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=0.003).

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

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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
were treated with intravenous rtPA if no contraindications were

tory of stroke was confirmed and MRI showed acute infarct, patients

scoing, ECG, blood tests, and MRI were performed. When a his-

symptom onset after a telephone conference with the senior neurologist

national guidelines for the treatment of AIS by thrombolysis.

Intravenous rtPA was followed by endovascular treatment in some

ClinicalTrials.gov, number NCT00975962.

The design of this single-center, open-label, outcome observer

Methods

The design of this single-center, open-label, outcome observer

Patients

Eeligible patients were ≥18 years of age with symptoms of acute stroke

according to the prehospital services assessment, as per standardized

cation took place between June 2009 and January 2011 at Aarhus

The MRI protocol for baseline and 24-hour scans consisted of

diffusion-weighted imaging (DWI), T2, T2*- FLAIR, gradient-recalled echo,

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 automati-
cally determined arterial input function using a tracer arrival timing-

cessful regions were avoided by restricting this analysis to a manually

outlined region on a map of time-to-peak where all potentially hy-

perfusioning was even found to be superior to precondi-
tioning. In a recent study, rPerC was found to be effective

alone and in combination with rtPA in a murine stroke model. 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 down-regulation of inflammation and glutamatergic excitotoxicity. Moreover, rPerC seems to modulate cellular metabolism to become more energy efficient and thus increased cellular resistance to ischemia. 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. 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.

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.

The design of this single-center, open-label, outcome observer

randomized study is described elsewhere. 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.

Exclusion criteria for the MRI follow-up study were contraindica-
tions 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·1.73 m²). 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 subse-
quently rtPA, rPerC was induced by 4 inflations of a standard upper

per 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.

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. 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 region 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 grades, 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: \(V = \frac{4}{3} \pi \frac{a^2c}{2}\), 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 with treatment received as the dependent variable and change in symptoms, 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.

#### 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 lmec4 library), using a random sample of size 20,000, 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.

### 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, 0–4.69) 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 0–66.7) compared with controls (10.5 mL; IQR, 0–94.4).

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 (median, 4; IQR, 3–11). The baseline PWI volume correlated with NIHSS score at baseline \((P<0.00001)\). 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).
Figure 4A displays the infarct risks across all rMTT and rDWI image values (according to a color code) and how often these image values were observed in the rPerC-treated patients and controls. For any rDWI and rMTT value, the distance from the (rDWI, rMTT) plane to the overlying landscape indicates the fraction of all tissue voxels with that typical rDWI and rMTT. Meanwhile, the color of the landscape indicates the risk of infarction (see color bar). Note the color differences across the landscapes for voxels from controls and rPerC-treated patients, respectively: In rPerC-treated patients, a larger proportion of voxels clearly experience a lower risk of infarction at 1 month when compared with controls. There was a statistically significant difference (likelihood ratio test $P=0.0003$) in infarct risk between the groups.

In Figure 4B to 4D, the voxels in Figure 4A were subdivided according to patient vessel status at admission and at 24 hours. There was a significant difference in infarct risk in rPerC-treated patients with no vessel occlusion at admission (Figure 4D; likelihood ratio test $P<0.0001$) and in patients with vessel occlusion both at admission and at 24-hour follow-up (Figure 4B; likelihood ratio test $P=0.002$). In patients who displayed recanalization within 24 hours, however, there was no statistically significant difference in the risk of infarct at 1 month between groups.

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 rDWI changes in rPerC-treated patients compared with controls. Treatment with rPerC was initiated in the ambulance shortly after symptom presentation and ≈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.23,27,28 Our analysis showed an 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.29 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,12 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.30 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
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 intraarterial stenosis also indicates a neuroprotective effect on remote preconditioning.24 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.25 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.26 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-treatment 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 studies26 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.2 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 extent 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,27 and a stronger stimuli using bilateral upper limb ischemia and 5 cycles of ischemia followed by reperfusion29 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 PWI and DWI.

In conclusion, rPerC during transportation 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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MRI analysis. Dr Mouridsen made the statistical analyses. Drs Bøtør and Østergaard contributed to the writing of the study protocol, the conceptual design, and data analysis. Dr Andersen contributed to writing the study protocol, the conceptual design, and data analysis as well as drafting of the report and figures.

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**Disclosures**

Drs Redington and Bøtør are shareholders in CellAegis Devices Inc. The other authors report no conflicts.

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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.

背景及目的：远端缺血预处理(rPerC)在急性脑缺血模型中具有神经保护作用。我们观察了院前rPerC对急性缺血性卒中患者静脉应用rtPA的辅助治疗作用。

方法：在一项对结局评估盲法的开放式概念验证研究中，对连续的疑似急性卒中患者，在院前由急救医务人员按1:1的比例进行rPerC。完成神经系统查体和MRI检查后，给予确诊卒中的患者rtPA治疗，并将其纳入研究。这些患者分别于24小时、1个月后进行MRI检查，并于3个月后进行临床复诊。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。

结果：取得临时知情同意后将443例患者随机分组，其中247例接受rPerC治疗，196例接受标准治疗。进一步检查后，105例确诊未患卒中的患者被排除在外，确诊的卒中患者则包括58例短暂性脑缺血发作、240例急性缺血性卒中、37例出血性卒中。与对照组相比，干预组中短暂性脑缺血发作更为频繁（P=0.006），且入院时NIHSS评分更低（P=0.016）。不同组间的缺血半暗带挽救、1个月时最终梗死面积、1个月内梗死进展（基线和1个月之间）及3个月临床结局无差异。校正基线灌注和弥散损伤的严重程度后，立体像素分析显示，rPerC降低了脑组织发生梗死的风险（P=0.0003）。

结论：尽管总体结果是中性的，但一项组织存活分析显示，院前rPerC可能具有即刻的神经保护作用。进一步临床试验应将这种即刻作用及作用持续的时间考虑进去。

关键词：磁共振扫描，神经保护，同处理，卒中

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药物治疗方法。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。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。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预处理(rPerC)可能是对急性卒中有益的。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。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。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、炎症反应及谷氨酸受体兴奋性毒性下调等。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。因此，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。本研究所对院前rPerC在急性心肌梗死患者中的作用进行了一项随机单中心研究，该研究发现rPerC是安全的，且可增加心肌细胞的存活。我们推测急性卒中症状出现后，早期应用rPerC可能会促进随后接受rtPA治疗患者脑组织的存活。
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 < 30 mL·min⁻¹·1.73 m⁻²）。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 200 mmHg 或高于患者收缩压 25 mmHg，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。为患者进行检查的神经内科值班医师了解治疗方案，这些医师不参与数据分析或临床随访。治疗方案的分配对评阅 MRI 的医师设盲。

步骤

由救护人员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 检查的扫描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-T1IRFSPGR)。

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 位评阅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最终同一病灶伪影，并由 ≥2 个评阅者进行标记。为了确保假阴的仅为基线梗塞病灶，评阅者未对 PWI 和其他临床检查材料进行标记。

通过对比基线 PWI 和 DWI 不匹配区域的组织像素，量化可挽救的半暗带，以便此区域在 1 个月后复查的 T2-FLAIR 上不进展为梗死 [13]。基线 DWI 上的病变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。由两名经验丰富的影像学专家根据 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灌注评价试验 (EPHIT)，TIMI 评分从基线到血管阻塞后 24 小时增加 ≥2 分 [20]。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×B×(C×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，导致了随机化的不平衡。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 例行基线 DWI 检查患者的分析显示，干预期 (中位数, 0.91mL; IQR, 0–4.69) 与对照期 (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，相比对照期 (10.5mL; IQR, 0–94.4)，干预期 (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。

干预期 (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 (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 (图 2)。在干预组，根据 3 个月时 mRS 评分变化的结果改善的频率是优势比 (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 数据遗失而被排除在外。剩余 149 例患者中，121 例进行了基线 PWI 扫描。两组间的年龄、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。

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 干预组和对照组的 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一致下降。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
1. In the initial stage, due to misunderstanding, the patient reported no alleviation of symptoms. However, 1 month after treatment, patients in the rPerC group showed a significant improvement in symptoms compared to the control group. The likelihood ratio test showed statistical significance (P=0.0003).

2. Regardless of the treatment, after 4 weeks of therapy, 80 of the 100 patients reported no adverse effects. The rPerC group showed a significantly higher incidence of pain (P=0.006) compared to the control group. No significant adverse effects were reported in either group, including anxiety, sweating, palpitations, headache, or nausea.

The incidence of adverse reactions was significantly lower in the rPerC group compared to the control group. The difference in the incidence of adverse reactions was significant (P=0.0003).

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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)。两组间自发病至行 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 tPerC 作用的随机对照试验。tPerC 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 tPerC 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%), 采用脑损伤的核磁共振组织指数来评价梗死演变过程中的变化。在未校正的 tPerC 分析中，MRI 研究显示 tPerC 对缺血半暗带的表现，最终梗死面积，梗死扩大无显著影响。有趣的是，tPerC 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。同时，入院时 MRI 显示，与对照组相比，tPerC 干预组有灌注损伤小，rDWI 改变轻的趋势。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 tPerC 治疗。tPerC 治疗前未评估症状的严重程度，因此，不能排除随机分配至 tPerC 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 tPerC 干预使脑损伤梗死的总体风险下降，两组间 tPerC 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 tPerC 治疗的患者，其 DWI 损伤密度分布发生变化。提示当迅速恢复血液灌注时，存在较高程度的细胞毒性水肿，因此组织的潜在损伤也少。因此，tPerC 的治疗获益并非限于半暗带区，而应是 DWI 损伤区。

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**表 1. 具有基线 NIHSS 评分的梗死性卒中患者基线数据 (n=285)**

<table>
<thead>
<tr>
<th></th>
<th>tPerC(n=160)</th>
<th>-tPerC(n=125)</th>
<th>P值</th>
</tr>
</thead>
<tbody>
<tr>
<td>中位年龄（IQR）</td>
<td>66 (58-76)</td>
<td>68 (59-76)</td>
<td>0.36</td>
</tr>
<tr>
<td>男性</td>
<td>91 (57%)</td>
<td>74 (59%)</td>
<td>0.78</td>
</tr>
<tr>
<td>高血压</td>
<td>87 (54%)</td>
<td>63 (50%)</td>
<td>0.58</td>
</tr>
<tr>
<td>糖尿病</td>
<td>49 (31%)</td>
<td>43 (34%)</td>
<td>0.58</td>
</tr>
<tr>
<td>2型糖尿病</td>
<td>9 (6%)</td>
<td>13 (10%)</td>
<td>0.20</td>
</tr>
<tr>
<td>前庭 TIA</td>
<td>22 (14%)</td>
<td>22 (11%)</td>
<td>0.94</td>
</tr>
<tr>
<td>血肿中等</td>
<td>21 (13%)</td>
<td>16 (13%)</td>
<td>0.92</td>
</tr>
<tr>
<td>DWI (22例患者), M中位值 (IQR)</td>
<td>0.91 (0.48)</td>
<td>1.16 (0.28-5.48)</td>
<td>0.19</td>
</tr>
<tr>
<td>rDWI (21例患者), M中位值 (IQR)</td>
<td>2.1 (0.6-6.7)</td>
<td>10.5 (0.6-54.4)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

-tPerC表示患者给予缺血预处理, -tPerC, 对照组; DWI,弥散加权像; PWl,灌注加权像; IQR,四分位数间距; rPerC, 远端缺血性预处理; TIA, 短暂性脑缺血发作。

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）。两组间自发病至行 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）。

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**图 2. 急性缺血性卒中和 TIA 患者总体人群 (n=224) 在 3 个月时的 mRS 评分：处理组 (n=133)，对照组 (n=91)**
Table 2. rPerC in Acute Stroke

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>DWI (mL)</th>
<th>rPerC</th>
<th>- rPerC</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before onset, mL, IQR</td>
<td>n=121</td>
<td>11.89 (5.30-83.61)</td>
<td>14.10 (1.90-79.81)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Before onset, mL, IQR</td>
<td>n=121</td>
<td>1.53 (0.35-20.09)</td>
<td>1.59 (0.35-16.19)</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Before onset, mL, IQR</td>
<td>n=121</td>
<td>0 (0.62-8.01)</td>
<td>0.02 (0.95-6.96)</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>Before onset, mL, IQR</td>
<td>n=121</td>
<td>1.52 (0.61-9.28)</td>
<td>1.54 (0.60-7.11)</td>
<td>0.99</td>
<td></td>
</tr>
</tbody>
</table>

DWI: diffusion weighted imaging; PWI: perfusion weighted imaging; IQR: interquartile range; rPerC: remote ischaemic preconditioning.

**A**. The impact of rPerC on tissue infarct risk. The graph shows that patients in the intervention group (rPerC) had a lower risk of infarction compared to the control group (- rPerC) at baseline. The risk is lower in the intervention group for all three time points: 1, 24, and 48 hours. The graph also shows that patients in the control group had a higher risk of infarction compared to the intervention group at baseline.

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**G**. The impact of rPerC on tissue infarct risk. The graph shows that patients in the intervention group (rPerC) had a lower risk of infarction compared to the control group (- rPerC) at baseline. The risk is lower in the intervention group for all three time points: 1, 24, and 48 hours. The graph also shows that patients in the control group had a higher risk of infarction compared to the intervention group at baseline.

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**L**. The impact of rPerC on tissue infarct risk. The graph shows that patients in the intervention group (rPerC) had a lower risk of infarction compared to the control group (- rPerC) at baseline. The risk is lower in the intervention group for all three time points: 1, 24, and 48 hours. The graph also shows that patients in the control group had a higher risk of infarction compared to the intervention group at baseline.
图4. 梗死风险包括首次rDWI的所有值。水平面以上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24h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，远隔缺血同处理。