). In the intention-to-treat analysis, the primary outcome occurred in 257 (16.9%) patients in RIC group compared with 288 (19.0%) patients in sham RIC group, with no significant difference between the two groups (hazard ratio [HR] 0.87, 95% CI 0.74-1.03; p=0.12). In the per-protocol analysis, there was a significant reduction in primary outcome with RIC compared to sham RIC (103 [14.7%] of 703 patients *vs.* 132 [18.7%] of 706 patients; HR 0.76, 95% CI 0.59-0.99, p=0.038). No RIC-related serious adverse events were observed.

Interpretation In the entire cohort, RIC did not lower the risk of ischemic stroke in patients with symptomatic ICAS. However, in patients who were at least 50% compliant with the treatment, RIC safely reduced the occurrence of ischemic stroke.

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Introduction

Stroke is the second leading cause of death and the third leading cause of death and disability combined globally,[1] and the disease burden is especially great in China.[2] Intracranial atherosclerotic stenosis (ICAS) is one of the most common causes of stroke worldwide and is associated with a substantial risk of recurrent stroke.[3] Effective treatments for ICAS are limited. Extracranial to intracranial bypass surgery and arterial stenting have both been associated with worse outcomes compared with aggressive medical treatment.[4-6] Even with aggressive medical treatment consisting of dual antiplatelet treatment and intensive management of vascular risk factor, patients are still at high risk of recurrent stroke.[7] Therefore, additional treatment is needed to reduce the recurrence of stroke in ICAS.

Remote ischemic conditioning (RIC) is a promising therapy that has been recommended for further investigation in subjects with ICAS.[8] RIC is an intervention producing repetitive transient ischemia of limbs by inflating blood pressure cuffs with the intention of protecting remote organs like the brain or heart from subsequent ischemic injury.[9,10] A small single-center randomized trial including 68 Chinese patients aged <80 years with ICAS who had an ischemic stroke or transient ischemic attack (TIA) within the previous 30 days showed that five cycles of RIC of both upper limbs for 300 days reduced the risk of recurrent stroke in the per-protocol analysis.[11] Another single-center clinical trial with 58 patients showed that RIC intervention for 180 days reduced stroke recurrence and ameliorated plasma biomarkers of inflammation in patients aged 80-95 years with symptomatic ICAS.[12] These two trials were single-center studies with small sample sizes that did not permit a definite conclusion regarding the effect of RIC on ICAS.

In the current multi-center, large-scale trial, we aimed to evaluate the efficacy of chronic RIC for preventing ischemic events in a broader range of patients with ischemic stroke or TIA attributable to stenosis of a major intracranial artery.

Methods

Study design and participants

RICA was an investigator-initiated, multi-center, randomized, double-blind, sham-controlled, parallel-group trial conducted at 84 clinical centers in China (appendix p 2-8). All sites participating in this study were comprehensive stroke centers certified by the China Stroke Prevention Project Committee affiliated with the National Health Commission of China (www.cnstroke.com). The trial protocol and the statistical analysis plan are provided in the appendix. Briefly, patients were eligible if aged 40-80 years, presented with ischemic stroke that occurred within 30 days or TIA within 15 days before randomization, and the stroke or TIA was attributable to 50 to 99 percent stenosis of a major intracranial artery (carotid, middle cerebral, vertebral, or basilar) verified by computed tomography angiography or magnetic

resonance angiography.[13] The site investigators were responsible for attributing the qualifying TIA or ischemic stroke to the stenotic artery as done in previous symptomatic intracranial stenosis trials.[5,7] Key exclusion criteria included cerebral venous thrombosis/stenosis, any cardiac source of embolism, extracranial carotid artery stenosis ≥50%, subclavian arterial stenosis ≥50% or subclavian steal syndrome, intracranial neoplasm, cerebral aneurysm or arteriovenous malformation, and a contraindication to remote ischemic conditioning therapy including severe soft tissue injury, fracture, or peripheral vascular disease in the upper limbs. The complete list of inclusion and exclusion criteria is provided in the appendix (p 10-11).

The study complied with the provisions of the Declaration of Helsinki and was approved by the ethics committee at each participating center. All patients or their legally authorized representatives provided written informed consent before enrollment. An independent data monitoring committee oversaw the study data.

Randomization and masking

Eligible patients were randomly assigned (1:1) to either the RIC group or the sham RIC group via an interactive web-based response system at Air Force Medical University (Shaanxi, China). Randomization was done with constant block size of four allocated to each center, stratified by qualifying event (ischemic stroke/TIA), to ensure balance between treatment groups. The randomization sequence was computer generated by an independent statistician who was not involved in the trial. After a patient's eligibility was determined, the investigator accessed the randomization interface to complete a randomization form, and then the web interface displayed a randomization code. The randomization code was used to link to the device (i.e., RIC device or sham RIC device) that was dispensed to the patient. For blinding purposes, RIC devices and sham RIC devices had identical appearance, and the patients could feel pressure on their arms when they used the devices whether the inflation pressure was 200mmHg or 60mmHg. All patients, investigators, and central study staff involved in the trial were masked to treatment assignment. After the end of the study, patients were contacted again to ask their knowledge of group allocation by answering the question: did you know which intervention you had received? If the answer was yes, the following question was asked: which intervention did you think you had received?

Procedures

Study patients were randomized to the RIC group or the sham RIC group. In both groups, the blood pressure cuffs of the automated device (appendix, p 12) were placed on a patient's bilateral upper arms to deliver the intervention which comprised of five programmed cycles of five-minute inflation of the cuffs followed by five-minute deflation.[11] Thus, one treatment cycle lasted for 45 minutes. In the RIC group the inflation pressure was 200mmHg whereas it was

60mmHg in the sham RIC group. The protocol required that both interventions were performed by patients once per day for the first 12 months after randomization because the risk of stroke from ICAS is highest during this period and then plateaus.[3,5,7] After each performance of the intervention, the signal was uploaded automatically by the RIC device to the central database. The calculation method for the compliance rate in the first 12 months is presented in the appendix (p 13). Use of the study interventions after 12 months was voluntary. Patients received other stroke preventive therapy as considered appropriate by the investigator at each participating site, including antiplatelet therapy, blood pressure management, lipid management, blood glucose management, following Chinese guidelines for secondary prevention of ischemic stroke and transient ischemic attack.[14]

Clinic visits were scheduled at months 1, 3, 6,12, and the end of the study, and these visits were supplemented by monthly follow-up telephone calls to the patient until the end of the study (appendix p 14). At each follow-up visit, any outcome events and adverse events were evaluated and recorded and counselling by the study team was conducted to improve compliance with use of the study device.

Outcomes

The primary outcome was the time to first occurrence of ischemic stroke. The pre-specified secondary outcomes included a composite of the time to first occurrence of any stroke (ischemic or hemorrhagic), TIA, or myocardial infarction; each component of the composite outcome; and the time to occurrence of all-cause death. Criteria for defining these outcome events are listed in the appendix (p 15). Patients were assessed until their last study visit, withdrawal from the study, lost to follow-up, or death. When an investigator suspected the occurrence of any outcome event or a patient reported a possible outcome event, the investigator evaluated the patient in person. If a stroke was suspected, the patient underwent neuroimaging (CT or MRI). All available source documentation including clinical records, results of neuroimaging, hospital discharge summary, and death certificate (if applicable) were provided to the event adjudication committee to determine whether the outcome event met the criteria for an endpoint. The final decision was made by the event adjudication committee, who were masked to treatment allocation. Prespecified adverse events of special interest during RIC or sham RIC based on our experience from previous single center studies included skin petechiae caused by cuff inflation, dizziness, and nausea.

Statistical analysis

We determined that the occurrence of 597 primary outcome events would provide the trial with 90% statistical power to detect a 22% lower relative risk in the RIC group than in the sham RIC group. We estimated that the target number of events would be obtained by enrolling

approximately 3000 patients over a period of 36 months with a minimum follow-up of 12 months, on the basis of an estimated rate of the primary outcome of 14% per year in the sham RIC group,[7] and accounting for 10% loss to follow-up and 5% noncompliance. Owing to the lower-than-expected primary outcome event rate and slower-than-expected patient recruitment, the study period was extended in order to achieve as close to the target number of events. Follow-up ended on Feb 28, 2021 and lasted approximately 64 months during which 545 primary outcome events occurred. This provided approximately 87% power for the primary outcome. Initially, one interim analysis of efficacy was planned with a stopping boundary corresponding to a two-sided alpha level of 0.003, and the final analysis was tested at a two-sided alpha level of 0.047 with the overall type I error preserved at 0.05. About a year after the start of the study, the planned interim analysis was cancelled in order to set a larger significance level (0.05 for two-sided alpha level) in the final analysis.

Efficacy analyses were done first on the intention-to-treat population that included all patients randomly assigned to treatment groups regardless of study treatment compliance, then on a per-protocol population that was prespecified as patients who were at least 50% compliant with the study intervention during the first 12 months of follow-up. Cumulative event rates were estimated with the Kaplan-Meier method and compared with stratified log-rank tests according to qualifying event (ischemic stroke/TIA). Hazard ratios (HRs) with 95% CIs were estimated with the Cox proportional-hazards model with stratification.[15] Event-free patients were censored at the time of study termination, withdrawal of consent, or lost to follow-up whichever occurred first. The proportional hazard assumptions were graphically inspected in the log-cumulative hazard plot and were also confirmed with the Schoenfeld residual test, which did not detect any significant violation. For the intention-to-treat analysis, an unadjusted Cox model was constructed with treatment group as the only explanatory variable. For the per-protocol analysis, an unadjusted Cox model and a second model adjusted for age, sex, body-mass index, time from qualifying event to randomization, previous ischemic stroke, previous TIA, previous myocardial infarction, systolic blood pressure, low-density lipoprotein cholesterol, fasting blood glucose, smoking status, symptomatic qualifying artery, and stenotic degree of qualifying artery were constructed to calculate the HRs. ~~Landmark analyses of the primary and secondary composite efficacy outcomes (post hoc analysis) were conducted separately for the periods from randomization to 12 months of follow-up during which the study intervention was required by the protocol and from 12 months to the end of the trial.~~ The effect of the intervention on the primary and secondary composite efficacy outcomes was tested in prespecified subgroups based on baseline characteristics (age, sex, qualifying event, previous ischemic stroke, previous TIA, previous myocardial infarction, hypertension, hyperlipidemia, diabetes mellitus, smoking status, and stenotic degree of qualifying artery) by including the subgroup and interaction term in the Cox model. Safety analyses were done on all patients with at least one

exposure to study intervention. The proportions of patients with adverse events in both treatment groups were compared using Fisher's Exact test. The effectiveness of blinding (post hoc analysis) was assessed by the distribution of patients who thought they received RIC, thought they received sham RIC, or did not know, using χ^2 test and Bang's blinding index.[16]

All tests were done at a two-sided 0.05 significance level. No adjustments were made for multiple comparisons. Statistical tests were done using R programming language version 4.0.3. This trial is registered with ClinicalTrials.gov, number NCT02534545.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. XJ, CH, JL, and YL had full access to all the data in the study, and XJ had final responsibility for the decision to submit for publication.

Results

Between Oct 28, 2015, and Feb 28, 2019, 5721 patients were screened at 84 sites in China. Of these, 3033 patients were randomly assigned to RIC group (n=1517) or sham RIC group (n=1516). 2688 patients were excluded, of whom the majority (n=1962 [73.0%]) did not meet eligibility criteria (figure 1). The median follow-up was 3.5 years (IQR 2.7-4.4), and 2919 (96.2%) patients completed follow-up (79 patients were lost to follow-up and 35 withdrew consent) (figure 1). In the study cohort, 46.5% of patients were at least 50% compliant with the study intervention in the first 12 months and the compliance was similar between the two groups (appendix figure S1).

Baseline demographic and clinical characteristics of the patients were balanced between the intervention groups (table 1) in the intention-to-treat population. The mean age was 61.1 years (SD 9.1), and 1079 patients (35.6%) were female. The most prevalent risk factors were hypertension (83.2%), hyperlipidemia (66.9%), and current or previous smoking (62.4%). The median time from the onset of the qualifying event to randomization was 9 days (IQR 6-11) for TIA and 12 days (IQR 8-16) for ischemic stroke. The qualifying event was an ischemic stroke in 2446 patients (80.6%). The baseline characteristics of the per-protocol population are shown in the appendix (table S1).

In the intention-to-treat analyses, the primary outcome occurred in 257 (16.9%) of 1517 patients in the RIC group and 288 (19.0%) of 1516 patients in the sham RIC group, with no significant difference between the two groups (HR 0.87, 95% CI 0.74-1.03, p=0.12; table 2, figure 2A). The incidence of the secondary composite outcome was significantly lower in patients assigned to RIC intervention than in those assigned to sham RIC intervention (318 [21.0%] *vs.* 376

[24.8%]; HR 0.82, 95% CI 0.71-0.95, p=0.0089; table 2, figure 2B), but there was no significant difference between the two groups in the individual components of the secondary composite outcome. All-cause death did not differ significantly between RIC and sham RIC group (79 [5.2%] *vs.* 84 [5.5%]; HR 0.93, 95% CI 0.68-1.27, p=0.65; table 2). In the per-protocol analyses, there was a significant reduction of the primary outcome with RIC intervention compared to sham RIC intervention (103 [14.7%] of 703 patients *vs.* 132 [18.7%] of 706 patients; HR 0.76, 95% CI 0.59-0.99, p=0.038; adjusted HR 0.76, 95% CI 0.59-0.98, p=0.037; table 2, and appendix table S2 and figure S2A). The statistical power was estimated based on the actual hazard ratio and sample size in the per-protocol analysis, which provided power of 72% for the primary outcome. ~~Post-hoc landmark analyses of the primary efficacy outcome in the per-protocol population revealed a statistical difference between groups within 12 months of follow-up (HR 0.70, 95% CI 0.51-0.98, p=0.037; appendix figure S4A), but no difference in the intention-to-treat population (appendix figure S3A). After 12 months, no significant difference was observed in either the intention-to-treat population or the per-protocol population. There was a significant decrease in the secondary composite outcome in the RIC group within 12 months in both the intention-to-treat and the per-protocol populations (appendix figure S3B and figure S4B).~~

The treatment effects of RIC compared with sham RIC on the primary outcome and the secondary composite outcome were consistent across all prespecified subgroups in both the intention-to-treat population and per-protocol population, with no significant interaction ($P_{interaction}$>0.10 for all comparisons; figure 3, and appendix figure S3~~5~~, figure S4~~6~~, and figure S5~~7~~).

More patients in the RIC group than in the sham RIC group had skin petechiae (104 [6.9%] of 1515 patients *vs.* 1 [0.1%] of 1514 patients; P<0.0001; table 3), and dizziness or nausea (19 [1.3%] *vs.* 2 [0.1%]; P=0.0002; table 3) at the time of the intervention; there were no long-term consequences in either group. The proportion of patients with serious adverse events was similar in both intervention groups (327 [21.6%] in RIC group *vs.* 312 [20.6%] in sham RIC group; appendix table S3). There were no serious adverse events reported related to use of the device.

A post-hoc analysis was performed to evaluate the effectiveness of blinding in 2524 patients (1280 in the RIC group and 1244 in the sham RIC group). Of the patients in the RIC group, 259 (20.2%) believed they received active RIC and 196 (15.3%) believed they received sham RIC. Of the patients in the sham RIC group, 240 (19.3%) believed they received active RIC and 231 (18.6%) believed they received sham RIC. The remaining patients in both groups responded that they did not know. There was no significant difference in the distribution of the response

between the two groups (P=0.092; appendix table S4). The Bang's blinding index demonstrated good effectiveness of blinding as well (RIC group score 0.049, 95% CI 0.017-0.082; sham RIC group score -0.007, 95% CI -0.041-0.027; appendix table S4).

Discussion

In this trial of 3033 patients with ischemic stroke or TIA attributable to stenosis of a major intracranial artery, chronic RIC did not significantly reduce ischemic stroke in the intention-to-treat population. However, a prespecified per-protocol analysis was done in patients performing the intervention on at least 50% of the potential treatment days in the first 12 months, and the result showed that chronic RIC significantly reduced the occurrence of ischemic stroke (p=0.038). Given the borderline statistical significance in the per-protocol analysis, we estimated the statistical power based on the actual hazard ratio and sample size in the per-protocol analysis. This showed power of 72% for the primary outcome which provides support that the positive result in the per-protocol analysis is likely reliable. Additionally, this result is consistent with our previous single-center clinical trial that repetitive RIC may be an effective way to reduce recurrent stroke in patients with symptomatic ICAS if patients are compliant with the therapy.[11] RIC also significantly reduced the secondary composite outcome of stroke (ischemic or hemorrhagic), TIA, or myocardial infarction in both the intention-to-treat and per-protocol populations. ~~Our landmark analyses showed that most of the benefit from RIC occurred within the first 12 months of follow-up. Although the number of primary and secondary outcomes beyond 12 months was lower in the RIC group, the difference between the two groups was not statistically significant. Possible reasons for the absence of substantial benefit beyond 12 months are that compliance with RIC was very low beyond 12 months when it was optional for study patients and the risk of stroke from ICAS is much lower beyond 12 months after a stroke or TIA.[3,5,7]~~

There are three main hypothesized mechanisms of stroke related to ICAS: hemodynamic compromise, artery-to-artery embolism, and branch occlusive disease.[3] There are several means by which RIC may lower the risk of stroke in ICAS subjects. In preclinical studies of chronic cerebral hypoperfusion, RIC once a day for 28 days or 4 months significantly improved cerebral blood flow and increased angiogenesis, arteriogenesis, capillary density, capillary diameter, and collateral formation.[17,18] In a previous clinical trial in ICAS, RIC for 300 days increased cerebral perfusion measured by SPECT imaging and improved collateral flow measured by transcranial Doppler sonography.[11] In patients with cerebral small vessel disease, RIC for one year improved cerebral perfusion and alleviated the resistance of distal vessels.[19,20] Based on these reports, long-term RIC could possibly improve cerebral blood flow in ICAS subjects with hemodynamic compromise via collateral remodeling, thereby lowering their risk of stroke.

In addition to improving hemodynamic compromise, chronic RIC might reduce the occurrence of artery-to-artery embolism. In patients with severe carotid artery stenosis, RIC for 2 weeks before carotid artery stenting lowered the incidence of new embolic infarctions.[21] Cerebrovascular embolic events were closely related to the instability of atherosclerotic plaque structure, which was shown to correlate with upregulation of several plasma markers such as high sensitivity C reactive protein (hs-CRP) and interleukin-6 (IL-6).[22] Previous clinical trials showed RIC for several months decreased plasma hs-CRP and IL-6,[12,23] suggesting RIC could possibly stabilize the structure of atherosclerotic plaque to reduce the occurrence of artery-to-artery embolism. Chronic RIC might also decrease the progression of branch occlusive disease. In a preclinical experiment, RIC for 12 months prevented atherosclerosis progression by reducing the percentage of plaque area in the aorta of hypercholesterolemic rabbits.[24] In patients with cerebral small vessel disease, RIC for 12 months significantly lowered plasma triglycerides, total cholesterol, and low-density lipoprotein,[20] which were related to the progression of atherosclerosis.[25] These results provided evidence that RIC might slow the progression of atherosclerosis to reduce the occurrence of ischemic stroke.

Although previous multi-center trials incorporating one-time use of RIC for neuroprotection or cardioprotection after acute stroke or myocardial infarction, or graft protection after renal transplant did not show a benefit of RIC,[26-30] this may be because the protection afforded by one-time RIC is limited. The mechanisms by which RIC might prevent stroke in patients with ICAS described above (i.e., improved collaterals, stabilization of plaque, slowing the progression of atherosclerosis) likely requires repeated RIC over a longer period. This is supported by the neutral results of our intention-to-treat analysis but positive results in per-protocol analysis, i.e., poor compliance was not associated with a reduction in ischemic stroke whereas good compliance was. The suggested effectiveness of repeated RIC observed in our trial may provide a rationale for cardio-protection trials in subjects with coronary disease and renal graft protection trials in subjects following renal transplant using repeated RIC over a longer period rather than one-time use of RIC.

This trial has many strengths including the large sample size, long follow-up, low drop-out rate, standardized device, automated upload of adherence data, and effective blinding. Nevertheless, there were some important limitations. First, compliance with RIC was much lower than expected largely because the device was considered inconvenient to use by many patients who had to sit quietly for 45 minutes with both upper arms confined by the device and who had to transport the device if patients were away from home. Further studies are needed to determine whether less frequent and shorter cycles of RIC are effective for the treatment of ICAS. Additionally, ongoing improvements in the design of the device to make it more practical to use

(e.g., making a wearable device - see appendix p 12) will help improve portability and compliance. When better compliance is achieved, further study should be conducted to provide more definitive evidence to support RIC treatment for ICAS. Second, we did not collect data on control of some risk factors (e.g., lipid and hemoglobin A1c levels) during follow-up so we cannot be certain that the differences in outcomes between the two arms was solely due to RIC and not to differences in other secondary prevention treatment in the trial. However, given the large sample size in this randomized trial with effective blinding it highly unlikely that there would have been significant differences in the other secondary prevention treatments in the two arms of the trial. Third, fewer women were enrolled in this study than men in large part because stroke is much more prevalent in men,[31] especially in ICAS related stroke.[7] The low proportion of women in our trial may also represent a bias against women in recruitment, and we will recruit more women in our future stroke trials. Importantly, there was no interaction between sex and outcome in this trial. Finally, there might be concerns about the generalizability of the trial results because all patients were Chinese. Confirmation in other populations is required.

In conclusion, although chronic RIC treatment did not lower the risk of ischemic stroke in patients with symptomatic ICAS in the entire cohort, it reduced the occurrence of ischemic stroke in patients who were compliant with the therapy. Also, RIC lowered the risk of combined cerebro- and cardio-vascular events in all patients and was associated with only minor adverse events. Further studies are needed to confirm these findings in non-Chinese populations.

Research in context

Evidence before this study

Intracranial atherosclerotic stenosis (ICAS) is one of the most common causes of stroke worldwide and is associated with a high risk of recurrent stroke on currently recommended treatments. Remote ischemic conditioning (RIC) is a promising therapy for secondary stroke prevention that warrants further investigation in large randomized trials. We searched PubMed, using the search terms “(intracranial arterial stenosis or intracranial atherosclerotic stenosis)” and “(conditioning or preconditioning or perconditioning or postconditioning)”, for studies published up to February 12, 2022, without language restrictions. 18 articles were retrieved, and 2 clinical studies were identified suggesting that RIC may safely reduce recurrent stroke in patients with symptomatic ICAS. However, the sample sizes in these two single-center trials were too small to draw any definite conclusions regarding the efficacy of RIC for preventing stroke in patients with ICAS.

Added value of this study

This RICA study is, to the best of our knowledge, the first multi-center, large-scale, randomized controlled trial to investigate the effect of RIC on clinical outcomes in patients with a recent cerebral ischemic event attributable to ICAS. The rigorous nature of this trial (large sample size, long follow-up, low drop-out rate, standardized device, automated upload of adherence data, and effective blinding) provides unique data on the potential role of RIC for secondary prevention of stroke in patients with symptomatic ICAS but also identifies challenges in patient compliance with the treatment that must be overcome to make RIC a practical and effective treatment for this disease.

Implications of all the available evidence

The results of this trial suggest that chronic RIC might be an efficacious therapy for patients with symptomatic ICAS in compliant patients. If confirmed in other trials of non-Chinese patients, together these findings will provide high level evidence to support using RIC for stroke prevention in patients with ICAS. Additionally, since the pathophysiology and treatment of ICAS are similar to coronary artery disease and atherosclerosis in other vascular beds (peripheral vascular, renal), the results of this trial also provide a strong rationale for clinical trials in patients with those diseases in which patients are treated with repeated RIC over a longer period.

Table 1: Baseline characteristics in the intention-to-treat population

	RIC group (n=1517)	Sham RIC group (n=1516)
Age, years	61.1 (9.1)	61.0 (9.1)
Sex		
Female	536 (35.3%)	543 (35.8%)
Male	981 (64.7%)	973 (64.2%)
Body-mass index, kg/m²	25.0 (3.1)	25.1 (3.1)
Qualifying event		
TIA	296 (19.5%)	291 (19.2%)
Ischemic stroke	1221 (80.5%)	1225 (80.8%)
Time from qualifying event to randomization, days		
TIA	9 (6-11)	9 (6-11)
Ischemic stroke	12 (8-16)	12 (8-16)
Neurologic score		
ABCD² for TIA*	4 (4-5)	4 (4-5)
mRS for ischemic stroke†	1 (1-2)	1 (1-2)
Comorbidities (Medical history and risk factors)		
Previous ischemic stroke	369 (24.3%)	360 (23.7%)
Previous TIA	172 (11.3%)	164 (10.8%)
Previous myocardial infarction	129 (8.5%)	127 (8.4%)
Hypertension	1254 (82.7%)	1268 (83.6%)
Hyperlipidemia	1000 (65.9%)	1030 (67.9%)
Diabetes mellitus	545 (35.9%)	553 (36.5%)
Current or previous smoking	954 (62.9%)	939 (61.9%)
Symptomatic qualifying artery		
Internal carotid	210 (13.8%)	207 (13.7%)
Middle cerebral	768 (50.6%)	774 (51.1%)
Basilar	266 (17.5%)	281 (18.5%)
Vertebral	205 (13.5%)	191 (12.6%)
Multiple arteries‡	68 (4.5%)	63 (4.2%)
Stenosis of qualifying artery ≥70%§	597 (39.4%)	605 (39.9%)
Fasting blood glucose, mmol/l	6.9 (2.8)	6.8 (2.8)
Blood pressure		
Systolic, mmHg	141.4 (16.7)	142.0 (16.4)
Diastolic, mmHg	84.8 (11.6)	85.2 (11.0)
Lipids		
LDL cholesterol, mg/dl	113.3 (32.9)	113.4 (32.5)
HDL cholesterol, mg/dl	43.5 (11.3)	43.7 (11.2)
Total cholesterol, mg/dl	184.1 (37.8)	183.8 (38.6)

Data are n (%), mean (SD), or median (IQR). RIC=remote ischemic conditioning. TIA=transient ischemic attack. mRS=modified Rankin scale. LDL=low-density lipoprotein. HDL=high-density lipoprotein. * Scores on the ABCD² scale range from 0 to 7, with higher scores indicating a

458 greater risk of stroke. † Scores on modified Rankin scale (mRS) range from 0 to 6, with higher
459 scores indicating a greater stroke severity. ‡ The affected arteries were a combination of the
460 internal carotid and middle cerebral arteries, the vertebral and basilar arteries, or the left and
461 right vertebral arteries. § Stenosis was quantified on the basis of a reading of the angiogram by
462 the site investigators.
463

Table 2: Efficacy outcomes in the intention-to-treat and per-protocol populations

	Intention-to-treat population				Per-protocol population			
	RIC group (n=1517)	Sham RIC group (n=1516)	HR (95% CI)	p value	RIC group (n=703)	Sham RIC group (n=706)	HR (95% CI)	p value
Primary outcome								
Ischemic stroke	257 (16.9%)	288 (19.0%)	0.87 (0.74-1.03)	0.12	103 (14.7%)	132 (18.7%)	0.76 (0.59-0.99)	0.038
Non-fatal	247 (16.3%)	277 (18.3%)			99 (14.1%)	127 (18.0%)		
Fatal	10 (0.7%)	11 (0.7%)			4 (0.6%)	5 (0.7%)		
Secondary outcomes								
Composite of stroke (ischemic or hemorrhagic), TIA, or myocardial infarction	318 (21.0%)	376 (24.8%)	0.82 (0.71-0.95)	0.0089	124 (17.6%)	170 (24.1%)	0.70 (0.56-0.88)	0.0026
Stroke (ischemic or hemorrhagic)	262 (17.3%)	294 (19.4%)	0.87 (0.74-1.03)	0.11	105 (14.9%)	134 (19.0%)	0.77 (0.59-0.99)	0.040
Non-fatal	251 (16.5%)	281 (18.5%)			101 (14.4%)	128 (18.1%)		
Fatal	11 (0.7%)	13 (0.9%)			4 (0.6%)	6 (0.8%)		
TIA	41 (2.7%)	53 (3.5%)	0.77 (0.51-1.16)	0.20	14 (2.0%)	21 (3.0%)	0.67 (0.34-1.31)	0.24
Myocardial infarction	43 (2.8%)	53 (3.5%)	0.80 (0.54-1.20)	0.28	17 (2.4%)	23 (3.3%)	0.73 (0.39-1.37)	0.33
Non-fatal	39 (2.6%)	47 (3.1%)			15 (2.1%)	21 (3.0%)		
Fatal	4 (0.3%)	6 (0.4%)			2 (0.3%)	2 (0.3%)		
All-cause death	79 (5.2%)	84 (5.5%)	0.93 (0.68-1.27)	0.65	33 (4.7%)	36 (5.1%)	0.92 (0.57-1.47)	0.72

Data are number of first events (%). RIC=remote ischemic conditioning.HR=hazard ratio. TIA=transient ischemic attack.

Table 3: Adverse events of special interest during RIC or sham RIC intervention

	RIC group (n=1515)	Sham RIC group (n=1514)	P value
Total	123 (8.1%)	3 (0.2%)	< 0.0001
Skin petechiae caused by cuff inflation	104 (6.9%)	1 (0.1%)	< 0.0001
Dizziness, or nausea	19 (1.3%)	2 (0.1%)	0.0002

Data are number (%) of patients, in those with at least one exposure to study intervention. RIC=remote ischemic conditioning.

Figure legends

Figure 1: Trial profile

RIC=remote ischemic conditioning. ITT=intention-to-treat.

Figure 2: Kaplan-Meier event curve for the primary outcome and the secondary composite outcome in the intention-to-treat population

(A) Primary outcome (ischemic stroke). (B) Secondary composite outcome (stroke, transient ischemic attack, or myocardial infarction). RIC=remote ischemic conditioning. HR=hazard ratio.

Figure 3: Prespecified subgroup analyses of the primary outcome in the intention-to-treat population

$p_{interaction}$ represents the likelihood of interaction between the subgroup variable and the intervention strategy. RIC=remote ischemic conditioning. TIA=transient ischemic attack. HR=hazard ratio.

Contributors

XJ, MIC, DCH, DSL, CH, JL, and RM conceived and designed the study. HS, YW, JHuang, ZL, XH, JY, JZ, PC, XZ, PH, HP, WC, HC, GL, DT, WY, and ZG participated in data collection. CH and YL did the statistical analyses. XJ, CH, JL, HS, WZ, SL, JHao, RM, YD, MIC, and HC interpreted the data. CH and JL wrote the first draft. XJ, JHao, HS, MIC, MF, DCH, ~~and~~ DSL, and DJH revised the manuscript. All authors approved the final version of the manuscript.

Declaration of interests

CH has received speaker fees from Novo Nordisk and Pfizer. HS has received speaker fees from AstraZeneca, Pzier, Amgen, Boehringer Ingelheim, Novartis, and Sanofi. WZ has received speaker fees from Tasly Pharma. RM has received grants from Ministry of Science and Technology of the People's Republic of China. MIC has received grants from NIH (CAPTIVA, U01 NS117450-01A1). MIC has received honoraria for lectures on Intracranial Stenosis at Taiwan Neurological Conference and grand rounds at other universities. DSL has served as CEC member. DJH has received consultant fees from Faraday Pharmaceuticals Inc. and Boehringer Ingelheim International GmbH; honoraria from Servier; and research funding from Astra Zeneca and Merck Sharp & Dohme Corp. XZ has received speaker fees from AstraZeneca and Bayer. GL has received speaker fees from Techpool, CSPC, and Boehringer Ingelheim. WY has participated in speakers bureaus and received speaker fees from Bayer, Sanofi, Novartis, Xian Janssen and Amgen. XJ has received grants from Beijing Municipal Education Commission and Beijing Municipal Finance Bureau. XJ has received speaker fees from AstraZeneca, Medtronic, and Sanofi. The remaining authors declare no competing interests.

Data sharing

RICA study is an investigator-initiated trial. Internal investigators who actively participated in the study and provide a methodologically sound study proposal will be granted priority access to the study data for a period of 48 months. After 48 months, individual, deidentified participant data underlying the findings described in this manuscript will be available by request to any external investigator following approval of a proposal by the steering committee. Study proposals can be filed at rica_group@ccmu.edu.cn.

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Remote Ischemic Conditioning for Avoiding Recurrence of Stroke in Patients with Symptomatic Intracranial Atherosclerotic Stenosis (RICA): a Multi-center, Randomized, Double-blind, Sham-controlled, Parallel-group Trial

STUDY PROTOCOL

Xuanwu Hospital Capital Medical University

Principal Investigator

Xunming Ji, MD, PhD, Professor of Neurosurgery

Version: RICA 1.4

30 August 2016

SIGNATURE PAGE

Declaration of Principal Investigator

Title: Remote Ischemic Conditioning for Avoiding Recurrence of Stroke in Patients with Symptomatic Intracranial Atherosclerotic Stenosis (RICA): a Multi-center, Randomized, Double-blind, Sham-controlled, Parallel-group Trial

Protocol version: RICA 1.4, dated 30 Aug. 2016

This study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki, and the International Council for Harmonisation Guidelines on Good Clinical Practice.

Principal Investigator

Xunming Ji, MD, PhD

Professor of Neurosurgery

Vice President

Xuanwu Hospital _____ _____

Capital Medical University Date Signature

SYNOPSIS

Title:	Remote ischemic conditioning for avoiding recurrence of stroke in patients with symptomatic intracranial atherosclerotic stenosis (RICA): a multi-center, randomized, double-blind, sham-controlled, parallel-group trial
Primary Objective:	To compare the effect of long-term treatment with remote ischemic conditioning (RIC) once daily for 12 months versus sham RIC for the prevention of ischemic stroke in patients with ischemic stroke or transient ischemic attack (TIA) attributable to 50-99% stenosis of a major intracranial artery.
Study Design:	An investigator-initiated, multi-center, randomized, double-blind, sham-controlled, parallel-group trial to evaluate the effect of RIC intervention compared to sham RIC intervention for the prevention of new ischemic stroke in patients with ischemic stroke or TIA attributable to stenosis of a major intracranial artery.
Patient Population:	Patients aged 40-80 years with ischemic stroke (mRS score ≤4) occurring within 30 days or TIA ($ABCD^2$ score ≥4) within 15 days before randomization, which is attributed to angiographically verified 50-99% stenosis of a major intracranial artery, including carotid, middle cerebral (M1 segment), vertebral, or basilar artery.
Inclusion/Exclusion Criteria:	Inclusion criteria: • Men and women between 40 and 80 years of age. • Patients who suffered an ischemic stroke or TIA prior to enrollment. ◆ Patients who suffered an ischemic stroke within 30 days prior to enrollment with a baseline mRS score ≤4. ◆ Patients who suffered a TIA within 15 days prior to enrollment with a baseline $ABCD^2$ score ≥4. • Qualifying event attributable to symptomatic intracranial atherosclerotic stenosis (50%-99%) of carotid artery, middle cerebral artery (M1 segment), vertebral artery, or basilar artery that has been documented by magnetic resonance angiography or computed tomography angiography.

- Informed consent obtained.

Exclusion Criteria

- Thrombolytic therapy within 24 hours prior to enrollment.
- Progressive neurological signs within 24 hours prior to enrollment.
- Cerebral venous thrombosis/stenosis.
- Intracranial arterial stenosis due to arterial dissection; Moyamoya disease; any known vasculitic disease; herpes zoster, varicella zoster, or other viral vasculopathy; neurosyphilis; any other intracranial infection; intracranial stenosis associated with cerebral spinal fluid pleocytosis; radiation induced vasculopathy; fibromuscular dysplasia; sickle cell disease; neurofibromatosis; benign angiopathy of central nervous system; postpartum angiopathy; suspected vasospastic process; or suspected recanalized embolus.
- Any of the following unequivocal cardiac source of embolism: rheumatic mitral disease with or without aortic stenosis, prosthetic heart valves, atrial fibrillation, atrial flutter, sick sinus syndrome, left atrial myxoma, patent foramen ovale, left ventricular mural thrombus or valvular vegetation, congestive heart failure, bacterial endocarditis, or any other severe cardiovascular condition.
- Uncontrolled severe hypertension, defined by sitting systolic blood pressure >180mmHg and/or sitting diastolic blood pressure >110mmHg after medication.
- Patients with any of the following abnormal laboratory parameters: aspartate aminotransferase and/or alanine aminotransferase >3 × the upper limit of the reference range; creatinine clearance <0.6mL/s and/or serum creatinine >265μmol/L (>3.0mg/dL); platelets $<100\times10^9$/L.
- Any intracranial hemorrhage (parenchymal, subarachnoid, subdural, or epidural) within 90 days prior to enrollment.
- Intracranial neoplasm, cerebral aneurysm, or arteriovenous malformation.

	• Retinal hemorrhage or visceral bleeding within 30 days prior to enrollment. • Severe hemostatic disorder or severe coagulation dysfunction. • Subclavian arterial stenosis ≥50% or subclavian steal syndrome. • Extracranial stenosis ≥50%. • Treatment of a target lesion with a stent, angioplasty, or other mechanical device prior to enrollment or intent to perform such a procedure within 12 months after enrollment. • Major surgery, including cardiac and open femoral, aortic, or carotid surgery, within 30 days prior to enrollment or intent to undergo within 12 months after enrollment. • Contraindication for remote ischemic conditioning, including severe soft tissue injury, fracture, or peripheral vascular disease in the upper limbs. • Life expectancy <3 years. • Women who were pregnant or breast-feeding at the time of enrollment or anytime during the study period. • Unwilling to comply with the treatment or follow-up assessments. • Participating in another clinical trial within 3 months prior to enrollment of this clinical trial. • Any patient deemed unsuitable for enrollment by the investigators.
Randomization:	Patients will be randomly assigned (1:1) to either RIC intervention group or sham RIC intervention group via an interactive web-based response system. Randomization will be performed by a computer-generated random code with constant block size allocated to each medical center, stratified by qualifying event (ischemic stroke/TIA).
Primary Endpoint:	Time from randomization to first occurrence of non-fatal or fatal ischemic stroke.
Study Duration:	The anticipated study duration is approximately 36 months. The expected minimum follow-up period is 12 months and the expected average follow-up period is 24 months for an

	individual patient. The actual duration of the study may vary depending on the enrollment rate and the primary efficacy event rate.
Number of Centers:	Approximately 80 medical centers in China.
Sample Size:	The study is event-driven, and it is estimated that approximately 3000 patients (1500 per treatment group) are to be enrolled in order to have 597 patients experiencing a primary efficacy event.
Statistical Methods:	The primary efficacy analysis will be based on the intent-to-treat population. Cumulative event rate will be estimated with the Kaplan-Meier method and compared with a stratified log-rank test according to qualifying event. Hazard ratio with 95% confidence intervals will be derived using a Cox proportional-hazards model with stratification. The treatment comparison will be tested at the two-sided significance level of 0.05. A corresponding sensitivity analysis will be performed on the per-protocol population.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse event
BI	Barthel Activities of Daily Living Index
CI	Confidence interval
CTA	Computed tomography angiography
eCRFs	electronic Case Report Forms
GCP	Good Clinical Practice
HR	Hazard ratio
ICAS	Intracranial atherosclerotic stenosis
ICE	Independent Ethics Committee
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
ITT	Intention-to-Treat
IWRS	Interactive web-based response system
MedDRA	Medical Dictionary for Regulatory Activities
MRA	Magnetic resonance angiography
mRS	modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
PP	Per-Protocol
RIC	Remote ischemic conditioning
SAE	Serious adverse event
TIA	Transient ischemic attack

1. BACKGROUND

Intracranial atherosclerotic stenosis (ICAS) is one of the most common causes of stroke worldwide and is associated with a high risk of recurrent stroke compared with other stroke subtypes.[1] Although extracranial large artery atherosclerosis may be a more common lesion in Caucasian populations, ICAS is more common in Chinese.[2] The percentage of Chinese with >50% luminal stenosis is approximately 30% in the sixth and seventh decades and approximately 50% in the eighth and ninth decades.[3] Patients with ICAS are at a high risk of recurrence up to 25-30% in 2 years after stroke.[4]

However, treatments for prevention of ischemic stroke in ICAS are not satisfied presently. Extracranial to intracranial arterial bypass surgery failed to show effectiveness in preventing cerebral ischemia in atherosclerotic arterial disease in carotid and middle cerebral arteries.[5] The 30-day rate of stroke or death was higher after percutaneous transluminal angioplasty and stenting, compared with aggressive medical therapy in ICAS.[6] Warfarin provided no benefit over aspirin for prevention of ischemic stroke, but was associated with significantly higher rate of adverse events such as death and major hemorrhage.[7] Multifaceted medical management that incorporated short-term dual antiplatelet treatment for 90 days followed by aspirin monotherapy, coupled with intensive management of vascular risk factors, was recommended for stroke prevention in ICAS,[1] however, a large subgroup of patients was still at high risk of recurrent stroke.[6,7]

Based on the unsatisfied treatments for ICAS right now, additional strategies are needed to explore for preventing ischemic stroke. Remote ischemic conditioning (RIC) is an intervention in which small doses of an injurious agent, such as ischemia, induce a tolerant phenotype that protects against subsequent larger injurious doses.[8] It involves repetitive inflation and deflation of a cuff around the limb to pressures above systolic blood pressure, with the intention of protecting distant organs such as the heart or brain. Neural and humoral mechanisms have been proposed as ways in which the peripheral signal from the limb is transmitted to a distant organ.[9] Many clinical trials have been performed in the fields of cardiology,[10-13] and emerging evidence indicates that RIC is safe for management of neurological conditions, such as acute ischemic stroke,[14] carotid endarterectomy,[15] and subarachnoid hemorrhage.[16,17]

Several clinical trials have been done by our team for safety and effectiveness of RIC in patients with ICAS. RIC consisting of five cycles of 5-min inflations of a blood pressure cuff to 200 mmHg around an upper limb followed by 5 min of reperfusion was

found to be safe and well tolerated in patients with unilateral middle cerebral artery stenosis, in whom RIC did not significantly affect blood pressure, heart rate, oxygenation index, and mean flow velocity in middle cerebral artery.[18] A randomized clinical trial included 68 Chinese patients aged <80 years with symptomatic ICAS to evaluate the effect of RIC intervention twice daily for 300 consecutive days. The incidence of recurrent stroke in RIC group was 5% at 90 days and 7.9% at 300 days, compared with 23.3% and 26.7% in control group, respectively. RIC also improved cerebral perfusion status measured by SPECT and transcranial Doppler sonography.[19] Another unpublished clinical trial evaluated the effect of RIC on symptomatic ICAS in octo- and nonagenarian Chinese patients. RIC might safely prevent stroke recurrence, accelerate stroke recovery, and ameliorate plasma biomarkers of inflammation and coagulation.

Based on the findings above, an investigator-initiated, multi-center, randomized, double-blind, sham-controlled, parallel-group trial is designed to evaluate the effect of RIC treatment on the prevention of new ischemic stroke in patients with symptomatic ICAS.

2. STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of the study is to compare the effect of long-term treatment with remote ischemic conditioning (RIC) once daily for 12 months versus sham RIC for the prevention of ischemic stroke in patients with ischemic stroke or transient ischemic attack (TIA) attributable to 50-99% stenosis of a major intracranial artery, including carotid artery, middle cerebral artery (M1 segment), vertebral artery, or basilar artery.

2.2 Secondary Objectives

The secondary objectives of the study are to compare the effect of long-term treatment with RIC versus sham RIC for:

- Prevention of the composite of stroke (ischemic and hemorrhagic), TIA, or myocardial infarction;
- Prevention of stroke (ischemic and hemorrhagic);
- Prevention of TIA;
- Prevention of myocardial infarction;
- Prevention of all-cause death.

2.3 Other Objectives

Other objectives are to compare the effect of long-term treatment with RIC versus sham RIC for:

- Change of National Institutes of Health Stroke Scale (NIHSS);
- Change of modified Rankin Scale (mRS);
- Change of Barthel Activities of Daily Living Index (BI).

3. STUDY PLAN AND PROCEDURES

3.1 Overall Study Design and Flow Chart

The study is an investigator-initiated, multi-center, randomized, double-blind, sham-controlled, parallel-group trial designed to evaluate the effect of RIC treatment for the prevention of new ischemic stroke in patients with ischemic stroke or TIA attributable to stenosis of a major intracranial artery. The schematic of study design is presented in Figure 1.

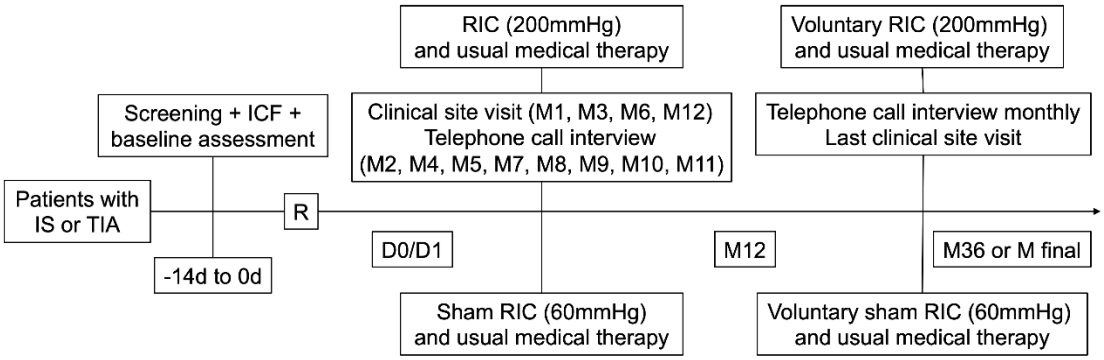

Figure 1. Study flow chart. ICF: Informed consent form; RIC: Remote ischemic conditioning; IS: Ischemic stroke; TIA: Transient ischemic attack; M: Month; D: Day; R: Randomization.

The study population will consist of patients who suffered ischemic stroke within 30 days prior to enrollment with baseline mRS score ≤4, or patients who suffered TIA within 15 days prior to enrollment with baseline $ABCD^2$ score ≥4. In addition, ischemic stroke or TIA should be attributed to 50-99% stenosis of a major intracranial artery documented by magnetic resonance angiography (MRA) and/or computed tomography angiography (CTA), including carotid artery, middle cerebral artery (M1 segment), vertebral artery, or basilar artery. Written consents should be provided by the patients or their legally authorized representatives to participate in the study.

Patients who meet all of the inclusion criteria and none of the exclusion criteria will be randomized via an interactive web-based response system (IWRS) in 1:1 ratio to either RIC group or sham RIC group. RIC intervention will be performed by an electric automated device (patent number ZL200820123637.X, China), inflated to 200mmHg, for 5 minutes inflation / 5 minutes deflation, 5 cycles, once a day, for 12 months. Sham RIC intervention will be performed by the electric automated device with the same procedure, except inflated to 60mmHg instead of 200mmHg.

Randomization will be stratified according to the qualifying event (ischemic stroke/TIA) with constant block size allocated to each medical center, to assure an equal distribution of treatments for either ischemic stroke or TIA patients at each medical center. After randomization, patients will receive double-blind treatment (RIC intervention or sham RIC intervention).

The primary efficacy outcome is the time to first occurrence of ischemic stroke after randomization. This study comprises two periods: an on-treatment period and a post-treatment period. In the on-treatment period, RIC intervention or sham RIC intervention will be performed for 12 months, followed by the post-treatment period, when intervention can be voluntarily performed by patients. Clinic visits will occur at month 1, 3, 6, 12, and the end of the study, which will be supplemented by monthly follow-up telephone calls to the patients until the end of the study. Outcome events and adverse events will be assessed at each clinical visit and telephone call.

Approximately 3000 patients will be recruited from about 80 medical centers in China. It is estimated that a total period of approximately 3 years will be necessary for this trial. The actual duration of the study may vary depending on the enrollment rate and the primary efficacy event rate. An independent data monitoring committee will oversee the data during the study.

3.2 Study Endpoints

3.2.1 Primary Efficacy Endpoint

- Time from randomization to first occurrence of non-fatal or fatal ischemic stroke.

3.2.2 Secondary Efficacy Endpoints

- Time from randomization to first occurrence of any event from the composite of non-fatal or fatal stroke (ischemic and hemorrhagic), TIA, or non-fatal or fatal myocardial infarction;

- Time from randomization to first occurrence of non-fatal or fatal stroke (ischemic and hemorrhagic);
- Time from randomization to first occurrence of TIA;
- Time from randomization to first occurrence of non-fatal or fatal myocardial infarction;
- Time from randomization to occurrence of all-cause death.

3.2.3 Other Efficacy Endpoints

- Change from baseline in NIHSS at 12 months after randomization;
- Change from baseline in mRS at 12 months after randomization;
- Change from baseline in BI at 12 months after randomization.

3.3 Randomization

Patients will be randomized via a IWRS in 1:1 ratio to either RIC group or sham RIC group, after they are verified eligible by the investigator. The random code will be maintained by a Contract Research Organization. The randomization scheme will be generated using validated software verified by a statistician who is not involved in this study. Randomization will be stratified according to the qualifying event with constant block size allocated to each medical center, to assure an equal distribution of treatments for either ischemic stroke or TIA patients at each medical center. All eligible patients will have an equal chance to be randomized to one of the treatment arms to ensure balance between two treatment arms.

After patient's eligibility is determined, the investigator will access the randomization interface in the IWRS with username and password to complete a randomization form. The computer will generate a random code based on the randomization scheme. The random code will appear on the screen and will be used to link the patient to a treatment arm. This code also links to the device that will be dispensed to the patient. If a patient withdraws from the study, his/her random code cannot be reused. Patients can only be randomized into the study once.

3.4 Blinding

3.4.1 Methods for Blinding

The treatment allocation in this study will be double blinded. The patients, the investigators and other staff at the medical centers, the principal investigator, the persons performing the assessments, data analysts, and other staff directly associated with the trial will be blinded.

The electric automated device inflated to 200mmHg (RIC device) and the device inflated to 60mmHg (sham RIC device) are identical in appearance and packaging. Each device will be labelled with a random code, which will be used to assign the device to the patient but will not indicate the treatment allocation.

3.4.2 Methods for Unblinding

The random code should not be broken, except in the cases of patient emergency, when knowledge of the treatment group is needed to provide appropriate management. The treatment allocation for the patient will be available to the investigator using the IWRS. If possible, contact should be made with the principal investigator to confirm the necessity before breaking the code. If the blindness is broken, the investigator must inform the principal investigator, and record the time and the reason for unblinding. The study treatment must be permanently discontinued after unblinding.

3.5 Withdrawal from Study

Withdrawal of consent occurs when a patient does not want to participate in the study anymore, does not want any form of follow-up, or does not want any further study related contacts. Patients have the right to withdraw from the study at any time without prejudice to their future treatment.

If a patient withdraws consent, the investigator must make every effort to determine the primary reason for this decision. If the withdrawal is due to adverse events, refer to the section **7. SAFETY MONITORING**. Patients who have withdrawn from the study cannot be included again in the study.

3.6 Lost to Follow-up

For patients whose status are unclear because they fail to appear for study visits without stating an intention to withdraw, the investigator should make every effort to contact the patient. A patient should not be considered lost to follow-up until the end of the study.

4. STUDY POPULATION

The study population will consist of patients who suffered symptomatic ischemic stroke or TIA attributable to 50-99% stenosis of a major intracranial artery, including carotid artery, middle cerebral artery (M1 segment), vertebral artery, or basilar artery. A total of 3000 patients who meet all of the inclusion criteria and none of the exclusion criteria are to be included in this study. Approval from local Institutional Review

Boards/Independent Ethics Committee should be obtained by the medical centers before patient enrollment.

4.1 Inclusion Criteria

- Men and women between 40 and 80 years of age.
- Patients who suffered an ischemic stroke or TIA prior to enrollment.
  - Patients who suffered an ischemic stroke within 30 days prior to enrollment with a baseline mRS score ≤4.
  - Patients who suffered a TIA within 15 days prior to enrollment with a baseline $ABCD^2$ score ≥4.
- Qualifying event attributable to symptomatic intracranial atherosclerotic stenosis (50%-99%) of carotid artery, middle cerebral artery (M1 segment), vertebral artery, or basilar artery that has been documented by magnetic resonance angiography or computed tomography angiography.
- Informed consent obtained.

4.2 Exclusion Criteria

- Thrombolytic therapy within 24 hours prior to enrollment.
- Progressive neurological signs within 24 hours prior to enrollment.
- Cerebral venous thrombosis/stenosis.
- Intracranial arterial stenosis due to arterial dissection; Moyamoya disease; any known vasculitic disease; herpes zoster, varicella zoster, or other viral vasculopathy; neurosyphilis; any other intracranial infection; intracranial stenosis associated with cerebral spinal fluid pleocytosis; radiation induced vasculopathy; fibromuscular dysplasia; sickle cell disease; neurofibromatosis; benign angiopathy of central nervous system; postpartum angiopathy; suspected vasospastic process; or suspected recanalized embolus.
- Any of the following unequivocal cardiac source of embolism: rheumatic mitral disease with or without aortic stenosis, prosthetic heart valves, atrial fibrillation, atrial flutter, sick sinus syndrome, left atrial myxoma, patent foramen ovale, left ventricular mural thrombus or valvular vegetation, congestive heart failure, bacterial endocarditis, or any other severe cardiovascular condition.
- Uncontrolled severe hypertension, defined by sitting systolic blood pressure >180mmHg and/or sitting diastolic blood pressure >110mmHg after medication.
- Patients with any of the following abnormal laboratory parameters: aspartate aminotransferase and/or alanine aminotransferase >3 × the upper limit of the reference range; creatinine clearance <0.6mL/s and/or serum

creatinine >265μmol/L (>3.0mg/dL); platelets <100×10^9/L.

- Any intracranial hemorrhage (parenchymal, subarachnoid, subdural, or epidural) within 90 days prior to enrollment.
- Intracranial neoplasm, cerebral aneurysm, or arteriovenous malformation.
- Retinal hemorrhage or visceral bleeding within 30 days prior to enrollment.
- Severe hemostatic disorder or severe coagulation dysfunction.
- Subclavian arterial stenosis ≥50% or subclavian steal syndrome.
- Extracranial stenosis ≥50%.
- Treatment of a target lesion with a stent, angioplasty, or other mechanical device prior to enrollment or intent to perform such a procedure within 12 months after enrollment.
- Major surgery, including cardiac and open femoral, aortic, or carotid surgery, within 30 days prior to enrollment or intent to undergo within 12 months after enrollment.
- Contraindication for remote ischemic conditioning, including severe soft tissue injury, fracture, or peripheral vascular disease in the upper limbs.
- Life expectancy <3 years.
- Women who were pregnant or breast-feeding at the time of enrollment or anytime during the study period.
- Unwilling to comply with the treatment or follow-up assessments.
- Participating in another clinical trial within 3 months prior to enrollment of this clinical trial.
- Any patient deemed unsuitable for enrollment by the investigators.

5. STUDY TREATMENT

All eligible patients will be randomized to either RIC group or sham RIC group and will be provided with the device by the investigator. The advantage of the device is that the standard RIC or sham RIC intervention is programmed and simply delivered by pressing a START button after the cuffs have been placed on bilateral upper arms.

5.1 Treatment Arms

Patients will be assigned to one of the following two treatment arms in the ratio of 1:1 after randomization:

- RIC intervention
- Sham RIC intervention

5.1.1 RIC Intervention

The cuffs of RIC device will be placed on bilateral upper arms and will be inflated to 200mmHg for 5 minutes and then deflated for 5 minutes. The programmed cycle will be repeated 5 times; therefore, the total length of RIC intervention is 45 minutes. RIC intervention will be performed by patients themselves at home once a day for 12 months and can be voluntarily performed by patients after 12 months.

5.1.2 Sham RIC Intervention

The components and external appearance of sham RIC device are visually identical to RIC device. The cuffs of sham RIC device will be placed on bilateral upper arms and will be inflated to 60mmHg for 5 minutes and then deflated for 5 minutes. The programmed cycle will be repeated 5 times; therefore, the total length of the sham RIC intervention is 45 minutes. Sham RIC intervention will be performed by patients themselves at home once a day for 12 months and can be voluntarily performed by patients after 12 months.

5.2 Handling and Dispensing

All RIC devices and sham RIC devices will be packaged and labeled in identical appearance, and then will be shipped to each medical center. A unique number is printed on the label which corresponds to one of the two treatment arms.

The devices must be received by a designated person at the medical center and be stored in a secure storage area under the appropriate storage conditions according to the instructions specified on the labels. When the shipment is received at the medical center, the designated person should verify the contents, sign the packing invoice provided with the shipment, and maintain the original copy for review by the principal investigator. The devices must only be dispensed to patients fulfilling the inclusion and exclusion criteria by the investigator.

5.3 Concomitant Treatment

The patients should be on optimal background medications to treat comorbidities, as considered appropriate by the investigator. No treatment is prohibited for this study.

5.4 Treatment Compliance

The performance of study treatment by the patients will be uploaded automatically to a central database and can be monitored by the investigator. If compliance is considered poor, the patients should be counselled on the importance of performing the study treatment.

5.5 Discontinuation of Study Treatment

The study treatment should be continued whenever possible in the first 12 months after randomization, unless the patient has a primary efficacy outcome. The investigators may choose to discontinue study treatment, if they think the continuation would be detrimental to the patients' well-being. Whenever possible, the patients should be re-challenged with study treatment if their conditions were considered appropriate by the investigator. Patients also have the right to discontinue study treatment at any time for any reason, without prejudice to further treatment. Such patients will always be asked about the primary reason for their decision to discontinue study treatment and the presence of adverse events if any. If the discontinuation was due to adverse events, refer to the section **7. SAFETY MONITORING**. It should be evaluated if the discontinuation can be made temporarily, and permanent discontinuation should be the last choice.

It is essential to collect as much data as possible for all patients throughout the study, especially all potential endpoint events. Discontinuation of study treatment does not mean discontinuation of follow-up or termination of study participation. Patients who have discontinued performance of study treatment are expected to, and should be encouraged to, remain in follow-up until the end of study. If they fail to attend follow-up, every effort should be made to contact them to determine if any adverse events or endpoints have occurred.

6. VISIT SCHEDULE AND ASSESSMENTS

Patients will visit the clinic at screening, baseline evaluation, month 1, 3, 6, 12 after randomization, and the end of the study. Visits for outcome events may also occur. The schedule for visits and the details of each visit are summarized in Table 1. All visit dates are calculated from the date of randomization.

	Day 0	Month 1±7 days	Month 3±7 days	Month 6±7 days	Month 12±7 days	Telephone contacts	Study end
Entry event assessment	*						
Patients demographic characteristics	*						
Inclusion and exclusion criteria	*						
Informed consent	*						
Randomization and treatment allocation	*						
Clinical and medical history	*						
Physical examination and vital signs	*	*	*	*	*		*
modified Rankin Scale	*	*	*	*	*		*
National Institutes of Health Stroke Scale	*	*	*	*	*		*
Barthel Activities of Daily Living Scale	*	*	*	*	*		*
Treatment compliance evaluation		*	*	*	*		*
Laboratory examination	*		*		*		*
Neuroimaging and electrocardiogram	*				*		
Smoking and alcohol consumption status	*	*	*	*	*		
Efficacy outcome events collection		*	*	*	*	*	*
Adverse events collection		*	*	*	*	*	*

Table 1. Entry and follow-up schedule

6.1 Screening and Baseline Evaluation

6.1.1 Screening

mRS for ischemic stroke patients and $ABCD^2$ score for TIA patients will be performed for screening. Head computed tomography or magnetic resonance imaging scan will be required to diagnose ischemic stroke or TIA. CTA or MRA will be required to measure the degree of stenosis in intracranial arteries (Appendix).[20]

Patients fulfilling all the inclusion and none of the exclusion criteria will be considered eligible for this trial and will be invited to participate. Written informed consent must be obtained prior to enrollment.

6.1.2 Baseline Evaluation

- Demographic information, medical history, tobacco, and alcohol use will be recorded.
- Physical examination will be evaluated, including measurement of weight, height, and waist circumference. Vital signs will be also evaluated, including systolic and diastolic blood pressure, heart rate, body temperature, and breathing rate.
- Laboratory assessments will include complete blood count, lipid profile, fasting glucose, prothrombin time, partial thromboplastin time, aspartate aminotransferase, alanine aminotransferase, creatinine, creatinine clearance.
- The NIHSS and the BI will be assessed.

6.1.3 Randomization

After eligibility has been confirmed, patients will be randomized to one of the treatment arms. The random code will be used to link the patient to a treatment arm and will be used to dispense the corresponding device to the patient.

6.2 On-treatment Period

6.2.1 Clinic Visits

Clinic visits will take place at month 1 ± 7 days, month 3 ± 7 days, month 6 ± 7 days, and month 12 ± 7 days. Unscheduled clinic visits will be possible at any time, in order to check the safety of the patient. Physical examination, vital signs, outcome events, and adverse events will be evaluated.

6.2.2 Phone Contacts

Phone contacts will be made to patients at month 2 ± 7 days, month 4 ± 7 days, month 5 ± 7 days, month 7 ± 7 days, month 8 ± 7 days, month 9 ± 7 days, month 10 ± 7 days, and month 11 ± 7 days. During the phone calls, outcome events and adverse events will be collected. A follow-up evaluation will be scheduled whenever an outcome event may have occurred.

6.3 Post-treatment Period

Patients can voluntarily perform the intervention in this period. They will be contacted by telephone every month until the end of the study. During the phone call, outcome events and adverse events will be collected. A follow-up evaluation will be scheduled whenever an outcome event may have occurred.

6.4 Outcome Assessment

Patients are requested to inform the investigator as soon as possible if they are hospitalized (regardless of reason) in order to ensure timely identification of potential outcome events. A follow-up evaluation will be scheduled whenever an outcome event may have occurred.

7. SAFETY MONITORING

7.1 Definitions

7.1.1 Adverse Event

An adverse event (AE) is defined as any new untoward medical occurrence or deterioration of a preexisting medical condition after providing written informed consent for participation in the study and does not necessarily have a causal relationship with the treatment. Efficacy endpoints will not be considered as adverse events except if, because of the course or severity or any other features of such events, the investigator considers these events as exceptional in this medical condition.

7.1.2 Serious Adverse Event

A serious adverse event (SAE) is defined as an AE that meets one or more of the following criteria:

- Results in death;
- Is life-threatening (Note: A life-threatening event is defined as an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.);
- Requires inpatient hospitalization or prolongation of existing hospitalization (Note: Hospitalization is defined as the admission to a hospital with at least one overnight stay. However, inpatient hospitalization for purely diagnostic reasons, routine health assessment requiring admission, elective surgery planned prior to signing consent, treatment of a pre-existing disease with no deterioration from baseline, or social reasons and respite care, are not considered as SAE.);
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect;
- Is an important medical event (Note: An important medical event is defined as a medical event that may not result in death, be life threatening, or require hospitalization, but may jeopardize the patient or may require medical or surgical intervention to prevent one of the other outcomes listed in the definition above.).

7.2 List of Interested Adverse Events

The benign nature of RIC excludes there being any expected SAEs. The following are expected non-serious AEs in response to RIC:

- Skin petechiae caused by cuff inflation;
- Headache, dizziness, nausea, or heart palpitation.

7.3 Procedure for Recording and Reporting of Adverse Events

Investigators have the responsibility for monitoring and recording AEs from the time of signing the informed consent to the time of last follow-up. If any AE occurs, investigators should specify the date when the AE started and ended, whether the event meets the criteria for a SAE, the severity of the AE, the causal relationship with study treatment, actions taken with respect to study treatment, and outcome.

All SAEs have to be reported to principal investigator by the investigator within 24 hours after first knowledge of the SAE, regardless of causality.

7.3.1 Severity

The severity of an AE is defined as the clinical determination of the intensity of the AE, and should be assessed by the investigator as mild, moderate, or severe using the following definitions:

- Mild: an event that patients are aware of signs or symptoms, but are easy to tolerate, which require minimal or no treatment and do not interfere with daily activities.
- Moderate: an event that patients feel sufficient discomfort to cause interference with normal activities.
- Severe: an event that patients are incapacitated with inability to perform normal activities, and may require systemic treatment.

7.3.2 Causality

All AEs should be assessed by the investigator to determine the causal relationship between the AE and the performance of study treatment, using the following definitions as guidelines:

- Unrelated: there is no evidence to suggest a causal relationship between study treatment and the AE. For example, the time course between study treatment and occurrence or worsening of the AE may rule out a causal relationship.
- Possibly related: study treatment administration may contribute to the AE, i.e., the AE occurred in a reasonable time after study treatment initiated, but the AE could also be produced by other factors, for example, an underlying disease, environmental factors, or other drugs or therapies.

- Probably related: the AE occurred in a reasonable time after study treatment initiated and is unlikely to be associated with other factors. This judgement by investigator may be based on the clinical experience or the knowledge of known AEs of study treatment.
- Definite: the AE occurred in a reasonable time after study treatment initiated and cannot be explained by other factors. The AE should be improved when discontinuation with study treatment and reappeared when re-challenge with study treatment.

7.4 Follow-up of Adverse Events

All AEs should be treated appropriately and be followed to resolution or stabilization, or be reported as SAEs if they become serious. Assessment should be made at each visit of any changes in severity, causality, seriousness, actions taken with respect to study treatment, and outcome.

For all SAEs, the investigator must follow up the patient until the SAE has resolved, stabilizes, or the patient dies. SAEs that are unresolved or not stabilized at the last visit in the trial should be followed up by the investigator for as long as medically indicated.

8. ETHICAL CONSIDERATIONS

This clinical trial will be conducted in accordance with "Good Clinical Practice" guidelines of the International Conference on Harmonisation (ICH-GCP) and the principles of the Declaration of Helsinki. This trial will also be conducted in compliance with all laws and regulations as well as any applicable guidelines.

8.1 Informed Consent Procedures

Prior to participation in this trial, the written informed consent must be signed by the patient or the patient's legal representatives. The signed consent form must be kept in the patient's file, and a copy of the signed form must be given to the patient or the patient's legal representatives.

All patients will be given a full explanation about the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved, and any discomfort it may entail, in language they are able to understand. The patients must be assured that participation in this study is voluntary, and they are free to withdraw from the study at any time for any reason, and the withdrawal will not affect subsequent medical treatment or relationship with the treating physician. The written

informed consent form should be signed and personally dated by the patient or the patient's legal representatives, and by the person who conducted the informed consent discussion.

8.2 Institutional Review Board/Independent Ethics Committee

Before enrollment of any patient into the study, the final version of the protocol, including the final version of the Informed Consent Form, must be submitted to and approved by a properly constituted Institutional Review Board/Independent Ethics Committee (IRB/IEC). During the study, any substantial amendments to the protocol must be submitted to the IRB/IEC for review and approval/favorable opinion. Reports of any serious and unexpected AEs should also be provided to the IRB/IEC. A progress report will be sent to the IRB/IEC annually and a summary of the trial will be submitted to the IRB/IEC at the end of the study.

9. DATA COLLECTION AND DATABASE MANAGEMENT

9.1 Data Collection

Data will be collected on the electronic Case Report Forms (eCRFs). All data entered in the eCRFs must be derived from the source documents, and the data must be consistent with the source documents. All eCRFs will be completed in their entirety to ensure accurate interpretation of data. The eCRF must be signed by the investigator, and the signing is considered to be the authorization of the eCRFs. Should a correction be made, the corrected information will be entered in the eCRFs overwriting the initial information. The eCRF is password protected so that any changes or alterations can be properly retraced and assigned to a certain person.

9.2 Confidentiality

Medical information of individual patient obtained as a result of this study is considered confidential. The patients will be assured that all findings will be stored in central database and handled in the strictest confidence. Data generated in this trial will be stored using a numeric code, without indicating the name of the patients. Disclosure to third parties is prohibited with the exceptions noted below. Data may be available for audit or inspection on request by principal investigator or the IRB/IEC. Any party with direct access to the data should maintain the confidentiality.

9.3 Database Management and Quality Control

Quality control will be applied to ensure that all data are reliable and have been processed correctly, both during and after the trial. After the data is entered in the

eCRFs, the principal investigator's designee must perform source data verification check systematically for accuracy, consistency, completeness, and reliability, including a comparison of the data in eCRFs with source documents. Data should only be included in the final analysis when checks have been satisfactorily completed. If data does not pass validation rules, data queries will be addressed to the investigator to request clarification or correction. The investigator is obliged to respond by confirming or modifying the data questioned.

10. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

10.1 General Considerations

A general description of the statistical methods is outlined below. A more detailed Statistical Analysis Plan will be provided in a separate document that will be finalized prior to database lock.

All summary statistics will be computed and displayed by treatment group. Descriptive statistics such as mean, median, standard deviation, inter-quartile range, minimum, and maximum will be used to summarize continuous variables. Counts and percentages will be used to summarize categorical variables. Graphical data displays may also be used as appropriate.

10.2 Analysis Sets

10.2.1 Intention-to-Treat Set

The intention-to-treat (ITT) set will be comprised of all subjects who have a signed valid informed consent, successfully complete the preliminary qualification procedures and are subsequently randomized to the RIC group or the sham RIC group.

10.2.2 Per-Protocol Set

The per-protocol (PP) set is a subset of the ITT set, consisting of subjects who do not have any major protocol deviations.

10.2.3 Safety Set

The safety set will be comprised of only those enrolled subjects in whom a study device was used, and will include the subjects as they are treated in the case where a subject is treated with a device that differs from their randomization assignment.

10.3 Sample Size Determination

The recruited sample size was determined to be up to 3000 (1500 for each group) based on the likelihood that approximately 10% loss to follow-up and 5% noncompliance. Statistical sample size estimation for the primary endpoint is as follows:

- Log-rank test
- 1:1 treatment assignment ratio
- 90% power
- 2-tailed $\alpha = 0.05$
- The primary event rate at 12 months in the control group = 14%
- Expected primary event rate at 12 months in the treatment group = 10.92%, i.e., an absolute reduction of 3.08%
- Exponential survival distribution
- Accrual duration = 2 years
- Total duration of the study = 3 years
- Therefore N = 2582 subjects (1291 subjects in each group), and 597 primary events are expected.

10.4 Efficacy Analyses

10.4.1 Analysis of Primary Efficacy Endpoint

The primary statistical null hypothesis is that there is no difference between treatment groups in survival distribution for time to first occurrence of non-fatal or fatal ischemic stroke after the randomization, and the alternative hypothesis is that there is a difference in the survival distribution between treatment groups.

The primary statistical hypothesis will be tested using a log-rank test, stratified by qualifying event (ischemic stroke/TIA). The primary analysis will be based on ITT set. The two-sided α level for the primary analysis will be 0.05. Hazard ratio (HR) and 95% confidence interval (CI) will be estimated using a Cox proportional hazards model, stratified by qualifying event, with treatment as the only explanatory variable.[21] Kaplan-Meier curve for both treatment groups will be presented.

A corresponding sensitivity analysis will be performed on the PP set.

Subgroup analyses of the primary efficacy endpoint will be performed on ITT set and PP set. For each subgroup, HR and 95% CI from the stratified Cox proportional hazards model as specified for the primary efficacy endpoint, will be performed by treatment group (except where the subgroup is the stratification factor, i.e., qualifying event).

The following subgroups will be included:

- Gender (male vs. female)
- Age (< 65 years vs. ≥65)
- Qualifying event (ischemic stroke vs. TIA)
- History of hypertension (yes vs. no)
- History of diabetes mellitus (yes vs. no)
- History of hyperlipidemia (yes vs. no)
- Previous ischemic stroke (yes vs. no)
- Previous TIA (yes vs. no)
- Previous myocardial infarction (yes vs. no)
- Smoking status (never vs. current or previous)
- Stenosis of qualifying artery (<70% vs. ≥70%)

Homogeneity of treatment effect in subgroups will be assessed via a test for treatment by subgroup interaction in the stratified Cox model with treatment, subgroup, and the treatment-by-subgroup interaction as the covariates. For qualifying event subgroups, unstratified Cox model will be used. P-values of interaction will be provided. Results will be graphically displayed using a forest plot.

10.4.2 Analyses of Secondary Efficacy Endpoints

Each secondary endpoint will be analyzed using the same statistical methods that are described for the primary efficacy endpoint on ITT set.

Other analyses described above for the primary efficacy endpoint may be performed for secondary efficacy endpoints.

10.4.3 Analyses of Other Efficacy Endpoints

Difference of mRS, NIHSS, and BI scores between baseline and month 12 in the mean with 95% CI will be calculated using analysis of covariance techniques with baseline value included in the ANCOVA model on ITT population.

10.5 Safety Analyses

All safety analyses will be conducted on the safety set. No formal statistical hypothesis testing between the treatment groups will be performed for the safety endpoints.

10.5.1 Adverse Events

The AEs and SAEs that occur after the initiation of study intervention will be included. All of them will be coded by system organ class using the Medical Dictionary for Regulatory Activities (MedDRA version 17.0 or a later release). An overall summary will be presented by treatment group for AEs and SAEs in terms of number and percentage of subjects having the event.

10.5.2 Adverse Events of Interest

AEs of interest will be summarized by treatment groups. These interested AEs are skin petechiae caused by cuff inflation, headache, dizziness, nausea, and heart palpitation.

11. STUDY MONITORING

Before enrollment of any patient in this trial, principal investigator will visit the medical center to determine the adequacy of the facilities. Appropriate training relevant to this trial should be provided to all the investigational staff prior to trial initiation.

The investigator is required to ensure compliance with all procedures required by the protocol. The investigator should not implement any deviation or change to the protocol without prior approval/favorable opinion from the IRB/IEC of an amendment.

The audit/inspection of this trial will be conducted by principal investigator or by the IRB/IEC, to systematically and independently examine that all related activities are conducted in compliance with the protocol. They will confirm that facilities remain acceptable, that investigational team is adhering to the protocol, and that data are being accurately and timely recorded in the eCRFs. They will also confirm AEs have been properly recorded in the eCRFs and any unexpected AEs and SAEs have been reported to principal investigator and the IRB/IEC.

In the event of health injury associated with this trial, principal investigator is responsible for compensation with a liability insurance in accordance with the legal requirements. This insurance provides cover for injury or death caused by the study. An insurance certificate will be provided to the IRB/IEC.

12. STUDY ORGANIZATION

12.1 Steering Committee

The steering committee will make ethical, scientific, and strategic decisions regarding the overall conduct of the trial, to ensure the study execution is of the highest quality. It will perform logistical coordination of different committees and will meet regularly to review the study progress. It will also review and approve the reporting and publications of the study.

Steering committee members: Xunming Ji (Chair), Longde Wang, Yang Hua, Junwei Hao, Jie Lu, Huisheng Chen, Wei Yue, Zhongrong Miao, Guozhong Li, Xiaobo Li, Xiaohong Chen, Zhenguang Li, Peifu Wang, Qi Fang, Wei Li, Lihua Wang, Runxiu Zhu, Ying Bai.

12.2 Executive Committee

The executive committee will help the steering committee maintain a high level of ethical, scientific, technical, and regulatory quality in all aspects of the trial. It will lead the successful implementation of the protocol according to the decisions made by the steering committee, and will monitor recruitment, compliance, and the adjudication process. It will meet more regularly to provide guidance for the day-to-day operations of the trial.

Executive committee members: Xunming Ji (Chair), Junwei Hao, Jiachun Feng, Wei Yue, Peimin Chen, Zongen Gao, Dingbo Tao, Huishan Du, Bozhuo Zhang.

12.3 Event Adjudication Committee

The event adjudication committee is comprised of clinicians who are not involved with the trial. The members will agree on standard definitions for the endpoints and will standardize adjudication procedures for assessing the endpoints. The members will also blindly review the endpoints reported by the investigators to determine whether the endpoints meet the criteria.

Event adjudication committee members: Qingfeng Ma, Yaou Liu, Rutai Hui, Yunyun Xiong.

12.4 Data Monitoring Committee

The data monitoring committee will meet periodically to monitor the progress of all aspects of the trial and ensure that the trial meets the highest standards of ethics and

patient safety. The members may suggest trial amendments regarding the safety of patients or early trial termination, but the final decision rests with the steering committee. Members of the data monitoring committee will not participate in the trial.

Data monitoring committee members: Gelin Xu, Chen Yao, Xiaokun Geng, Jun Chen (USA), Cesario V. Borlongan (USA).

13. PUBLICATIONS

Principal investigator has ownership of all data and results collected during this trial. All decisions regarding the use of data and results for public presentations and publications must be approved by principal investigator. The publication of the principal results from any single-center experience within the trial is not allowed before publication of the primary results.

All presentations and publications of the results will be based on clean, checked, and validated data in order to ensure the accuracy of the results. The results of this trial will be published irrespective of whether the results are regarded positive or negative.

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APPENDIX

Appendix 1 Definition of Endpoints

1. Stroke

Stroke is defined as an acute episode of focal neurological dysfunction associated with cerebral vascular injury, with no apparent non-vascular cause such as brain infection, trauma, tumor, seizure, severe metabolic disease, or degenerative neurological disease.

1.1 Ischemic Stroke

Ischemic stroke is defined as an acute episode of a new focal neurological deficit lasting >24 hours, an increase in existing focal neurological deficit lasting >24 hours, or a focal neurological deficit lasting <24 hours that is associated with evidence of new ischemic changes based on neuroimaging. The focal neurological deficit is not attributable to a non-ischemic etiology. Hemorrhage may be a consequence of ischemic stroke, and in this situation, the stroke is an ischemic stroke with hemorrhagic transformation but not a hemorrhagic stroke.

1.2 Hemorrhagic Stroke

Hemorrhagic stroke is defined as a new focal neurological deficit caused by a non-traumatic intraparenchymal hemorrhage, with neuroimaging evidence of corresponding intraparenchymal hemorrhage. It does not include the hemorrhagic transformation of an ischemic stroke.

2. Transient Ischemic Attack (TIA)

TIA is defined as a transient episode of a focal ischemic neurological deficit resolving completely within 24 hours, caused by focal cerebral or retinal ischemia, without evidence of corresponding cerebral infarction based on neuroimaging.

3. Myocardial Infarction

Myocardial infarction is defined as an episode of myocardial necrosis in a clinical setting consistent with myocardial ischemia. The diagnosis requires both the evidence of myocardial necrosis (changes in cardiac biomarkers or pathological findings) and the supporting information (clinical presentation, electrocardiographic changes, or myocardial or coronary artery imaging results).

4. Fatal Stroke or Fatal Myocardial Infarction

Fatal stroke or fatal myocardial infarction is defined as death occurring within 28 days following a confirmed outcome event in the absence of other definitive causes.

Appendix 2 Evaluation of Stenosis in Intracranial Artery

To measure the percent diameter stenosis, the investigator will measure the diameters of the stenosis ($D_{stenosis}$) of the target lesion and the reference normal vessel (D_{normal}) according to the WASID rules. The equation used for determining percent stenosis of a major intracranial artery is as follows: percent stenosis = $[(1 - (D_{stenosis}/D_{normal}))] \times 100$, where $D_{stenosis}$ = the diameter of the artery at the site of the most severe degree of stenosis and D_{normal} = the diameter of the proximal normal artery.

D_{normal} for the middle cerebral, intracranial vertebral, and basilar arteries: the diameter of the proximal part of the artery at its widest, nontortuous, normal segment is chosen (first choice). If the proximal artery is diseased (eg, middle cerebral artery origin stenosis), the diameter of the distal portion of the artery at its widest, parallel, non-tortuous normal segment is substituted (second choice). If the entire intracranial artery is diseased, the most distal, parallel, non-tortuous normal segment of the feeding artery is measured (third choice).

D_{normal} for the intracranial carotid artery: D_{normal} for the precavernous, cavernous, and postcavernous stenoses is measured at the widest, non-tortuous, normal portion of the petrous carotid artery that has parallel margins (first choice). If the entire petrous carotid is diseased, the most distal, parallel part of the extracranial internal carotid artery is substituted (second choice).

If tandem intracranial lesions are present, percent stenosis of both sites is measured and the more severe stenosis is selected. When a "gap sign" is present (ie, the lumen of the vessel cannot be visualized at the site of severe stenosis), $D_{stenosis}$ cannot be measured with calipers. In these cases, percent stenosis is defined as 99% luminal stenosis.

CONSORT Checklist