Remote Ischemic Conditioning as an Additional Treatment for Acute Ischemic Stroke.

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Remote ischemic conditioning as an additional treatment for acute ischemic stroke: the preclinical and clinical evidence

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Introduction

Acute ischemic stroke (AIS) is the leading cause of disability in adults worldwide and has the second highest mortality of all cardiovascular diseases\(^1\). The burden of stroke is likely to increase significantly during the next decades, primarily due to population growth and aging\(^2\). Given the detrimental impact of stroke on healthcare (costs) and patient well-being, it is imperative to explore opportunities for novel therapies to add to the current treatment to further minimize neurological injury.

During an ischemic stroke, occlusion of a cerebral artery abrogates cerebral perfusion, causing brain tissue distal from the occlusion to become deprived of oxygen and nutrients, ultimately leading to ischemic injury. Surrounding the ischemic core an area called the penumbra contains potentially reversible injured brain tissue, which may remain viable for several hours. Whilst the time window to attenuate the detrimental impact of an ischemic stroke seems limited to six hours after onset of AIS\(^3, 4\), recent research suggests that subgroups may benefit up to 24 hours\(^5, 6\). This time window of 6-24 hours offers perspective for hospital-based, additional therapies to reduce ischemic injury and minimize clinical deterioration in AIS patients.

This review focuses on remote ischemic conditioning (RIC) as an additive therapy to improve clinical outcomes in AIS patients, both when applied as a single as well as repeated bouts. RIC refers to the application of several cycles of brief ischemia and reperfusion to a limb (using a blood pressure cuff). Pre-clinical work revealed this stimulus to reduce neural damage after reperfusion\(^7\text{-}11\), validating the concept that RIC may have clinical potential in AIS. RIC therefore represents a simple, low cost therapeutic strategy that may salvage brain tissue in the penumbral area. In this review, we will summarize (pre)clinical evidence for the efficacy of RIC as an additional therapy in AIS patients.
**Methods**

A formal systematic review was not performed because of the heterogeneity of the studies and recently published systematic reviews on preclinical\(^{[12]}\) and clinical studies\(^{[13]}\). Nonetheless this review tested for the rigor, quality and appropriateness of the available studies that examined the (pre)clinical efficacy of RIC in AIS patients by providing detailed information for each individual study. In addition, this narrative review also highlights remaining knowledge gaps to give directives for future research. The primary search originally occurred in September 2018, and was repeated in March 2019, and used keywords related to ischemic conditioning and stroke in Pubmed (i.e., "ischemic conditioning" OR "ischemic conditioning" AND "stroke") and were included if they (1) were written in English, (2) were performed in either humans or animals, and (3) primarily focused on the application of remote ischemic conditioning as a therapeutic strategy in stroke (models). From these initial articles, reference lists were scanned for additional suitable articles to include in this review. Eventually, this yielded 34 suitable articles, of which 27 were performed in a preclinical setting and 7 were performed in humans.

**What is remote ischemic conditioning?**

Ischemic conditioning was first introduced in the field of cardiology in 1986\(^{[14]}\) by Murry *et al.*, who found that short repetitive bouts of occlusion and reperfusion of a coronary artery in dogs subsequently protected the heart against a myocardial infarction. The first evidence for the remote application of ischemic conditioning was discovered in 1993 in a study that showed that ischemic conditioning of a coronary artery also protected remote cardiac tissue not directly supplied by this artery.\(^{[15]}\) This initiated research that allowed the application of RIC to become clinically applicable, especially since the observation that also RIC applied to a limb (using a
blood pressure cuff) effectively protected remote tissue, such as the brain, against prolonged ischemia (e.g. during/after AIS) and ischemia reperfusion (I/R) injury (e.g. induced by the revascularization procedure)\cite{16}. Whilst initial studies have primarily explored the effects of RIC in patients with coronary heart disease, with (pre)clinical studies showing conflicting results \cite{17-22}, more recent studies have also explored the potential of RIC in AIS patients\cite{7-11}.

The application of RIC can be divided into three variants that differ based on the timing in relation to AIS: before, during or after an ischemic event\cite{23}, which are respectively called remote ischemic pre-conditioning (rIPreC), per-conditioning (rIPerC) and post-conditioning (rIPostC). Although the timing of these three types of RIC differ, previous meta-analyses suggest that the neuroprotective effects of the distinct types of RIC are comparable\cite{24,25} (figure 1). Furthermore, even though the exact mechanisms by which RIC reduces I/R injury in the brain remain unclear, the currently accepted hypothesis is that transient I/R injury induced by pre-, per- and post-conditioning all induce the release of humoral factors and local autacoids (e.g. nitric oxide, nitrite and adenosine), which activate afferent neural and/or humoral pathways\cite{9}. After signal transmission\cite{9,26}, RIC reduces I/R-induced oxidative damage\cite{11} and suppresses inflammatory responses in the brain which can last up to days after revascularization\cite{16}. More detailed discussion of potential mechanisms explaining the potential benefits of RIC to reduce I/R in the brain can be found elsewhere in excellent and detailed reviews covering this topic\cite{9,12}. Given this comparable mechanism and the sparsity of data in the (clinical) field, we have included all three variants of RIC in our review.

What is the evidence for RIC as an additional therapy in AIS?

Evidence for conditioning of the brain from preclinical studies in animals

Is a single bout of RIC effective in the animal brain?
A single bout of RIC activates at least two distinct time frames of protection against I/R injury of the brain\cite{27}. The initial protection is short lasting (~2 hours) and occurs immediately after RIC. The delayed form of protection reappears after 12-24 hours and lasts 48-72 hours\cite{28}. A substantial amount of preclinical studies has investigated the protective effect of single RIC in focal ischemia models using direct cerebral artery occlusion. The first evidence for the protective effects for RIC in cerebral ischemia originates from 2008, when Ren et al.\cite{27} found that induction of a remote RIC-stimulus to the femoral artery prior to cerebral ischemia (rIPreC) reduced infarct size after focal cerebral ischemia in rats. The potential acute protective effect of rIPreC has thereafter been confirmed by numerous other studies in animals (Table 1).

Whilst these previous studies highlight the potential of RIC to salvage brain injury, the unpredictability of AIS makes rIPreC not feasible for implementation as an additional therapy in stroke patients. Therefore, after the confirmation that rIPreC is a safe and effective method to protect against cerebral ischemia, the focus of researchers shifted towards the application of ischemic conditioning during (i.e. rIPerC) and after (i.e. rIPostC) AIS in animal models. One of the first studies investigating the effect of rIPostC in rats showed a reduction in infarct size of 63% when RIC was applied immediately after reperfusion, whilst a 43% reduction in infarct size was present when RIC was applied 3 hours post-stroke induction \cite{29}. The majority of subsequent studies supported RIC’s ability to significantly reduce infarct size and improve neurological scores in rats when applied during or after focal cerebral ischemia (Table 2).

*Is repeated RIC effective in the animal brain?*

Hess et al. postulated that, in addition to the short-lasting benefits of acute RIC, long-term benefits may be induced with repeated daily conditioning \cite{9}. A limited number of published studies have explored the effect of repeated RIC in an animal model for brain ischemia. One study found that a single episode of rIPerC afforded short-term protection, whilst brain infarct
size was further ameliorated when combined with repeated rIPostC during the 14 days after reperfusion. Recently, another study provided further support for the benefits of repeated rIPostC, in that daily repeated rIPostC in a mice model was associated with a smaller infarct size and transiently improved neurological function when conditioning started up to 24 hours after reperfusion. Interestingly, even when rIPostC was started 5 days from injury and was repeated for 14 consecutive days, neurological improvement was sustained at least for 3 months.

Evidence for conditioning of the human brain

Despite the potent effects of RIC to reduce infarct size in animal studies, only few clinical trials explored the effect of RIC in stroke patients (Table 3). At least, these studies show that RIC is well tolerated and has no severe adverse effects in AIS patients. The clinical effects of RIC in humans are discussed below.

Is a single bout of RIC effective in the human brain?

The first study investigating the effect of single RIC in stroke patients was performed by Hougaard et al., who applied a single bout of rIPerC in ischemic stroke patients during transportation to the hospital (where they received thrombolysis within 4.5 hours). Although no effects on infarct size and growth (measured with MRI) was found, a tissue survival analysis suggested that prehospital rIPerC may have immediate neuroprotective effects. An important practical limitation is that 18% of the patients had a transportation time too short for the full rIPerC protocol. Consequently, patients may have received a sub-optimal dose of RIC, underestimating the potential effect size of RIC. In a follow-up study (i.e. RECAST), 26 patients with an ischemic stroke received rIPostC within 24 hours after AIS. Interestingly, a significantly lower NIHSS after 90 days was found after rIPostC compared to placebo. Since
this study was not powered \textit{a priori} to detect changes in clinical outcome (i.e. NIHSS), no definitive conclusions of the effect of rIPostC on clinical outcome can be made.

\textbf{Is repeated RIC effective in the human brain?}

Additional benefits of conditioning may be achieved by repeatedly applying RIC in stroke patients. Two randomized controlled trials examined the effect of repeated RIC in patients with intracerebral artery stenosis (ICAS). One RCT included 68 patients with stroke or TIA within the previous 30 days\cite{36}, with the intervention group receiving RIC to the upper arm twice daily for 300 consecutive days. Incidence of recurrent stroke after 300 days in the intervention group was 7.9\% versus 26.7\% in the control group. RIC also significantly improved the rate of recovery, with 65.8\% showing a modified Rankin Scale-score of 0-1 after 90 days versus 13.3\% in the control group. Another RCT, performed by the same researchers, supported the findings of the first trial in a population of 58 symptomatic ICAS patients\cite{37}. Two subsequent studies, performed in patients with small vessel disease, found that repeated RIC resulted in a decrease in white matter hyperintensities after one year\cite{38, 39}. Taken together these clinical studies performed in ICAS and small vessel disease suggest that repeated RIC effectively and safely reduces the risk of recurrent stroke and supports the hypothesis that the brain demonstrates remodeling that may protect against continued cerebral ischemia.

\textbf{Knowledge gaps and future directions}

Although the preclinical evidence from studies in animals is promising and beneficial effects have been observed in clinical trials, some considerations should be discussed. First, caution is warranted for translation or extrapolation of (pre)clinical results. Related to pre-clinical studies several problems make translation to the human clinical situation difficult, including
homogeneity of the animals as opposed to heterogenous humans and the duration/severity of
the ischemic lesion. To support this notion, many neuroprotectants that appeared promising in
pre-clinical models have failed in clinical translation\cite{40}. For clinical trials, it is important to
realize that results from distinct subgroups of stroke patients (Table 3) cannot be simply
extrapolated to the “average” stroke patient.

Although some of the results from clinical studies are promising, we judged a substantial
amount of these studies to be at high risk for bias (Table 4). This interpretation is in line with
the assessment that was performed by Zhao et al.\cite{13}. Important to note is that six out of the
seven trials are at high risk for bias because one or two investigators had potential conflict of
interest related to the automated RIC device\cite{33,35-39}. This leads to only one clinical study that
seems to be at low risk for bias on all criteria\cite{32}. Additionally, some form of publication bias
may be present in our review. Interestingly, all studies with a relatively small sample size show
a positive effect on different measures of clinical outcome (e.g. NIHSS, mRS and stroke
incidence), while studies with a larger sample size show no significant effect on clinical
outcome (Table 3). Therefore, we cannot exclude the potential for publication bias in this field.

A final consideration is the selection of the most effective RIC protocol for AIS patients.
Currently, most clinical trials adopt 3-5 cycles of 5-minutes upper-arm ischemia, with 5 minutes
of reperfusion between the cycles. Although this protocol remains pragmatic,\cite{41} it should be
realized that this protocol is ‘copied’ from the area of cardiology. Whether differences in the
number of cycles, duration of ischemia, location of ischemia, and/or the timing of a single RIC
in relation to the ischemic event impact efficacy of RIC is currently unknown. Somewhat
related is the timing of subsequent bouts to optimally benefit from repeated RIC. The current
lack of knowledge in this area highlights the need for further research, but also suggests that the optimal benefits of (repeated) RIC have yet to be determined.

What can we learn from Cardiology?

Since research on RIC in the field of Cardiology is a few steps ahead of Neurology, this provides an opportunity to guide the development of RIC in our area. Despite the initial successes of pre-clinical work in cardiac ischemia, translation to the clinical setting in humans appeared challenging. For example, large randomized controlled trials found no improvement in clinical outcome and mortality in patients undergoing coronary bypass grafting (CABG). Likely explanations relate to the interference between RIC versus medication (e.g. statins), anesthetics used in surgical procedures), aging and presence of (cardiovascular) co-morbidities. Another important observation is that most patients scheduled for CABG have a history of angina pectoris or myocardial infarction, clinical conditions associated with short exposure to cardiac ischemia. Therefore, patients may have already been “naturally” conditioned. These subject- and treatment-related factors may interfere with efficacy of RIC, and should therefore be taken into account for (ongoing) RIC trials in AIS patients. Indeed, prior TIA is associated with a reduced severity of and disability from stroke. In line with angina pectoris, prior TIA may lead to a “naturally” conditioned status and therefore these patients may be less likely to receive additional benefits from RIC.

What answers will be provided in the near future?

In light of some of the evidence gaps raised above, several trials are currently ongoing to explore the effects of RIC. Upon demonstrating the feasibility and safety of RIC in AIS patients, follow-up trials RECAST-2 (n=60, single vs repeated RIC, NCT02779712) and REVISE-2
(n=180, CT-scan as primary outcome, NCT03045055) focus on clinical effectiveness of RIC in patients and likely provide meaningful insight into the clinical effects and/or optimal protocol for conditioning. In addition, studies also explore the benefits of applying repeated RIC in the first week after stroke onset (France; NCT02189928,[^48] the Netherlands; NTR6880). Finally, Hougaard and coworkers currently perform a large (n=2,500) follow-up study of their earlier conducted trial[^35]: the RESIST trial (NCT03481777), which primarily focuses on the effect of RIC on clinical parameters and control for between-patient variability. Interestingly, in addition to single RIC, the RESIST-trial will also perform repeated RIC in a subgroup of patients to explore the potential difference between single and repeated application. Individual data from these trials will help to better understand the effectiveness of RIC in AIS patients and will guide potential future implementation of RIC in clinical practice.

**Conclusion**

Recent evidence from animals and humans, including various patient groups, demonstrated that remote ischemic conditioning is a feasible and safe strategy. Moreover, pre-clinical studies in animals and initial studies in humans (including in patients), support the ability of RIC to reduce infarct size and improve clinical status when applied during (per-conditioning) or immediately after (post-conditioning) AIS. Given the hypothesis that RIC could prevent cerebral damage after the ischemic event by targeting I/R injury (which lasts for several days), RIC could even be implemented after the currently accepted treatment window for AIS of 6-24 hours. In fact, (pre)clinical studies show promising results for single and repeated conditioning, both *during* and *after* AIS. This relatively new area in stroke warrants further attention and (clinical) follow-up studies, especially given the simplicity, low costs, non-invasive character and the ability of RIC to be applied without interfering with current treatment guidelines.
Disclosures

None
References:


**Figure Legends**

Figure 1. The different variants of remote ischemic conditioning and the observed effects in the brain.

**Tables**

Table 1. Summarized description of preclinical studies in ischemic preconditioning

*Studies reviewed for reporting of 3 measures of study quality: Randomization, blinding of endpoints and whether a sample size analysis was performed for a hypothesized effect size.

Table 2. Summarized description of preclinical studies in remote ischemic per- and postconditioning.

*Studies reviewed for reporting of 3 measures of study quality: Randomization, blinding of endpoints and whether a sample size analysis was performed for a hypothesized effect size.

Table 3. Summarized description of clinical studies into the effect of remote ischemic conditioning.

Table 4. Risk of bias assessment of clinical studies into the effect of remote ischemic conditioning
<table>
<thead>
<tr>
<th>Study</th>
<th>Animals</th>
<th>Randomization groups</th>
<th>Stroke model</th>
<th>RIC location and cycles</th>
<th>Time of RIC (before stroke)</th>
<th>Infarct size</th>
<th>Neurological outcomes</th>
<th>Quality*</th>
<th>Physiological mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhao et al. 2006</td>
<td>SHR rats Male 250-350 g. N=87</td>
<td>1: Preconditioning 2: Sham 3: Control</td>
<td>Permanent occlusion of the right MCA and CCA.</td>
<td>MCA, 1x10 min</td>
<td>24 hours</td>
<td>↓ Severity of perfusion deficits ↓ infarct volume</td>
<td>None described</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ren et al. 2008</td>
<td>SD rats Male 270-330 g. N=60</td>
<td>Different preconditioning protocols at different time windows.</td>
<td>Permanent occlusion left distal MCA + occlusion bilateral CCA (30 min.)</td>
<td>Femoral artery 1: 2x5 min 2: 2x15 min 3: 3x15 min.</td>
<td>1: 12 hours, 2: 48 hours 3: immediately before</td>
<td>↓ infarct size with 2x15 min and 3x15 min.</td>
<td>Randomized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malhotra et al. 2011</td>
<td>Adult Wistar rats Male 200-225 g.</td>
<td>1: rIPreC 2: Sham surgery</td>
<td>MCA occlusion (120 min.)</td>
<td>Abdominal aorta 3x10 min.</td>
<td>1: 24 hours 2: 48 hours 3: 72 hours</td>
<td>1: ↓ infarct size 2: No effect 3: No effect</td>
<td>Randomized Blinded</td>
<td>A ganglion blocker attenuated the neuroprotective effect.</td>
<td></td>
</tr>
<tr>
<td>Yuan et al. 2012</td>
<td>Wistar rats Male 250-280 g.</td>
<td>1: Sham group 2: Control group 3: IC of the CCA 4: rIPreC</td>
<td>Occlusion left CCA (30 min.) + permanent occlusion left distal MCA</td>
<td>Left hind limb 3x5 min.</td>
<td>Daily during the three days before stroke</td>
<td>↓ infarct size</td>
<td>↑ Neurological scores</td>
<td>Randomized</td>
<td>Increased cerebral anti-oxidative abilities.</td>
</tr>
<tr>
<td>Wei et al. 2012</td>
<td>SD Rats Male 250-350 g.</td>
<td>1: rIPreC 2: Control</td>
<td>Occlusion bilateral CCA + distal left MCA (30 min.)</td>
<td>Femoral artery 3x15 min.</td>
<td>Immediately before</td>
<td>↓ infarct size</td>
<td>↑ behavioral outcomes</td>
<td>Randomized Blinded</td>
<td>Through sensory nerves</td>
</tr>
<tr>
<td>Hu et al. 2012</td>
<td>SD rats Male 280-320 g. N=128</td>
<td>Eight different groups</td>
<td>Occlusion right MCA (120 min.)</td>
<td>Right hind limb 3x5 min.</td>
<td>1 hour</td>
<td>↓ infarct size on DWI imaging</td>
<td>↓ NDS Randomized</td>
<td>Through adenosine pathway.</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Animals</td>
<td>Randomization groups</td>
<td>Stroke model</td>
<td>RIC location and cycles</td>
<td>Time of RIC</td>
<td>Infarct size</td>
<td>Neurological outcomes</td>
<td>Quality*</td>
<td>Physiological mechanism</td>
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<td>Hahn et al. 2011, [54]</td>
<td>SD rats (p60) Male 270-330 g. N=39</td>
<td>1: rlPreC 2: rlPerC 3: Sham conditioning</td>
<td>MCA occlusion (120 min.)</td>
<td>rlPreC: 40 minutes before ischemia rlPerC: during reperfusion</td>
<td>↓ in rlPreC ↓ in rlPerC</td>
<td>Randomized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ren et al. 2011, [55]</td>
<td>Adult SD rats Male 280-320 g. N=54</td>
<td>1: rlPerC 2: Sham conditioning.</td>
<td>MCA occlusion (90 min.)</td>
<td>Immediately after stroke and before reperfusion</td>
<td>↓ Infarct size ↓ Brain edema</td>
<td>Randomized</td>
<td>↓ Blood-brain barrier leakage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sun et al. 2012, [56]</td>
<td>Adult SD rats Male 290-310 g. N=56</td>
<td>7 different serials of RIC</td>
<td>MCA occlusion (90 min.)</td>
<td>Femoral artery, bilateral. 3x10 min.</td>
<td>1: at 72 hours 2: at 72 hours</td>
<td>↓ NDS ↓ NDS</td>
<td>Randomized Blinded</td>
<td>Through opening of KATP channels.</td>
<td></td>
</tr>
<tr>
<td>Hoda et al. 2012, [57]</td>
<td>C57BL/6J Mice Male, 20 weeks old N=90</td>
<td>1: rlPerC + tPA 2: rlPerC without tPA 3: tPA only 4: Sham treatment</td>
<td>Thromboembolic with/without tPA after 4 hours</td>
<td>Left hind limb. 5x5 min.</td>
<td>2 hours after (embolic) stroke and 2 hours before reperfusion.</td>
<td>RIC alone: ↓ 25.7% RIC+tPA : ↓ 50%</td>
<td>Randomized Blinded Sample size estimation</td>
<td>Increased relative CBF</td>
<td></td>
</tr>
<tr>
<td>Peng et al. 2012, [58]</td>
<td>Adult SD rats Male 200-250 g.</td>
<td>1: Sham conditioning 2: Control 3: rlPostC</td>
<td>Four vessel occlusion (8 min.)</td>
<td>Bilateral femoral artery, 3x15 min.</td>
<td>Immediately after global cerebral ischemia</td>
<td>↓ neuronal death ↑ spatial learning ↑ memory</td>
<td>Randomized Blinded</td>
<td>Upregulation of eNOS through the P13K/Akt pathway.</td>
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</tr>
<tr>
<td>Study</td>
<td>Animals</td>
<td>Randomization groups</td>
<td>Stroke model</td>
<td>RIC location and cycles</td>
<td>Time of RIC</td>
<td>Infarct size</td>
<td>Neurological outcomes</td>
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<td>Physiological mechanism</td>
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<tr>
<td>Hoda et al. 2014[60]</td>
<td>C57BL/6J mice Female, 20 weeks old</td>
<td>N=140</td>
<td>1: rIPerC + tPA 2: rIPerC without tPA 3: tPA only 4. Sham treatment</td>
<td>Thromboembolic with/without tPA after 4 hours</td>
<td>Left hind limb 4x10 min.</td>
<td>↓ infarct size ↓ hemorrhage ↓ edema</td>
<td>↑ sensorimotor function ↓ NDS ↓ mortality</td>
<td>Randomized Blinded Sample size estimation</td>
<td>RIC improved CBF</td>
</tr>
<tr>
<td>Cheng et al. 2014[61]</td>
<td>Adult SD rats Male 250-300 g. N=45</td>
<td>1: Sham operation 2: Control 3: rIPostC</td>
<td>MCA occlusion (90 min.)</td>
<td>Right hind limb 3x5 min.</td>
<td>At the beginning of reperfusion</td>
<td>↓ infarct size</td>
<td>No improvement</td>
<td>Randomized</td>
<td>Related to neuronal apoptosis and inflammation.</td>
</tr>
<tr>
<td>Su et al. 2014[62]</td>
<td>SD Rats Male 28-320 g. N=168</td>
<td>Seven experimental groups</td>
<td>MCA occlusion (120 min.)</td>
<td>Bilateral femoral artery 4x10 min.</td>
<td>At the beginning of MCA occlusion.</td>
<td>↓ infarct size ↓ edema</td>
<td>↓ NDS</td>
<td>Randomized Blinded</td>
<td>Through the autophagy-lysosome pathway</td>
</tr>
<tr>
<td>Khan et al. 2015[63]</td>
<td>C57BL/6J mice Male, 10 weeks old</td>
<td>N=20</td>
<td>1: Sham group 2: Control group 3: rIPostC</td>
<td>BCAS induced by microcoils around both CCA’s.</td>
<td>Hind limb 4x10 min.</td>
<td>1 week after induction of BCAS. Daily for 2 weeks.</td>
<td>↑ Cognitive function</td>
<td>Randomized Blinded Sample size estimation</td>
<td>Increased cerebral perfusion.</td>
</tr>
<tr>
<td>Li et al. 2015[64]</td>
<td>SD rats Male 220-280 g. 8-10 weeks old</td>
<td>1: Sham surgery 2: Control 3: rIPostC</td>
<td>MCA occlusion (120 min.)</td>
<td>Bilateral femoral artery 3x10 min.</td>
<td>Immediately after reperfusion.</td>
<td>↓ NDS</td>
<td>Randomized Blinded</td>
<td>Attenuation of neuronal apoptosis and suppression of p38 MAPk-AFT2 pathway.</td>
<td></td>
</tr>
<tr>
<td>Ren et al. 2015[65]</td>
<td>Adult SD rats Male 280-320 g.</td>
<td>1: Single rPerC 2: rPerC + repeated rPostC 3: Sham stroke 4: Ischemic control</td>
<td>MCA occlusion (90 min.)</td>
<td>Bilateral hind limb 3x10 min.</td>
<td>1: Single RIC: Immediately after stroke 2: Repeated RIC: Immediately after stroke + daily repeated RIC during 14 days</td>
<td>↓ infarct size after 7 days 2: ↓ infarct size after 7 and 14 days</td>
<td>↑ neurological outcome</td>
<td>Blinded</td>
<td>Increased expression of neuroglobin.</td>
</tr>
<tr>
<td>Li et al. 2015[66]</td>
<td>Adult SD rats Male 250-280 g. N=185</td>
<td>1: Sham group 2: Control group 3: rIPostC</td>
<td>MCA occlusion (60 min.)</td>
<td>Bilateral hind limb. 3x10 min.</td>
<td>During reperfusion.</td>
<td>↓ infarct volume ↓ edema</td>
<td>↑ neurological function</td>
<td>Randomized Blinded</td>
<td>Elevation of the integrity of blood-brain barrier.</td>
</tr>
<tr>
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<td>Neurological outcomes</td>
<td>Quality</td>
<td>Physiological mechanism</td>
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<tr>
<td>Li et al. 2015</td>
<td>CD1 mice Male 25-30 g. N=18</td>
<td>1: Sham group 2: Control group 3: rIPostC</td>
<td>MCA occlusion (60 min.)</td>
<td>Bilateral femoral artery.</td>
<td>Immediately after reperfusion</td>
<td>↓ infarct volume ↓ edema</td>
<td>↑ neurological outcome</td>
<td>Randomized Blinded</td>
<td>Reduction of oxidative stress.</td>
</tr>
<tr>
<td>Zong et al. 2015</td>
<td>SD rats Male 250-280 g.</td>
<td>1: Sham 2: Control 3: rIPostC</td>
<td>MCA occlusion (60 min.)</td>
<td>Bilateral hind limb.</td>
<td>At the beginning of reperfusion</td>
<td>↓ infarct volume ↓ edema</td>
<td>↓ NDS</td>
<td>Randomized Blinded</td>
<td>Inhibition of HIF-1α.</td>
</tr>
<tr>
<td>Chen et al. 2016</td>
<td>SD rats Male 250-280 g.</td>
<td>1: rIPostC 2: Sham conditioning</td>
<td>MCA occlusion (90 min.)</td>
<td>Left femoral artery.</td>
<td>1: Immediately after reperfusion 2: 1 hour after reperfusion 3: 3 hours after reperfusion</td>
<td>1: ↓ infarct volume 2: No effect 3: No effect</td>
<td>1: ↑ Neurobehavioral scores 2: No effect 3: No effect</td>
<td>Randomized Blinded</td>
<td>Downregulation of the activation of NADPH oxidase in neutrophils.</td>
</tr>
<tr>
<td>Wang et al. 2016</td>
<td>Adult SD rats Male 250-280 g.</td>
<td>1: Sham 2: Control 3: rIPerC 4: IPOC 5: rIPerC + IPOC</td>
<td>MCA occlusion (120 min.)</td>
<td>rIPerC: left hind limb IPOC: MCA</td>
<td>rIPerC: 40 min prior to reperfusion IPOC: At the beginning of reperfusion</td>
<td>rIPerC + IPOC: ↓ infarct volume by &gt;50% rIPerC alone: ↓ infarct volume by 25%</td>
<td>↓ NDS</td>
<td>Blinded</td>
<td>Inhibition of autophagy</td>
</tr>
<tr>
<td>Zhang et al. 2017</td>
<td>SD rats Male 300-320 g.</td>
<td>1: Sham 2: Control 3: rIPostC</td>
<td>MCA occlusion (120 min.)</td>
<td>Bilateral femoral artery</td>
<td>At the beginning of reperfusion</td>
<td>↓ infarct volume</td>
<td>↑ Neurobehavioral scores</td>
<td>Blinded</td>
<td>Suppression of blood brain barrier leakage.</td>
</tr>
<tr>
<td>Li et al. 2018</td>
<td>SD rats Female 250-280 g. 15-16 weeks N=81</td>
<td>1: rIPostC 2: Sham-stroke 3: ischemic control</td>
<td>MCA occlusion (60 min.)</td>
<td>Bilateral hind limb</td>
<td>Immediately after reperfusion</td>
<td>↓ infarct size by 41.9% ↓ edema by 27.6%</td>
<td>↓ NDS</td>
<td>Randomized Blinded</td>
<td>Reduction of blood-brain barrier injury and leakage.</td>
</tr>
<tr>
<td>Doeppner et al. 2018</td>
<td>C57BL6 mice Male 24-28 g.</td>
<td>1: rIPostC 2: Control</td>
<td>MCA occlusion (60 min.)</td>
<td>Bilateral hind limb</td>
<td>1: 12 hours after reperfusion, repeated daily for 3-7 days. 2: 24 hours after reperfusion 3: 120 hours after reperfusion, repeated for 14 days</td>
<td>1: ↓39.8% 2: ↓26% 3: ↑ neuronal density by 60.1%</td>
<td>1: Transient improvement 2: transient improvement 3: Sustained improvement</td>
<td>Randomized Blinded</td>
<td>Mediated via HSP-70.</td>
</tr>
</tbody>
</table>
Table 3. Summarized description of clinical studies into the effect of remote ischemic conditioning.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Randomization groups</th>
<th>Location of RIC</th>
<th>Cycles (occlusion/reperfusion)</th>
<th>Time of RIC</th>
<th>Effect on infarct size</th>
<th>Effect on neurological outcomes</th>
<th>Physiological mechanism</th>
</tr>
</thead>
</table>
| Meng et al. 2012[36]   | Patients with Intracranial arterial stenosis (N=68).                   | 1: Standard treatment only (N=30) 2: RIC (N=38)                                      | Bilateral upper arm | 5x5 min                       | -Within 30 days after stroke  
- Twice daily for 300 consecutive days                                         | ↓ Stroke recurrency  
↑ recovery in mRS                    |                    | Improvement in cerebral perfusion                                    |
| Hougaard et al. 2014[35] | Patients suspected of an ischemic stroke (N=443).                     | 1: Standard treatment (N=196) 2: rIPerC (N=247)                                       | Upper limb         | 4x5 min.                      | During transportation to the hospital                                         | No effect on penumbral salvage or infarct size.  
No effect (NIHSS and mRS)     |                    |                   |                               |
| Meng et al. 2015[31]   | Patients with intracranial arterial stenosis (N=58).                   | 1: RIC (N=30) 2: Sham (N=28)                                                        | Bilateral upper arm | 5x5 min.                      | - Within 7 days after an ischemic stroke or TIA  
- Twice daily for 180 consecutive days                                         | ↓ Stroke recurrency  
↓ NIHSS  
↓ mRS                    |                    | Reduction of inflammation and coagulation                                    |
| Mi et al. 2016[39]     | Patients with cerebral small vessel disease (N=17).                    | 1: RIC (N=9) 2: Sham (N=8)                                                          | Bilateral upper arm | 5x5 min.                      | Twice daily for 1 year                                                      | ↓ White matter lesions  
↓ Dizziness handicap inventory  
No effect on number of lacunar infarcts   |                    | Accelerated flow velocity in MCA.                                          |
Table 3. Continued.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Randomization groups</th>
<th>Location of RIC</th>
<th>Cycles (occlusion/reperfusion)</th>
<th>Time of RIC</th>
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<th>Effect on neurological outcomes</th>
<th>Physiological mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang et al. 2017.[38]</td>
<td>Patients with cerebral small vessel disease-related mild cognitive impairment (N=30).</td>
<td>1: RIC (N=14) 2: Sham (N=16)</td>
<td>Bilateral upper arm</td>
<td>5x5 min.</td>
<td>Twice daily for 1 year</td>
<td>↓ White matter hyperintensities</td>
<td>↑ visuospatial and executive abilities</td>
<td>Effect on triglycerides, cholesterol and homocysteine</td>
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<tr>
<td>Zhao et al. 2017.[33]</td>
<td>Patients undergoing carotid artery stenting (N=189).</td>
<td>1: rIPreC (N=63) 2: Sham (N=63) 3: No intervention (N=63)</td>
<td>Bilateral upper arm</td>
<td>5x5 min.</td>
<td>Twice daily during two weeks before carotid artery stenting.</td>
<td>↓ new DWI lesions ↓ DWI lesions volume</td>
<td>No effect on clinical ischemic events</td>
<td>No changes in Enolase or S-100B levels.</td>
</tr>
<tr>
<td>Study</td>
<td>Random sequence generation</td>
<td>Allocation concealment</td>
<td>Blinding of participants and personnel</td>
<td>Blinding of outcome assessment</td>
<td>Incomplete outcome data</td>
<td>Selective reporting</td>
<td>Other sources of bias</td>
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<td>Meng et al. 2015 [71]</td>
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