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;45:00-00.)

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).1 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.2 Remote ischemic preconditioning, by which ischemia induced in 1 organ leads to ischemic tolerance in other organs, is a potent endogenous protective mechanism.3 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.4 Both remote ischemic pre- and perconditioning have now been proven effective in animal models, and remote
perconditioning was even found to be superior to preconditioning.\textsuperscript{5,6} In a recent study, rPerC was found to be effective alone and in combination with rtPA in a murine stroke model.\textsuperscript{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 down-regulation of inflammation and glutamatergic excitotoxicity.\textsuperscript{8} Moreover, rPerC seems to modulate cellular metabolism to become more energy efficient and thus increased cellular resistance to ischemia.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 hyperperfusion at admission.

### Methods

The design of this single-center, open-label, outcome observer blinded, randomized study is described elsewhere.\textsuperscript{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).\textsuperscript{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\textsuperscript{−1}·1.73 m\textsuperscript{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, accessible to the investigator until closure of the database.

The MRI protocol for baseline and 24-hour scans consisted of diffusion-weighted imaging (DWI), T2\textsuperscript{*}, 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\textsuperscript{*}, T2-FLAIR, and 3-dimensional T1 inversion recovery fast spoiled gradient-recalled sequence.\textsuperscript{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 function\textsuperscript{16} using a tracer arrival timing-insensitive method.\textsuperscript{16} The PWI lesion was defined as areas with a Tmax value exceeding 6 seconds.\textsuperscript{17} Spurious hyperintensities in unaffected regions were avoided by restricting this analysis to a manually outlined region on a map of time-to-peak\textsuperscript{15} 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 22 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.\textsuperscript{13} 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\textsuperscript{8,10} by 2 trained neuroradiologists. Recanalization was defined as improvement of Thrombolysis in Myocardial Ischemia grading from baseline to 24-hour postarterial obstruction by 2+ signs, as used in Echoplanar Imaging Thrombolytic Evaluation Trial (EPIThET; the adapted Thrombolysis in Myocardial Ischemia scale).\textsuperscript{11} 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: \((A \times B \times C \times \text{slice thickness})/2\), where \(A\) is the greatest hemorrhage diameter, \(B\) is the diameter \(90°\) to \(A\), and \(C\) is the number of slices which signs show up of hemorrhage.\textsuperscript{21}

### 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.\textsuperscript{22} 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.\textsuperscript{16,23} 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.\textsuperscript{24}

The mixed effects models were fitted using penalized quasi-likelihood in the statistical software R (package MASS).\textsuperscript{25} 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).\textsuperscript{26} 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.

### 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, 0–4.69) 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 (5; 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 intravenous 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 $\approx 1$ 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

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**Table 1. Baseline Data for Patients With Ischemic Stroke With Available Baseline NIHSS Score (n=285)**

<table>
<thead>
<tr>
<th></th>
<th>+rPerC (n=160)</th>
<th>−rPerC (n=125)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (IQR)</td>
<td>66 (58–76)</td>
<td>68 (59–76)</td>
<td>0.36</td>
</tr>
<tr>
<td>Men</td>
<td>91 (57%)</td>
<td>74 (59%)</td>
<td>0.78</td>
</tr>
<tr>
<td>Hypertension</td>
<td>87 (54%)</td>
<td>63 (50%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Smoking</td>
<td>49 (31%)</td>
<td>43 (34%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Diabetes mellitus type 2</td>
<td>9 (6%)</td>
<td>13 (10%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Former TIA</td>
<td>22 (14%)</td>
<td>14 (11%)</td>
<td>0.64</td>
</tr>
<tr>
<td>Former Stroke</td>
<td>21 (13%)</td>
<td>16 (13%)</td>
<td>0.92</td>
</tr>
<tr>
<td>DWI (262 patients), mL, median (IQR)</td>
<td>0.91 (0–4.96)</td>
<td>1.16 (0.26–5.40)</td>
<td>0.19</td>
</tr>
<tr>
<td>PWI (201 patients), mL, median (IQR)</td>
<td>2.1 (0.6–6.7)</td>
<td>10.5 (0–94.4)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

+rPerC indicates patients treated with perconditioning; −rPerC, controls; DWI, diffusion-weighted imaging; IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; PWI, perfusion-weighted imaging; rPerC, remote ischemic perconditioning; and TIA, transient ischemic attack.

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**Figure 2. Modified Rankin Scale (mRS) after 3 months for the whole population of patients with acute ischemic stroke and transient ischemic attack (n=224): treated group (n=133) controls (n=91).**

Treated: mRS=0: 58% mRS=1: 14% mRS=2: 8% mRS=3: 9% mRS=4: 5% mRS=5: 0% mRS=6: 4%
Uncertained: mRS=0: 47% mRS=1: 26% mRS=2: 15% mRS=3: 3% mRS=4: 5% mRS=5: 1% mRS=6: 1%
among the 2 groups. This approach has previously been shown to increase the sensitivity to treatment-related effects during that of patient-level analysis. 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. 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 DWI 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 DWI 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) |
|----------------------------------------|----------------------------------------|-----------------|
| Penumbral salvage, mL, median (IQR)   |
| (n=121)                               | +rPerC 11.89 (0.53 to 63.39)           | −rPerC 14.10 (1.60 to 79.82) | 0.20 |
| Final infarct size after 1 month, mL, |
| median (IQR) (n=149)                   | 1.63 (0.35 to 20.09)                   | 1.99 (0.35 to 16.19)          | 0.97 |
| Infarct growth, mL, median (IQR) (n=149) | 0 (--0.62 to 8.01)                     | 0.02 (--0.95 to 4.96)         | 0.79 |
| Acute DWI volume, mL, median (IQR) (n=149) | 1.52 (0.61 to 9.28)                     | 1.54 (0.60 to 7.11)          | 0.99 |
| Acute PWI volume, mL, median (IQR) (n=121) | 17.35 (1.01 to 87.18)                   | 16.55 (1.71 to 170.16)       | 0.26 |

DWI indicates diffusion-weighted imaging; IQR, interquartile range; PWI, perfusion-weighted imaging; and rPerC, remote ischemic perconditioning.

Overall, the findings of our study are consistent with findings in animal studies testing remote ischemic perconditioning.

Figure 3. A. Infarct risk for tissue with increased diffusion-weighted imaging (DWI) values (rDWI=1.2) across tissue mean transit time (MTT) values. The curves show overall infarct risk in the remote ischemic perconditioning (rPerC)-treated and rPerC-untreated groups at admission. B. Occlusion and recanalization were rated on Thrombolysis in Myocardial Ischemia scale at baseline and 24 hours. DWI indicates relative diffusion-weighted imaging; and rMTT, relative mean transit time.
Figure 4. Infarct risk including all values of initial \( r\text{DWI} \). The frequency of tissue voxels with a given combination of \( r\text{MTT} \) and \( r\text{DWI} \) 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 \( r\text{DWI} \) values 0.5, 1.0, and 1.5 and \( r\text{MTT} \) 0, 5, 10, 15 s. A, Infarct risk for all values of \( r\text{DWI} \). 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. \( r\text{DWI} \) indicates relative diffusion-weighted imaging; \( r\text{MTT} \), relative mean transit time; and \( r\text{PerC} \), remote ischemic perconditioning.
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. 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. 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. 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.

Neither the primary nor any of the secondary outcomes could be achieved in our study. Although previous clinical studies 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. 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, and a stronger stimuli using bilateral upper limb ischemia and 5 cycles of ischemia followed by reperfusion 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. Bøtker 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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