Remote Ischemic Perconditioning as an Adjunct Therapy to Thrombolysis in Patients With Acute Ischemic Stroke

A Randomized Trial

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Background and Purpose—Remote ischemic preconditioning is neuroprotective in models of acute cerebral ischemia. We tested the effect of prehospital rPerC as an adjunct to treatment with intravenous alteplase in patients with acute ischemic stroke.

Methods—Open-label blinded outcome proof-of-concept study of prehospital, paramedic-administered rPerC at a 1:1 ratio in consecutive patients with suspected acute stroke. After neurological examination and MRI, patients with verified stroke receiving alteplase treatment were included and received MRI at 24 hours and 1 month and clinical re-examination after 3 months. The primary end point was penumbral salvage, defined as the volume of the perfusion–diffusion mismatch not progressing to infarction after 1 month.

Results—Four hundred forty-three patients were randomized after provisional consent, 247 received rPerC and 196 received standard treatment. Patients with a nonstroke diagnosis (n=105) were excluded from further examinations. The remaining patients had transient ischemic attack (n=58), acute ischemic stroke (n=240), or hemorrhagic stroke (n=37). Transient ischemic attack was more frequent (P=0.006), and National Institutes of Health Stroke Scale score on admission was lower (P=0.016) in the intervention group compared with controls. Penumbral salvage, final infarct size at 1 month, infarct growth between baseline and 1 month, and clinical outcome after 3 months did not differ among groups. After adjustment for baseline perfusion and diffusion lesion severity, voxelwise analysis showed that rPerC reduced tissue risk of infarction (P=0.0003).

Conclusions—Although the overall results were neutral, a tissue survival analysis suggests that prehospital rPerC may have immediate neuroprotective effects. Future clinical trials should take such immediate effects, and their duration, into account.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00975962. (Stroke. 2014;45:159-167.)

Key Words: magnetic resonance scan • neuroprotection • perconditioning • stroke

Stroke is a leading cause of death and disability worldwide. Intravenous administration of alteplase (rtPA) within 4.5 hours of symptom onset is currently the only approved drug treatment of acute ischemic stroke (AIS). Various forms of neuroprotection have proven effective in animal models of acute ischemia, but their translation into successful therapies in human stroke has so far been unsuccessful. Remote ischemic preconditioning, by which ischemia induced in 1 organ leads to ischemic tolerance in other organs, is a potent endogenous protective mechanism. Preconditioning is not practical in a clinical setting because it must be initiated before the ischemic event. Remote ischemic perconditioning (rPerC) by which the sublethal ischemic stimulus is administered during the ischemic event may, however, be beneficial after an acute stroke. Both remote ischemic pre- and perconditioning have now been proven effective in animal models, and remote

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perconditioning was even found to be superior to preconditioning.6,7 In a recent study, rPerC was found to be effective alone and in combination with rtPA in a murine stroke model.7 The protective effect seemingly involves the activation of multiple endogenous defense mechanisms. These include an upregulation of nitric oxide levels, improved cerebral blood flow in the ischemic penumbra, and downregulation of inflammation and glutamatergic excitotoxicity.8 Moreover, rPerC seems to modulate cellular metabolism to become more energy efficient and thus increased cellular resistance to ischemia.9 rPerC confers maximal organ protection during 2 time windows. In animal studies, the first period starts immediately after the application of rPerC and lasts 3 to 6 hours, whereas the second period begins at 24 hours and lasts up to 4 days.10,11 The efficacy of prehospital rPerC has been tested in a randomized single-center study in patients with acute myocardial infarction at our institution, and the study showed rPerC to be safe and to increase myocardial salvage.12

We hypothesize that rPerC administered early after the onset of acute stroke symptoms would improve tissue survival in patients subsequently treated for an ischemic stroke by intravenous rtPA. The study was designed as an exploratory study, using MRI lesion volumes as surrogates to evaluate size of effect and mechanism of action. Primary outcome was defined as the penumbral salvage. Final infarct size, infarct growth, and clinical outcome at 3 months were secondary outcomes. In addition, we examined the risk of infarction in treated and untreated tissue according to the severity of the level of hypoperfusion at admission.

Methods

The design of this single-center, open-label, outcome observer blinded, randomized study is described elsewhere.13 Patient inclusion took place between June 2009 and January 2011 at Aarhus University Hospital, Aarhus, Denmark. The study complied with the Helsinki II declaration and was approved by the regional ethics committee (Protocol No.: M-20080148). The study was registered with ClinicalTrials.gov, number NCT00975962.

Before the study, regional ambulance personnel received training in obtaining provisional informed consent and in administering rPerC.

Patients

Eligible patients were ≥18 years of age with symptoms of acute stroke according to the prehospital services assessment, as per standardized national guidelines (modified Face, Arm, Speech Test [FAST] criteria).14 Patients deemed candidates for rtPA treatment within 4.5 hours of symptom onset after a telephone conference with the senior neurologist on call were then randomized after their provisional written consent.

On admission, patients were evaluated by a short neurological examination, and National Institutes of Health Stroke Scale (NIHSS) scoring, ECG, blood tests, and MRI were performed. When a history of stroke was confirmed and MRI showed acute infarct, patients were treated with intravenous rtPA if no contraindications were recorded. The patient’s signed consent to participate in the MRI follow-up study was obtained after the initiation of intravenous rtPA. Intravenous rtPA was followed by endovascular treatment in some cases. Follow-up MRI and NIHSS were performed after 24 hours and 1 month, and a clinical examination, including NIHSS and modified Rankin Scale, was performed after 3 months. For the patients lost to follow-up, last observation was carried forward either from baseline or from 1-month follow-up. All patients were treated according to national guidelines for the treatment of AIS by thrombolysis.

Exclusion criteria for the MRI follow-up study were contraindications to rtPA or MRI, known hypersensitivity to Gadovist or any of its ingredients, and acute or chronic severe renal impairment (glomerular filtration rate <30 mL·min⁻¹·1.73 m⁻²). Patients with a nonstroke diagnosis were excluded after the medical examination, and MRI scan was performed.

Randomization and Masking

For patients randomized to rPerC treatment prehospital before subsequent rtPA, rPerC was induced by 4 inflations of a standard upper limb blood pressure cuff to either 200 or 25 mmHg above the patient’s systolic blood pressure, each lasting 5 minutes and separated by 5 minutes of cuff deflation. The randomization was made via telephone by a staff nurse or an on-call physician not involved in the study. The randomization was performed by drawing from a large number of sealed opaque envelopes containing treatment instructions for the ambulance staff. The treatment allocation code was stored in a way such that they remained inaccessible to staff participating in the clinical follow-up or subsequent data analysis. On-call physicians examining patients on arrival at the department of neurology were not blinded to treatment instructions. These physicians did not participate in data analysis or follow-up clinical ratings. The readers of MRI scans were blinded to treatment allocation.

Procedures

rPerC was performed by ambulance staff during transportation. For patients with a transportation time too short for 4 cycles of inflation and deflation (n=45), the procedure was discontinued on arrival to the stroke unit. The number of inflations was recorded and stored with the randomization code.

A questionnaire was designed to detect any discomfort created by rPerC for all patients continuing in the MRI follow-up study on the day after admission. Pain in the relevant upper limb, nausea, headache, palpitations, anxiety, and sweating were recorded. The questionnaires were stored together with the randomization envelopes, inaccessible to the investigator until closure of the database.

The MRI protocol for baseline and 24-hour scans consisted of diffusion-weighted imaging (DWI), T2*, gradient-recalled echo, T2 fluid-attenuated inversion recovery (T2-FLAIR), time-of-flight magnetic resonance angiography, and perfusion-weighted imaging (PWI) using bolus injection (5 mL/s) of 0.1 mmol/kg body weight Gadovist (Gadobutrol, Bayer Pharma AG, Berlin, Germany). One-month follow-up MRI consisted of DWI, T2*, T2, T2-FLAIR, and 3-dimensional T1 inversion recovery fast spoiled gradient-recalled sequence.15

Postprocessing of perfusion data was performed using in-house software. Maps of mean transit time (MTT) were calculated by deconvolution of the tissue concentration curves with an automatically determined arterial input function16 using a tracer arrival time-insensitive method.16 The PWI lesion was defined as areas with a Tmax value exceeding 6 seconds.17 Spurious hyperintensities in unaffected regions were avoided by restricting this analysis to a manually outlined region on a map of time-to-peak15 where all potentially hypoperfused tissue was included.

Final lesions were outlined on 1 month T2-FLAIR images by 4 raters using semiautomated software. The 4 sets of lesion masks were combined to create a final common lesion mask comprising voxels marked by ≥2 readers. To ensure that only baseline infarcts were outlined, baseline T2-FLAIR, DWI, and apparent diffusion coefficient images were available to the readers. Readers were blinded to PWI and clinical data except for the lateralization of symptoms.

Penumbral salvage was quantified by identifying the tissue voxels in the volume difference between PWI and DWI at baseline which did not proceed to infarction according to the 1-month follow-up T2-FLAIR MRI.15 Infarct growth was defined as the difference between the baseline DWI lesion volume and the final infarct volume. Baseline DWI lesions were outlined semiautomatically by 1 rater. DWI and PWI were coregistered within subjects. Coregistration was performed in MATLAB 2010b (MathWorks Inc, Natick, MA).
using SPM8 (Wellcome Trust Centre for Neuroimaging, UCL, United Kingdom). DWI and MTT values were normalized to normal-appearing contralateral white matter in an area of interest above the brain ventricles (rDWI and rMTT). Vessel recanalization was rated on the Thrombolysis in Myocardial Ischemia (TIMI) scale by 2 trained neuroradiologists. Recanalization was defined as improvement of Thrombolysis in Myocardial Ischemia grading from baseline to 24-hour postarterial obstruction by ≥2 points, as used in Echoplanar Imaging Thrombolytic Evaluation Trial (EPITHET; the adapted Thrombolysis in Myocardial Ischemia scale). Patients scoring 3 on the Thrombolysis in Myocardial Ischemia scale at baseline were considered to have a normal time-of-flight magnetic resonance angiography.

The volume of intracerebral hemorrhage was found on either MRI or computed tomography depending on which scan was obtained at baseline. The volume was calculated by the formula: \( \frac{A \times B \times C}{2} \), where \( A \) is the greatest hemorrhage diameter, \( B \) is the diameter 90° to \( A \), and \( C \) is the number of slices which show signs of hemorrhage.

Calculations and Statistical Analyses

Patient-Level Analysis
Baseline patient data in the intervention and control group, respectively, were compared using \( \chi^2 \) tests. The effect of rPerC on modified Rankin Scale was analyzed using proportional odds logistic regression, suitable for neuroprotection. To assess whether rPerC was associated with increased discomfort, a logistic regression was fitted with treatment received as the dependent variable and change in discomfort (after minus before) as predictors. Death in patients with primary intracerebral hemorrhage (PICH) at 3 months was analyzed using Fisher exact test. Logistic regression was used to detect any association between the PICH volume and the time from symptom onset. Baseline DWI and PWI lesion volume, PICH volume, and time delay, penumbral salvage, final infarct size, and infarct growth were compared across groups using Wilcoxon rank-sum test.

Tissue-Level Analysis
To quantify the risk of infarction in treated and untreated tissue in relation to the severity of the level of hyperperfusion at admission, a logistic regression model was fitted using voxelwise DWI and PWI values as covariates. This model allows quantification of the risk of infarction across the range of DWI and PWI values. By including rPerC as a factor in the model, the effect of treatment can be quantified as a change in the risk of tissue infarction adjusted for the severity of initial DWI and PWI abnormalities. The model included interactions to allow treatment to affect the change in infarct in different ways in, for example, core and penumbra. Correlation among voxels from the same subject was modeled by adding a random effect for subjects. Balance between infarcting and noninfarcting voxels was ensured by sampling noninfarcting voxels from the PWI and DWI lesions as well as contralateral normal tissue to match the number of infarcting voxels.

The mixed effects models were fitted using penalized quasi-likelihood in the statistical software R (package MASS). Because rPerC was allowed to interact with baseline PWI and DWI values, a maximum likelihood test was used to assess the overall significance of rPerC. To ensure that proper values of the maximum likelihood were obtained, we used a Laplace approximation as the basis for these tests (package lme4 library), using a random sample of size 200,000, because this algorithm was unable to fit the entire data set (exceeding 380,000 data points). Data are presented as median and interquartile range (IQR). A \( P \) value <0.05 was considered statistically significant. An analysis plan was prepared before unblinding of clinical and MRI data.

Role of the Source of Funding
The sponsors were not involved in the study design, collection, analysis, and interpretation of data, writing of the report or the decision to submit the report for publication. The corresponding author had full access to all the data in the study and had the final responsibility for the decision to submit for publication.

Results

Figure 1 shows the trial profile. A total of 443 patients were randomized in the ambulance: 247 were treated with rPerC and 196 were not treated. The randomization imbalance was caused by a misunderstanding: accordingly, final written consent was not obtained in patients randomized to no rPerC in the initial period, and their data were therefore lost to follow-up. Of the randomized patients, 105 (24%) patients were found to have a nonstroke diagnosis and were excluded from the analysis; 298 had an ischemic event (transient ischemic attack [TIA], \( n=58 \); AIS, \( n=240 \)), 184 were treated with intravenous rtPA, and 37 had a PICH (1 subarachnoid hemorrhage excluded from the analysis). Of the 298 patients with an ischemic event, 32 were examined by computed tomography at baseline. Clinical data at 3 months were available for 285 (96%) patients. Baseline MRI was performed in 266 (89%) patients, and 171 (60%) gave consent to continue in the MRI follow-up study. For the patients with ischemic events, there were no significant differences in baseline characteristics whether they continued in the MRI or not (Table 1). DWI lesions at baseline (available for 262 patients) showed no statistical difference; \( P=0.19 \) between the intervention group (median, 0.91 mL; IQR, 0.4–6.69) and the control group (1.16 mL; IQR, 0.26–5.40). PWI lesions at baseline (available for 201 patients) showed a nonsignificant \( (P=0.08 \) tendency toward smaller lesion volumes in the intervention group (median, 2.1 mL; IQR 0–6.67) compared with controls (10.5 mL; IQR, 0–9.44).

The number of patients with TIA was significantly higher \( (P=0.006 \) in the intervention group \( n=42 \) compared with the control group \( n=16 \). In the TIA group, 7 of the 42 rPerC-treated patients were DWI positive, compared with 5 DWI-positive patients of 16 patients with TIA who did not receive rPerC. Baseline NIHSS score in patients with AIS and TIA was significantly lower \( (P=0.016 \) in the intervention group (median, 4; IQR, 2–7) compared with controls (5; IQR, 3–11). The baseline PWI volume correlated with NIHSS score at baseline (Pearson \( r <0.00001 \). Three-month modified Rankin Scale score was available for 224 of 240 patients with AIS and showed no significant difference between the 2 groups (Figure 2). The probability of improved outcome on modified Rankin Scale at 3 months in the intervention group was an odds ratio of 1.19 (0.69–2.09; \( P=0.54 \).

Of the 154 patients undergoing follow-up MRI, 3 patients were post hoc reclassified as not having had a stroke and 2 patients were excluded because of missing MRI data. Of the remaining 149 patients (50%), 81 were treated by rtPA and rPerC (number of inflations, 4; \( n=33 \); 3; \( n=22 \); 2; \( n=18 \); 1; \( n=5 \); not known: \( n=3 \) ) and 68 were treated by rtPA and acted as the control group. Of the 149 patients, 121 had a baseline PWI scan. There were no differences in demographic and clinical characteristics between the 2 groups regarding age, onset to treatment time, NIHSS score at baseline, systolic and diastolic blood pressure, hypertension, smoking, diabetes mellitus, former TIA, or stroke.
We found no significant difference in penumbral salvage, final infarct size, and infarct growth between the intervention and control group, respectively. Information on recanalization was not included in these analyses. The MRI data are shown in Table 2.

The tissue-level analysis showed a treatment-dependent change in infarct risk when correcting for the differences in baseline values of MTT and PWI among the intervention group and controls. Figure 3 shows the overall infarct risk for tissue with a fixed degree of DWI elevation at admission (rDWI=1.2) across all values of rMTT for rPerC-treated patients and controls. The figure shows a uniform decrease in tissue infarct risk across all values of rMTT, that is, for all levels of (hypo)perfusion. Figure 3B shows tissue infarct risk according to patient vessel status at arrival and after rtPA treatment (24 hours). Notably, rPerC is associated with a reduction of infarct risk in DWI-positive tissue for patients with no baseline occlusion (n=68). In patients with persisting occlusion (n=13), there is a reduction of infarct risk except for severely prolonged MTT. However, there is an increase in tissue infarct risk for patients in whom recanalization was achieved during the initial 24 hours (n=44).
A complete questionnaire was filled in by 80 patients. Of these, 30 answered that they did not remember anything from the ambulance transportation. There were no reports of significant discomfort in any of the 2 groups, and most patients did not recall discomfort at all. Recall of pain was significantly higher in patients treated with rPerC ($P=0.006$). rPerC did not induce significant anxiety, sweating, palpitations, headache, or nausea.

In patients diagnosed with a PICH, there was no difference in hematoma volume ($P=0.7$) between the intervention (n=22; 18.9 mL; IQR, 8–40) and the control group (n=14; 22.9 mL; IQR, 5–55). The time from onset to time of MRI did not differ (median, 104 minutes; IQR, 84–139 versus 122 minutes; IQR, 91–202; $P=0.5$), and there was no difference in mortality after 3 months (6/22=27% versus 5/14=36%; $P=0.7$).

**Discussion**

This study is the first randomized controlled trial to examine the effects of rPerC in patients with acute stroke. The primary mechanism of action was suspected to be neuroprotection during the reperfusion phase. Accordingly, the study was designed as a proof-of-concept study to test the efficacy of rPerC as an adjunct to rtPA. In the subgroup of randomized patients who were diagnosed with ischemic stroke and treated with rtPA (149/240; 62%) on arrival, MRI-derived tissue indices of cerebral damage were used to evaluate any differences in infarct evolution. The MRI study showed no significant effect in the unadjusted analysis of rPerC on penumbral salvage, final infarct size, and infarct growth. Surprisingly, rPerC-treated patients showed significantly lower NIHSS scores and higher frequency of TIA diagnosis than controls. Meanwhile, MRI at admission showed a tendency toward smaller perfusion deficits and milder DWI changes in rPerC-treated patients compared with controls. Treatment with rPerC was initiated in the ambulance shortly after symptom presentation and $\approx$1 hour before MRI. Symptom severity was not assessed before rPerC treatment, and therefore, an initial imbalance in symptom severity among patients randomized to rPerC and non-rPerC cannot be ruled out.

The voxel-based logistic regression analysis permitted us to address treatment-related changes in infarct risk, factoring in any imbalances in baseline DWI and PWI lesion severity.
among the 2 groups. This approach has previously been shown to increase the sensitivity to treatment-related effects during that of patient-level analysis. Overall reduction in the risk of infarction for tissue subjected to rPerC, and the likelihood ratio test for effect of rPerC in the whole group was \( P = 0.0003 \) (Figures 3A and 4A).

The distribution of DWI lesion intensities seems left-shifted for patients treated with rPerC during transportation to the hospital, suggesting a lower degree of cytotoxic edema and therefore potentially less tissue damage when perfusion is promptly restored. Consequently, the beneficial effects of rPerC may not be limited to penumbral tissue, but seems to pertain to tissue within the DWI lesion.

The study was not powered to show effect in clinical outcome at 3 months. Before the study was initiated and based on the results from a study in patients with acute myocardial infarction, it was thought that the mechanism of action of rPerC would be protection in the reperfusion phase in patients with proven arterial occlusions at admission. Surprisingly, clinical and MRI data suggested an immediately acting neuroprotective effect of the rPerC treatment. The rPerC-treated patients with favorable status at admission were less likely to receive rtPA and to be included in MRI follow-up. To account for the difference in the distribution of perfusion (rMTT) and rDWI values between rPerC-treated and rPerC-untreated groups on arrival to the hospital at baseline, a model of tissue risk of infarction was used.

rPerC was associated with a reduced risk of infarction in tissue with elevated DWI image intensity (Figure 4B); a radiological sign otherwise associated with poor tissue outcome. To account for the effects of recanalization as a result of the parallel treatment by rtPA, the tissue-level analysis was also performed according to vessel status before and after rtPA treatment. This analysis showed a clear reduction in infarct risk across all DWI and MTT values for patients in whom no vessel occlusion was found at admission. Furthermore, there was a clear reduction in infarct risk irrespective of baseline MTT and rDWI in the patients in whom parallel rtPA treatment did not change vessel status. We interpret this finding consistent with the significant lower NIHSS score at admission before if at all rtPA was administered that rPerC may be efficient in AIS even in patients in whom rtPA cannot be administered, irrespective of vessel status.

The overall findings of our study are therefore consistent with findings in animal studies testing remote ischemic

Table 2. MRI Results for Patients Treated With Preconditioning (+rPerC) and Controls (−rPerC)

<table>
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<tr>
<th></th>
<th>+rPerC</th>
<th>−rPerC</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penumbral salvage, mL, median (IQR) (n=121)</td>
<td>11.89 (0.53 to 63.39)</td>
<td>14.10 (1.60 to 79.82)</td>
<td>0.20</td>
</tr>
<tr>
<td>Final infarct size after 1 month, mL, median (IQR) (n=149)</td>
<td>1.63 (0.35 to 20.09)</td>
<td>1.99 (0.35 to 16.19)</td>
<td>0.97</td>
</tr>
<tr>
<td>Infarct growth, mL, median (IQR) (n=149)</td>
<td>0 (−0.62 to 8.01)</td>
<td>0.02 (−0.95 to 4.96)</td>
<td>0.79</td>
</tr>
<tr>
<td>Acute DWI volume, mL, median (IQR) (n=149)</td>
<td>1.52 (0.61 to 9.28)</td>
<td>1.54 (0.60 to 7.11)</td>
<td>0.99</td>
</tr>
<tr>
<td>Acute PWI volume, mL, median (IQR) (n=121)</td>
<td>17.35 (1.01 to 87.18)</td>
<td>16.55 (1.71 to 170.16)</td>
<td>0.26</td>
</tr>
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DWI indicates diffusion-weighted imaging; IQR, interquartile range; PWI, perfusion-weighted imaging; and rPerC, remote ischemic perconditioning.
Figure 4. Infarct risk including all values of initial rDWI. The frequency of tissue voxels with a given combination of rMTT and rDWI is shown as the elevation of the 3-dimensional surface above the horizontal plane, and the corresponding infarct risk is shown by a surface color code. To aid interpretation, gridlines have been added at rDWI values 0.5, 1.0, and 1.5 and rMTT 0, 5, 10, 15 s. A, Infarct risk for all values of rDWI. B, Infarct risk for patients with no recanalization at 24 hours. C, Infarct risk for patients with recanalization at 24 hours. D, Infarct risk for patients with no vessel occlusion at baseline or 24 hours. rDWI indicates relative diffusion-weighted imaging; rMTT, relative mean transit time; and rPerC, remote ischemic perconditioning.
pre- and perconditioning in stroke models and showed infarct reduction as a result of these treatments. 5–7 The translation into a possible clinical benefit remains to be shown. Recently, a clinical study in the poststroke phase in patients with intrar-arterial stenosis also indicates a neuroprotective effect on remote preconditioning. 23 Interestingly, this study also found improved perfusion status after bilateral upper limb induced ischemia.

This study shows that rPerC induced by intermittent upper arm ischemia, given to all patients with suspected AIS, is safe. A recent meta-analysis has shown that a nonstroke diagnosis is found in 26% of patients admitted under suspicion for stroke regardless of the referral source. 32 In the present study, 24% had a nonstroke diagnosis, and probably the telephone conference before admission and use of acute MRI helped to improve diagnostic accuracy. 33 rPerC was well tolerated and did not induce intolerable discomfort in the subgroup of patients with stroke. The results of our questionnaire analysis are consistent with other studies. 12,34,35 We could not detect any adverse effect on hematoma volume in patients presenting with PICH, and there were no excess mortality in the group treated with rPerC. Other studies have not reported on bleeding problems caused by or worsened by rPerC.

Our study has some methodological limitations. The follow-up group comprised only patients with MRI-proven stroke subsequently treated with intravenous rtPA and not randomized stroke mimic or patients with TIA. A classical intention-to-treat analysis could therefore not be performed, and the results are based on per-protocol analysis. Also, because of the small number of patients in each group, we have not adjusted for a reduced number of inflations in the analysis. Furthermore, because the voxel survival analysis was not a prespecified primary or secondary end point, the results must be interpreted with caution and can only be used as a hypothesis-generating result. Because patients with TIA may have had an effect of rPerC before baseline, the MRI study was a selected group of rPerC-treated patients at follow-up consisting of patients with more severe strokes compared with those excluded at baseline. The number of patients with large vessel occlusion in the study cohort was small, and therefore the presence of a protective effect in this subgroup of patients could not be ruled out. Furthermore, in patients, who recanalize late after symptom onset, the effect of rPerC may have been worn out. The effect of rPerC is described in 2 windows: one starting when the treatment is given and lasting 3 to 6 hours, and the second window starting after ≈24 hours and lasting 96 hours. 11

Neither the primary nor any of the secondary outcomes could be achieved in our study. Although previous clinical studies 36 of the beneficial effect of rPerC on reperfusion injury in the acute myocardial infarction have been conducted in patients who were mechanically revascularized, this is the first study to investigate the efficacy of rPerC in patients who achieved reperfusion by thrombolysis. It may be speculated whether reperfusion achieved by thrombolysis may be gentle because recanalization is often gradual or partial and hence more inherently protective via a postconditioning-like mechanism than abrupt mechanical reperfusion, hence attenuating the potential for a maximum rPerC effect. In a recent experimental model using rPerC at 2 hours after embolic middle cerebral artery occlusion in the mouse with and without intravenous rtPA at 4 hours, individual treatments with rPerC and intravenous rtPA reduced the infarct size similarly. Combination therapy of rPerC and rtPA resulted in additive effects in further improving the neurological outcome and reducing the infarct size. 7 In accordance with our findings the study also revealed early improvement of cerebral perfusion by rPerC independent of reperfusion. These findings indicate not only that the mechanisms of rPerC may not be confined to reperfusion injury, but also that additional proof-of-concept studies are needed to identify timing and extension of and mechanisms behind this intervention. Recanalization after 7 to 8 hours is in the gap between the 2 windows, when the treatment may no longer be effective. rPerC might be administered repeatedly to prolong the effect of rPerC, 37 and a stronger stimuli using bilateral upper limb ischemia and 5 cycles of ischemia followed by reperfusion 29 may further improve the effect of rPerC.

The randomization in this study was unequal. Because of a procedural error during the initial study period, final consent was not obtained from patients randomized to no rPerC treatment. Hence, these control patients were initially not registered as study participants. This imbalance may have affected the clinical outcome data but does not affect the tissue-level results in that this approach is inherently adjusted for any imbalance in baseline FWI and DWI.

In conclusion, rPerC during transport to hospital had no statistically significant effect on salvage, infarct size, or infarct progression as measured by MRI in a subgroup of patients and 3-month clinical outcome. However, when adjusted for baseline severity of hypoperfusion, a voxel-by-voxel analysis demonstrated increased tissue survival after 1 month suggesting that prehospital rPerC may be neuroprotective. Baseline clinical data from all patients randomized in ambulance whether or not they continued in the MRI follow-up study or were treated with rtPA support the positive signal of rPerC treatment seen in the tissue survival analysis. Further studies and an optimized study design taking the results from this explorative study into consideration are needed to prove a meaningful clinical effect.

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Disclosures
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Remote Ischemic Preconditioning as an Adjunct Therapy to Thrombolysis in Patients With Acute Ischemic Stroke: A Randomized Trial

Kristina Dupont Hougaard; Niels Hjort; et al.

卒中具有高致死率、高致残率的特点。发病4.5小时内静脉应用rtPA是目前唯一被批准的急性缺血性卒中治疗方法1。多种神经保护剂虽在急性缺血性动物模型中被证实有效，但到目前为止，都尚未成功用于人类卒中的治疗2。远端缺血预处理，即通过诱导一个脏器缺血引起其他脏器的缺血耐受，是一种有效的内源性保护机制3。由于须在缺血事件发生前给予，预处理并不适用于临床。然而，在缺血事件中给予远端缺血同处理(rPerC)可能对急性卒中有益4。远端缺血预处理和同处理均在动物模型中被证实有效，且同处理优于预处理5,6。近期对鼠卒中模型进行的一项研究发现，rPerC单独或与rtPA联合应用均有效7。其保护作用可能与多种内源性防御机制的激活有关，包括NO水平上调、缺血半暗带区血流量增加、炎症反应及谷氨酸兴奋毒性下调等8。此外，rPerC还可调节细胞代谢，提高能量利用率，从而增强细胞对缺血的耐受能力9。rPerC在2个时间窗内发挥最大的器官保护作用。在动物研究中，第一个时间窗始于给予rPerC即刻，并持续3~6小时，第二个时间窗则始于24小时后并持续4天10,11。本研究所对院前rPerC可能对于急性卒中患者静脉应用rtPA的辅助治疗作用进行了探索性研究，采用MRI损伤容积来评价其作用效果和机制。主要终点为缺血半暗带的挽救情况，即1个月后灌注-弥散不匹配区不进展为梗死。

方法

该单中心、开放、对结果观察者盲法、随机研究的设计方法见文献13。研究对象为2009.06-2011.01就诊于丹麦奥胡斯大学医院的患者。该研究遵守Helsinki II宣言，并获得了地区伦理委员会的批准。在研究开始前，对本地区救护人员进行了相关培训，包括获取临时知情同意和实施rPerC。

患者

入选的患者为≥18岁、院前根据标准的国家指南(modified Face, Arm, Speech Test[FAST]标准)评估有急性卒中症状者14。发病时间在4.5小时以内、准备接受rtPA治疗的患者，与高年资神经科专家进行电话会议并签署临时知情同意书后，进行随机分组。入院时，对患者进行神经系统检查评估、NIHSS评分、ECG、血液检测及MRI检查。一旦明确卒中类型且MRI显示急性梗死，即给予无使用禁忌症的患者静脉rtPA治疗。启动静脉rtPA治疗后，患者签署参与MRI随访研究的同意书。部分患者在静脉rtPA后还接受血管内治疗。分别在24小时、1个月后对患者进行MRI及NIHSS评分随访，并在3个月后对患者进行包括NIHSS和改良的
Rankin 评分在内的临床检查。对于失访患者,采用在基线或 1 个月随访时最后一次的观察结果。所有患者根据 AIS 国家治疗指南的溶栓推荐进行治疗。

MRI 随访研究的排除标准为存在 rtPA 或 MRI 禁忌、对造影剂钆布醇或其中任何成分过敏及急慢性肾损害(肾小球滤过率 < 30mL·min⁻¹·1.73m²⁻¹)。临床检查、MRI 扫描后确定为非卒中的患者被排除在外。

随机化和盲法

对于 rtPA 前随机接受院前 rPerC 治疗的患者,使用标准上肢血压套囊进行 4 次充气,使血压上升至 200mmHg 或高于患者收缩压 25mmHg,每次持续 5 分钟后放气 5 分钟。随机化由不参与该研究的护士或值班医师通过电话进行。通过随机抽取装有治疗方案的密封不透明信封实现随机化。治疗方案分配编码对参与临床随访或后期数据分析的人员设盲。治疗方案的分配对评估 MRI 的医师设盲。

步骤

由救护车在转运过程中为患者实施 rPerC。对于转运时间过短,不能完成 4 个充气、放气循环的患者(n=45),进入卒中单元后即终止操作。记录充气次数并与随机编码一起保存。

入院当日,为所有继续 MRI 随访研究的患者建立调查问卷,以记录由 rPerC 引起的任何不适。记录内容包括使用侧上肢疼痛、恶心、头痛、心悸、焦虑、出汗等。调查表与随机化信封一起存放,对调查者设盲直至数据库关闭。

首次和 24 小时后 MRI 的检查序列包括扩散加权成像 (DWI)、T2* 梯度回波序列(T2*GRE)、T2 液体衰减翻转恢复序列(T2-FLAIR)、时间飞跃法(TOF)磁共振血管成像(MRA)和灌注加权成像(PWI)(对比剂:钆布醇,0.1 mmol 体重,团注速度:5 mL/s,德国柏林拜耳制药公司)。1 月后复查的 MR 扫描序列包括:DWI、T2*WI、T2WI、T2-FLAIR 和 3D-T1 翻转恢复快速梯度回波序列(3D-T1RFSGR)。

采用自编的软件对灌注图像进行后处理,采用示踪剂到达时间不敏感的方法来自动确定动脉输入功能,再通过组织浓度曲线的去卷积法计算平均通过时间图(MTT)。在 PWI 上,将最大平均通过时间超过 6 秒的区域定义为病灶。为避免未受累区域的高信号伪影区,在包含所有潜在低灌注区的达峰时间(TTP)图上人工手绘兴趣区。

在 1 月后随访的 T2-FLAIR 图像上,4 位评审者采用半自动软件绘出最终病灶。这四套病灶伪影结合形成一套包含像素的同一病灶伪影,并由≥ 2 个评审者进行标记。为了确保检查的仅为基线梗塞灶,评审者可以参阅 T2-FLAIR,DWI 和表观扩散系数(ADC)图像。除了知晓患者症状出现的侧别,评审者对 PWI 和其他临床资料并不知晓。

通过绘制基线 PWI 和 DWI 不匹配区域的组织像素,量化可挽救的半暗带,以此区域在 1 个月后复査的 T2-FLAIR 上不进展为梗死[13]。基线 DWI 上的病灶体积与最终梗塞体积有差异时,定义为梗死扩大。DWI 和 MTT 值的标准化(rDWI 和 rMTT),是根据表现正常的对侧侧脑室以上层面的白质兴趣区。由两名经培训的影像学专家根据 TIMI 分级对血管再通情况进行评价[18,19]。血管再通的定义是,根据回波平面成像溶栓评价试验(EPIPHET),TIMI 评分从基线到血管阻塞后 24 小时增加 ≥ 2 分[18]。基线 TIMI 评分为 3 分的患者具有正常的时间飞跃法 MRA 表现。

根据基线 MRI 或 CT 来计算脑出血体积。出血体积依据公式: 

\[ A \times B \times (C \times \text{层面厚度})/2 \]

其中 A 为最大出血直径, B 为与 A 垂直的直径, C 为出血的层面数。

结果

图 1 为研究流程图。在救护车上将 443 例患者随机分组,其中 247 例给予 rPerC 干预治疗,另 196 例为对照组。在初始阶段由于误解,未能获取随机分配至非 rPerC 干预组患者的同意书,使得这部分患者失访,导致了随机化的不均衡。在入组的患者中,105 例(24%)因最终确定未患卒中而被排除在外,298 例存在缺血事件(短暂性脑缺血发作[TIA],n=58; AIS,n=240),184 例接受静脉 rtPA 治疗,37 例患有原发性脑出血(PICH,其中 1 例蛛网膜下腔出血者被排除在外)。存在缺血事件的 298 例患者中,32 例接受基线 CT 扫描。3 个月时的临床数据来自 285 例患者(96%)。266 例患者(89%)接受基线 MRI 检查,171 例患者(60%)签署参加后续 MRI 随访研究的同意书。对于存在缺血事件的患者,无论是否继续 MRI 检查,他们的基线特征无显著差异(表 1)。对 202 例执行基线 DWI 检查患者的分析显示,干抗组(中位数,0.91mL;IQR,0–4.69)与对照组(1.16mL;IQR,0.26–5.4)的基线 DWI 损伤无统计学差异(P=0.19)。对 201 例患者行基线 PWI 检查,相比对照组(10.5mL;IQR,0–94.4),干预组(中位数,2.1mL;IQR,0–66.7)基线 PWI 损伤体积并未显示出显著缩小的趋势。

干预组(n=42)中的 TIA 患者明显多于对照组(n=16)。在 TIA 亚组,7/42 例 rPerC 干预患者 DWI 病灶阳性,而 5/16 例无 rPerC 干预患者 DWI 病灶阳性。干预组(中位数,4;IQR,2–7)中 AIS 和 TIA 患者的基线 NIHSS 评分显著低于对照组(中位数,5;IQR,3–11)。在基线水平,基线 PWI 体积与 NIHSS 评分具有相关性(Pearson P<0.00001),3 个月时,对 240 例 AIS 患者中 224 例进行改良 Rankin 评分,两组间无统计学差异(表 1)。在干预组,根据 3 个月时 mRS 评分变化的趋同改善的率是优势比(OR)为 1.19(0.69–2.09;P=0.54)。

在进行 MRI 随访的 154 例患者中,3 例患者由于未患卒中,事后被重新分组,2 例患者由于 MRI 数据丢失而被排除在外。剩余 149 例患者中,121 例进行了基线 PWI 扫描。两组间在年龄、治疗时间、基线 NIHSS 评分、收缩/舒张压、高血压、吸烟、糖尿病、TIA 或卒中病史无统计学差异。

我们发现干预组与对照组间缺血半暗带挽救、最终梗死体积及梗死扩大无统计学差异意义。这些分析未包括血管再通的相关数据。MRI 数据详见表 2。

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校正干预组和对照组 MTT 及 PWI 间基线值差异后,对梗死风险的组织水平分析显示了治疗依赖性改变。在入院时 rPerC 干预组和对照组所有 rMTT 值中,图 3 以固定的 DWI 信号增高(rDWI=1.2)表示组织的总体梗死风险。该图显示在所有 rMTT 值也就是所有水平的低灌注中, rPerC 干预组组织梗死风险显著性下降。图 3B 根据患者入院时及 rtPA 治疗(24 小时)后的血管状况显示组织梗死风险。无基线梗死的患者(n=68), rPerC 干预与 DWI 阳性组织的

计算及统计分析(略)
梗死风险下降显著相关。在持续血管闭塞的患者中（n=13），除了 MTT 显著延长，其余接受 rPerC 干预的梗死风险均降低。然而，虽给予 rPerC 干预，最初 24 小时内血管再通患者（n=44）的组织梗死风险仍有所增加。

图 4A 以所有 rMTT 和 rDWI 像素值（根据色码）表示梗死风险及 rPerC 干预组和对照组中这些像素值的出现频率。对于任何 rDWI 和 rMTT 值，从平面（rDWI, rMTT）到叠加地形的距离表示组织像素具有这种典型 rDWI 和 rMTT 的比例。同时，地形的颜色表示梗死风险（见色带）。注意对照组和 rPerC 干预组地形像素颜色的差异：1 个月时，相比对照组，rPerC 干预患者大部分像素明显显示了较低的梗死风险，两组间的梗死风险具有显著的统计学差异（似然比检验 P=0.0003）。

图 4B 至 4D 中，根据患者入院和 24 小时的血管状态，将图 4A 中的像素进行细分。入院时无血管闭塞的 rPerC 干预患者（图 4D，似然比检验 P<0.0001）与入院及 24 小时随访时存在血管闭塞的患者（图 4B，似然比检验 P=0.002）的梗死风险具有显著的统计学差异。24 小时内血管再通患者与 1 个月随访时的梗死风险无显著统计学差异。

80 例患者填写了调查问卷。其中 30 例不能回忆救护车转运过程中的任何事。两组患者均无明显不适，且大部分患者无任何不适。rPerC 干预组患者回忆起疼痛的比例显著高于对照组（P=0.006）。rPerC 不引起明显的焦虑、出汗、心悸、头痛或恶心等不适。

注：1. 在初始化阶段，由于误解，随机分配到无 rPerC 治的患者，未获得最终结果而被剔除。患者数目未知。
2. 无论是否给予 rPA, 3 个月内追踪了 288/298 (96.6%) 的 AIS 或 TIA 患者。
在 PICH 患者中，干预组（n=22；18.9mL；IQR, 8-40）和对照组（n=14；22.9mL；IQR, 5-55）间的血肿体积无统计学差异（P=0.7）。

两组间自发病至 MRI 检查的时间无统计学差异（中位数，104 分钟；IQR, 84-139 vs 122 分钟；IQR, 91-202；P=0.5），且 3 个月后的死亡率亦无统计学差异（6/22=27% vs 5/14=36%；P=0.7）。

**讨论**

本研究是第一项在急性卒中患者中，观察 rPerC 作用的随机对照试验。rPerC 的主要作用机制可能是在再灌注阶段发挥神经保护作用。因此，本研究是一项观察 rPerC 作为 rtPA 辅助治疗的概念验证性研究。对诊断为缺血性卒中且入院时接受 rtPA 治疗的患者（149/240; 62%），采用脑损伤的核磁系列组织指数来评价梗死演变过程中的变化。在未校正的 rPerC 分析中，MRI 研究显示 rPerC 对缺血半暗带挽救、最终梗死面积、梗死扩大无显著影响。有趣的是，rPerC 干预组 NIHSS 评分显著低于对照组，而 TIA 的发生率则高于对照组。症状出现后不久在救护车上，即大约行 MRI 检查前 1 小时，就给予 rPerC 治疗。rPerC 治疗前未评估症状的严重程度，因此，不能排除随机分配至 rPerC 干预和对照组患者间症状严重程度的不平衡。

我们采用基于像素的 logistic 回归分析来处理干预相关的梗死风险改变，包含两组间基线 DWI 和 PWI 损伤严重程度的不平衡。这种方法以前已被证实可以提高对患者治疗作用的敏感性 [23,27,28]

本研究显示 rPerC 干预使脑组织梗死的总体风险下降，两组间 rPerC 疗效的似然比检验为 P=0.0003（图 3A 和 4A）。在转运至医院过程中接受 rPerC 治疗的患者，其 DWI 损伤密度分布看起来左移，提示当迅速恢复血流灌注时，存在较轻程度的细胞毒性水肿，因此组织的潜在损伤也少。因此，rPerC 的治疗获益并非仅限于半暗带区，而延及至DWI损伤区。

本研究未能观察 rPerC 对卒中后 3 个月临床转归的影响。在本研究开始前及基于以前急性心肌梗死患者的研究结果，对人院时需诊断 DAT 闭塞的患者，通常认为 rPerC 是在再灌注阶段发挥保护作用。令人惊奇的是，临床和 MRI 数据均表明 rPerC 在给予即刻就产生神经保护作用。给予 rPerC 治疗的患者，当入院时症状良好时，很少接受 rtPA 治疗，因而很少纳入 MRI 研究。为了说明人院时基线 rPerC 干预与非干预组间灌注分布（rMTT）和 rDWI 值的差异，我们使用了一个组织梗死风险模型。

rPerC 干预与 DWI 高信号组织区梗死风险的下降相关（图 4B）； DWI 高信号征提示组织转归不良 [30]。血管再通作为 rtPA 平行治疗的结果，为了评估血管再通的作用，根据 rtPA 治疗前后的血管状态，进行了组织水平的分析。对所有 DWI 和 MTT 值的分析显示，入院时无血管闭塞患者发生梗死的风险明显下降。不受血管基线 MTT 和 rDWI，在 rtPA 平行治疗不改变血管状态的患者，梗死风险也明显下降。该结果与患者入院时较低的 NIHSS 分值一致，如果给予 rtPA 治疗，则 rPerC 对 AIS 患者可能有效，甚至在未给予 rtPA 治疗的患者也有效，不考虑血管状态。

本研究总的结果与以动物卒中模型观察远端缺血预处理和同侧处理作用的实验结果一致，均表明这些干预可能减少梗死。[5-7] 接下来要证明的是这些干预是否可转换成可能的临床获益。最近，一项对颅内动脉狭窄患者卒中后阶段的临床研究显示，远端缺血预处理具有神经保护作用 [31]。有趣的是，这项研究还发现诱导双侧上肢缺血可改善预后。

本研究表明，给予疑似 AIS 患者的 rPerC 干预（通过间歇性上肢缺血实现）是安全的。近期一项 meta 分析显示，不考虑诊断参考资源，疑似卒中而收入院的患者中 26% 未患卒中 [32]。本研究中 24% 的患者未患卒中，入院前的电话会议及早期 MRI 检查有助于提高诊断的准确性 [33]。患者能很好的耐受 rPerC，其治疗未引起卒中的不良反应，不考虑患者的卒中率。

我们研究总的结果与一些方法学上的局限性。随访组仅由 MRI 证实患有梗死且接受 rtPA 治疗的患者组成，不包括类似卒中患者或 TIA 患者。研究结果是在按试验方案分析基础上进行，因此这不是经典的影响 – 治疗分析。另外，由于每组患者样本量较小，对于某些罕见的事件，如死亡或主要不良事件，可能有统计学上的限制。
图3. A, 随着DWI值增加 (rDWI=1.2)，根据组织平均通过时间 (MTT) 表示的组织梗死风险。曲线表示患者入院时远隔缺血同处理组 (rPerC) 和对照组的总体梗死风险。B, 溶栓组在基线和24 h时记录的血管闭塞和再通表示心肌缺血状况。rDWI 表示相对弥散加权成像; rMTT 表示相对平均通过时间。

Hougaard et al rPerC in Acute Stroke

<table>
<thead>
<tr>
<th></th>
<th>+ rPerC</th>
<th>- rPerC</th>
<th>P值</th>
</tr>
</thead>
<tbody>
<tr>
<td>可挽救半暗带, mL中位值（IQR） (n=121)</td>
<td>11.89 (0.53–83.89)</td>
<td>14.10 (1.90–79.82)</td>
<td>0.2</td>
</tr>
<tr>
<td>1个月后梗死面积, mL中位值（IQR） (n=149)</td>
<td>1.53 (0.35–20.09)</td>
<td>1.99 (0.35–16.19)</td>
<td>0.97</td>
</tr>
<tr>
<td>坏死灶, mL中位值（IQR） (n=149)</td>
<td>0.02 (–0.95–4.96)</td>
<td>0.02 (–0.95–4.96)</td>
<td>0.79</td>
</tr>
<tr>
<td>急性DWI缺损, mL中位值（IQR） (n=149)</td>
<td>1.52 (0.61–9.28)</td>
<td>1.54 (0.60–7.11)</td>
<td>0.99</td>
</tr>
<tr>
<td>急性PWI缺损, mL中位值（IQR） (n=121)</td>
<td>17.35 (1.01–87.18)</td>
<td>16.55 (1.71–170.16)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

DWI:弥散加权成像; PWI:灌注加权成像; IQR:百分位间距; rPerC:远隔缺血同处理。
图4. 梗死风险包括首次rDWI的所有值。水平面上3D图像呈现的是伴有rMTT和rDWI的组织像素频次，图像色码表示梗死风险。为了更好地说明，在rDWI值0.5、1.0、1.5以及rMTT值0.5、10、15s处分别加入了网格线。A，用rDWI值表示的梗死风险；B, 24h血管无再通患者的梗死风险；C，24h血管再通患者的梗死风险；D, 基线或24 h无血管闭塞患者的梗死风险。rDWI, 相对弥散加权成像；rMTT, 相对平均通过时间；rPerC, 远隔缺血处理。