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

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[7-11], 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)[16]. Whilst initial studies have primarily explored the effects of RIC in patients with coronary heart disease, with (pre)clinical studies showing conflicting results [17-22], more recent studies have also explored the potential of RIC in AIS patients[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[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[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 [9]. After signal transmission[9, 26], RIC reduces I/R-induced oxidative damage[11] and suppresses inflammatory responses in the brain which can last up to days after revascularization[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[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.

<u>What is the evidence for RIC as an additional therapy in AIS?</u>

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[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[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*.[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 [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 [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 [30]. 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[31].

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.[32-34] 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)[35]. 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) [32], 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 *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.

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,[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 [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.[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.

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[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.[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[33, 35-39]. This leads to only one clinical study that seems to be at low risk for bias on all criteria[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,[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 [23], 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)[17-19]. Likely explanations relate to the interference between RIC versus medication (e.g. statins, [21] anesthetics used in surgical procedures), aging and presence of (cardiovascular) co-morbidities[42, 43]. 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[44]. 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.[45-47] 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,[32, 33] 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:

1. Roth, G.A., et al., *Demographic and epidemiologic drivers of global cardiovascular mortality.* N Engl J Med, 2015. **372**(14): p. 1333-41.
2. Howard, G. and D.C. Goff, *Population shifts and the future of stroke: forecasts of the future burden of stroke.* Ann N Y Acad Sci, 2012. **1268**: p. 14-20.
3. Berkhemer, O.A., C.B. Majoie, and D.W. Dippel, *Intraarterial treatment for acute ischemic stroke.* N Engl J Med, 2015. **372**(12): p. 1178-9.
4. Saver, J.L., et al., *Time to Treatment With Endovascular Thrombectomy and Outcomes From Ischemic Stroke: A Meta-analysis.* JAMA, 2016. **316**(12): p. 1279-88.
5. Nogueira, R.G., et al., *Thrombectomy 6 to 24 Hours after Stroke with a Mismatch between Deficit and Infarct.* N Engl J Med, 2018. **378**(1): p. 11-21.
6. Albers, G.W., et al., *Thrombectomy for Stroke at 6 to 16 Hours with Selection by Perfusion Imaging.* N Engl J Med, 2018. **378**(8): p. 708-718.
7. Iadecola, C. and J. Anrather, *Stroke research at a crossroad: asking the brain for directions.* Nat Neurosci, 2011. **14**(11): p. 1363-8.
8. Wang, Y., et al., *Ischemic conditioning-induced endogenous brain protection: Applications pre-, per- or post-stroke.* Exp Neurol, 2015. **272**: p. 26-40.
9. Hess, D.C., et al., *Remote ischaemic conditioning-a new paradigm of self-protection in the brain.* Nat Rev Neurol, 2015. **11**(12): p. 698-710.
10. Bai, J. and P.D. Lyden, *Revisiting cerebral postischemic reperfusion injury: new insights in understanding reperfusion failure, hemorrhage, and edema.* Int J Stroke, 2015. **10**(2): p. 143-52.
11. Chen, G., et al., *Limb Remote Ischemic Postconditioning Reduces Ischemia-Reperfusion Injury by Inhibiting NADPH Oxidase Activation and MyD88-TRAF6-P38MAP-Kinase Pathway of Neutrophils.* Int J Mol Sci, 2016. **17**(12).
12. Chen, G., et al., *Limb Remote Ischemic Conditioning: Mechanisms, Anesthetics, and the Potential for Expanding Therapeutic Options.* Front Neurol, 2018. **9**: p. 40.
13. Zhao, J.J., et al., *Remote Ischemic Postconditioning for Ischemic Stroke: A Systematic Review and Meta-Analysis of Randomized Controlled Trials.* Chin Med J (Engl), 2018. **131**(8): p. 956-965.
14. Murry, C.E., R.B. Jennings, and K.A. Reimer, *Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium.* Circulation, 1986. **74**(5): p. 1124-36.
15. Przyklenk, K., et al., *Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion.* Circulation, 1993. **87**(3): p. 893-9.
16. Wu, M.Y., et al., *Current Mechanistic Concepts in Ischemia and Reperfusion Injury.* Cellular Physiology and Biochemistry, 2018. **46**(4): p. 1650-1667.
17. Hausenloy, D.J., et al., *Remote Ischemic Preconditioning and Outcomes of Cardiac Surgery.* N Engl J Med, 2015. **373**(15): p. 1408-17.
18. Meybohm, P., et al., *A Multicenter Trial of Remote Ischemic Preconditioning for Heart Surgery.* N Engl J Med, 2015. **373**(15): p. 1397-407.
19. Hong, D.M., et al., *Does remote ischaemic preconditioning with postconditioning improve clinical outcomes of patients undergoing cardiac surgery? Remote Ischaemic Preconditioning with Postconditioning Outcome Trial.* Eur Heart J, 2014. **35**(3): p. 176-83.
20. Haller, P.M., et al., *Remote ischaemic conditioning for myocardial infarction or elective PCI: systematic review and meta-analyses of randomised trials.* Eur Heart J Acute Cardiovasc Care, 2018: p. 2048872618784150.
21. Heusch, G., et al., *Remote ischemic conditioning.* J Am Coll Cardiol, 2015. **65**(2): p. 177-95.
22. Wever, K.E., et al., *Determinants of the Efficacy of Cardiac Ischemic Preconditioning: A Systematic Review and Meta-Analysis of Animal Studies.* Plos One, 2015. **10**(11).

23. Hausenloy, D.J. and D.M. Yellon, *The therapeutic potential of ischemic conditioning: an update.* Nat Rev Cardiol, 2011. **8**(11): p. 619-29.
24. Dirnagl, U., K. Becker, and A. Meisel, *Preconditioning and tolerance against cerebral ischaemia: from experimental strategies to clinical use.* Lancet Neurol, 2009. **8**(4): p. 398-412.
25. Dezfulian, C., M. Garrett, and N.R. Gonzalez, *Clinical application of preconditioning and postconditioning to achieve neuroprotection.* Transl Stroke Res, 2013. **4**(1): p. 19-24.
26. Hess, D.C., M.N. Hoda, and M.B. Khan, *Humoral Mediators of Remote Ischemic Conditioning: Important Role of eNOS/NO/Nitrite.* Acta Neurochir Suppl, 2016. **121**: p. 45-8.
27. Ren, C., et al., *Limb remote-preconditioning protects against focal ischemia in rats and contradicts the dogma of therapeutic time windows for preconditioning.* Neuroscience, 2008. **151**(4): p. 1099-103.
28. Hausenloy, D.J. and D.M. Yellon, *The second window of preconditioning (SWOP) where are we now?* Cardiovasc Drugs Ther, 2010. **24**(3): p. 235-54.
29. Ren, C., et al., *Limb remote ischemic postconditioning protects against focal ischemia in rats.* Brain Res, 2009. **1288**: p. 88-94.
30. Ren, C., et al., *Limb remote ischemic per-conditioning in combination with post-conditioning reduces brain damage and promotes neuroglobin expression in the rat brain after ischemic stroke.* Restor Neurol Neurosci, 2015. **33**(3): p. 369-79.
31. Doeppner, T.R., et al., *Very Delayed Remote Ischemic Post-conditioning Induces Sustained Neurological Recovery by Mechanisms Involving Enhanced Angioneurogenesis and Peripheral Immunosuppression Reversal.* Front Cell Neurosci, 2018. **12**: p. 383.
32. England, T.J., et al., *RECAST (Remote Ischemic Conditioning After Stroke Trial): A Pilot Randomized Placebo Controlled Phase II Trial in Acute Ischemic Stroke.* Stroke, 2017. **48**(5): p. 1412-1415.
33. Zhao, W., et al., *Safety and Efficacy of Remote Ischemic Preconditioning in Patients With Severe Carotid Artery Stenosis Before Carotid Artery Stenting: A Proof-of-Concept, Randomized Controlled Trial.* Circulation, 2017. **135**(14): p. 1325-1335.
34. Zhao, W., et al., *Remote ischemic conditioning for acute stroke patients treated with thrombectomy.* Ann Clin Transl Neurol, 2018. **5**(7): p. 850-856.
35. Hougaard, K.D., et al., *Remote ischemic perconditioning as an adjunct therapy to thrombolysis in patients with acute ischemic stroke: a randomized trial.* Stroke, 2014. **45**(1): p. 159-67.
36. Meng, R., et al., *Upper limb ischemic preconditioning prevents recurrent stroke in intracranial arterial stenosis.* Neurology, 2012. **79**(18): p. 1853-61.
37. Meng, R., et al., *Ischemic Conditioning Is Safe and Effective for Octo- and Nonagenarians in Stroke Prevention and Treatment.* Neurotherapeutics, 2015. **12**(3): p. 667-77.
38. Wang, Y., et al., *Remote Ischemic Conditioning May Improve Outcomes of Patients With Cerebral Small-Vessel Disease.* Stroke, 2017. **48**(11): p. 3064-3072.
39. Mi, T., et al., *The Interventional Effect of Remote Ischemic Preconditioning on Cerebral Small Vessel Disease: A Pilot Randomized Clinical Trial.* Eur Neurol, 2016. **76**(1-2): p. 28-34.
40. Dirnagl, U. and M. Endres, *Found in translation: preclinical stroke research predicts human pathophysiology, clinical phenotypes, and therapeutic outcomes.* Stroke, 2014. **45**(5): p. 1510-8.
41. Skyschally, A., et al., *Ischemic postconditioning: experimental models and protocol algorithms.* Basic Res Cardiol, 2009. **104**(5): p. 469-83.
42. Thijssen, D.H., et al., *Repeated ischaemic preconditioning: a novel therapeutic intervention and potential underlying mechanisms.* Exp Physiol, 2016. **101**(6): p. 677-92.
43. Ferdinandy, P., et al., *Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning.* Pharmacol Rev, 2014. **66**(4): p. 1142-74.

44. Abete, P., et al., *Angina-induced protection against myocardial infarction in adult and elderly patients: a loss of preconditioning mechanism in the aging heart?* J Am Coll Cardiol, 1997. **30**(4): p. 947-54.
45. Moncayo, J., et al., *Do transient ischemic attacks have a neuroprotective effect?* Neurology, 2000. **54**(11): p. 2089-94.
46. Weih, M., et al., *Attenuated stroke severity after prodromal TIA: a role for ischemic tolerance in the brain?* Stroke, 1999. **30**(9): p. 1851-4.
47. Wegener, S., et al., *Transient ischemic attacks before ischemic stroke: preconditioning the human brain? A multicenter magnetic resonance imaging study.* Stroke, 2004. **35**(3): p. 616-21.
48. Pico, F., et al., *A multicenter, randomized trial on neuroprotection with remote ischemic per-conditioning during acute ischemic stroke: the REmote iSchemic Conditioning in acUtE BRAin INfarction study protocol.* Int J Stroke, 2016. **11**(8): p. 938-943.
49. Zhao, L. and T.S. Nowak, Jr., *CBF changes associated with focal ischemic preconditioning in the spontaneously hypertensive rat.* J Cereb Blood Flow Metab, 2006. **26**(9): p. 1128-40.
50. Malhotra, S., et al., *Neurogenic pathway mediated remote preconditioning protects the brain from transient focal ischemic injury.* Brain Res, 2011. **1386**: p. 184-90.
51. Yuan, H.J., et al., *Noninvasive delayed limb ischemic preconditioning in rats increases antioxidant activities in cerebral tissue during severe ischemia-reperfusion injury.* J Surg Res, 2012. **174**(1): p. 176-83.
52. Wei, D., et al., *The chronic protective effects of limb remote preconditioning and the underlying mechanisms involved in inflammatory factors in rat stroke.* PLoS One, 2012. **7**(2): p. e30892.
53. Hu, S., et al., *Noninvasive limb remote ischemic preconditioning contributes neuroprotective effects via activation of adenosine A1 receptor and redox status after transient focal cerebral ischemia in rats.* Brain Res, 2012. **1459**: p. 81-90.
54. Hahn, C.D., et al., *Remote ischemic per-conditioning: a novel therapy for acute stroke?* Stroke, 2011. **42**(10): p. 2960-2.
55. Ren, C., et al., *Remote ischemic post-conditioning reduced brain damage in experimental ischemia/reperfusion injury.* Neurol Res, 2011. **33**(5): p. 514-9.
56. Sun, J., et al., *Protective effect of delayed remote limb ischemic postconditioning: role of mitochondrial K(ATP) channels in a rat model of focal cerebral ischemic reperfusion injury.* J Cereb Blood Flow Metab, 2012. **32**(5): p. 851-9.
57. Hoda, M.N., et al., *Remote ischemic perconditioning is effective alone and in combination with intravenous tissue-type plasminogen activator in murine model of embolic stroke.* Stroke, 2012. **43**(10): p. 2794-9.
58. Peng, B., et al., *Remote ischemic postconditioning protects the brain from global cerebral ischemia/reperfusion injury by up-regulating endothelial nitric oxide synthase through the PI3K/Akt pathway.* Brain Res, 2012. **1445**: p. 92-102.
59. Qi, Z.F., et al., *AKT/GSK3beta-dependent autophagy contributes to the neuroprotection of limb remote ischemic postconditioning in the transient cerebral ischemic rat model.* CNS Neurosci Ther, 2012. **18**(12): p. 965-73.
60. Hoda, M.N., et al., *Remote ischemic perconditioning is effective after embolic stroke in ovariectomized female mice.* Transl Stroke Res, 2014. **5**(4): p. 484-90.
61. Cheng, Z., et al., *Non-invasive remote limb ischemic postconditioning protects rats against focal cerebral ischemia by upregulating STAT3 and reducing apoptosis.* Int J Mol Med, 2014. **34**(4): p. 957-66.
62. Su, J., et al., *Autophagy activation contributes to the neuroprotection of remote ischemic perconditioning against focal cerebral ischemia in rats.* Neurochem Res, 2014. **39**(11): p. 2068-77.
63. Khan, M.B., et al., *Remote ischemic postconditioning: harnessing endogenous protection in a murine model of vascular cognitive impairment.* Transl Stroke Res, 2015. **6**(1): p. 69-77.

64. Li, H., et al., *The role of p38MAPK signal pathway in the neuroprotective mechanism of limb postconditioning against rat cerebral ischemia/reperfusion injury.* J Neurol Sci, 2015. **357**(1-2): p. 270-5.
65. Li, S., et al., *Remote ischemic post-conditioning improves neurological function by AQP4 down-regulation in astrocytes.* Behav Brain Res, 2015. **289**: p. 1-8.
66. Li, P., et al., *Remote limb ischemic postconditioning protects mouse brain against cerebral ischemia/reperfusion injury via upregulating expression of Nrf2, HO-1 and NQO-1 in mice.* Int J Neurosci, 2016. **126**(6): p. 552-559.
67. Zong, Y., et al., *Limb remote ischemic postconditioning protects cerebral ischemia from injury associated with expression of HIF-1alpha in rats.* BMC Neurosci, 2015. **16**: p. 97.
68. Wang, J., et al., *A Combination of Remote Ischemic Perconditioning and Cerebral Ischemic Postconditioning Inhibits Autophagy to Attenuate Plasma HMGB1 and Induce Neuroprotection Against Stroke in Rat.* J Mol Neurosci, 2016. **58**(4): p. 424-31.
69. Zhang, W., Y. Wang, and G. Bi, *Limb remote ischaemic postconditioning-induced elevation of fibulin-5 confers neuroprotection to rats with cerebral ischaemia/reperfusion injury: Activation of the AKT pathway.* Clin Exp Pharmacol Physiol, 2017. **44**(6): p. 656-663.
70. Li, J., et al., *Limb remote ischemic postconditioning protects integrity of the blood-brain barrier after stroke.* Neural Regen Res, 2018. **13**(9): p. 1585-1593.
71. Meng, H., et al., *Epidural injections with or without steroids in managing chronic low back pain secondary to lumbar spinal stenosis: a meta-analysis of 13 randomized controlled trials.* Drug Des Devel Ther, 2015. **9**: p. 4657-67.

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 1. Summarized description of preclinical studies in ischemic preconditioning

Study	Animals	Randomization groups	Stroke model	RIC location and cycles	Time of RIC (before stroke)	Infarct size	Neurological outcomes	Quality*	Physiological mechanism
Zhao et al. 2006.[49]	SHR rats Male 250-350 g. N=87	1: Preconditioning 2: Sham 3: Control	Permanent occlusion of the right MCA and CCA.	MCA. 1x10 min	24 hours	↓ Severity of perfusion deficits ↓ infarct volume		None described	
Ren et al. 2008.[27]	SD rats Male 270-330 g. N=60	Different preconditioning protocols at different time windows.	Permanent occlusion left distal MCA + occlusion bilateral CCA (30 min.)	Femoral artery 1: 2x5 min 2: 2x15 min 3: 3x15 min.	1: 12 hours, 2: 48 hours 3: immediately before	↓ infarct size with 2x15 min and 3x15 min.		Randomized	
Malhotra et al. 2011.[50]	Adult Wistar rats Male 200-225 g.	1: rIPreC 2: Sham surgery	MCA occlusion (120 min.)	Abdominal aorta 3x10 min.	1: 24 hours 2: 48 hours 3: 72 hours	1: ↓ infarct size 2: No effect 3: No effect	1: ↓ Neurological deficit scores (NDS) 2: No effect 3: No effect	Randomized Blinded	A ganglion blocker attenuated the neuroprotective effect.
Yuan et al. 2012.[51]	Wistar rats Male 250-280 g.	1: Sham group 2: Control group 3: IC of the CCA 4: rIPreC	Occlusion left CCA (30 min.) + permanent occlusion left distal MCA	Left hind limb 3x5 min.	Daily during the three days before stroke	↓ infarct size	↑ Neurological scores	Randomized	Increased cerebral anti-oxidative abilities.
Wei et al. 2012.[52]	SD Rats Male 250-350 g.	1: rIPreC 2: Control	Occlusion bilateral CCA + distal left MCA (30 min.)	Femoral artery 3x15 min.	Immediately before	↓ infarct size	↑ behavioral outcomes	Randomized Blinded	Through sensory nerves
Hu et al. 2012.[53]	SD rats Male 280-320 g. N=128	Eight different groups	Occlusion right MCA (120 min.)	Right hind limb 3x5 min.	1 hour	↓ infarct size on DWI imaging	↓ NDS	Randomized	Through adenosine pathway.

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

Study	Animals	Randomization groups	Stroke model	RIC location and cycles	Time of RIC	Infarct size	Neurological outcomes	Quality*	Physiological mechanism
Ren et al. 2009.[29]	SD rats Male 270-330 g. N=37	1: rIPostC 2: Control 3: Sham conditioning	Permanent occlusion left distal MCA + occlusion bilateral CCA (30 min.)	Femoral artery 3x15 min.	1: Immediately after reperfusion 2: 3 hours after stroke 3: 6 hours after stroke	1: ↓ 67% 2: ↓ 43% 3: No effect		Randomized Blinded	Through afferent nerves
Hahn et al. 2011.[54]	SD rats (p60) Male 270-330 g. N=39	1: rIPreC 2: rIPerC 3: Sham conditioning	MCA occlusion (120 min).	Left hind limb 4x5 min.	rIPreC: 40 minutes before ischemia rIPerC: during reperfusion	↓ in rIPreC ↓ in rIPerC		Randomized	
Ren et al. 2011.[55]	Adult SD rats Male 280-320 g. N=54	1: rIPerC 2: Sham conditioning.	MCA occlusion (90 min.)	Femoral artery of the lower limb, bilateral. 3x10 min.	Immediately after stroke and before reperfusion	↓ Infarct size ↓ Brain edema		Randomized	↓ Blood-brain barrier leakage
Sun et al. 2012.[56]	Adult SD rats Male 290-310 g. N=56	7 different serials of RIC	MCA occlusion (90 min.)	Femoral artery, bilateral. 3x5 min.	1: 3 hours after reperfusion 2: 6 hours after reperfusion	1: ↓ at 72 hours 2: ↓ at 72 hours	1: ↓ NDS 2: ↓ NDS	Randomized Blinded	Through opening of K_{ATP} channels.
Hoda et al. 2012.[57]	C57BL/6J Mice Male, 20 weeks old N=90	1: rIPerC + tPA 2: rIPerC without tPA 3: tPA only 4. Sham treatment	Thromboembolic with/without tPA after 4 hours	Left hind limb. 5x5 min.	2 hours after (embolic) stroke and 2 hours before reperfusion.	RIC alone: ↓ 25.7% RIC+tPA : ↓ 50%	RIC alone: ↓ NDS RIC+tPA : ↓ NDS	Randomized Blinded Sample size estimation	Increased relative CBF
Peng et al. 2012.[58]	Adult SD rats Male 200-250 g.	1: Sham conditioning 2: Control 3: rIPostC	Four vessel occlusion (8 min.)	Bilateral femoral artery. 3x15 min.	Immediately after global cerebral ischemia	↓ neuronal death	↑ spatial learning ↑ memory	Randomized Blinded	Upregulation of eNOS through the P13K/Akt pathway.
Qi et al. 2012.[59]	SD rats Male 300-320 g.	1: Control 2: rIPostC	MCA occlusion (120 min.)	Bilateral femoral artery 3x10 min.	1: Immediately after reperfusion 2: 10 min after reperfusion 3: 30 min after reperfusion	↓ Infarct volume	rIPostC within 10 min: ↑ neurological function	Blinded	A critical role for AKT/GSK3β-dependent autophagy in reducing cell death.

Table 2. Continued.

Study	Animals	Randomization groups	Stroke model	RIC location and cycles	Time of RIC	Infarct size	Neurological outcomes	Quality*	Physiological mechanism
Hoda et al. 2014.[60]	C57BL/6J mice Ovariectomized Female, 20 weeks old N=140	1: rIPerC + tPA 2: rIPerC without tPA 3: tPA only 4. Sham treatment	Thromboembolic with/without tPA after 4 hours	Left hind limb 4x10 min.	2 hours after stroke and 2 hours before reperfusion.	↓ infarct size ↓ hemorrhage ↓ edema	↑ sensorimotor function ↓ NDS ↓ mortality	Randomized Blinded Sample size estimation	RIC improved CBF
Cheng et al. 2014.[61]	Adult SD rats Male 250-300 g. N=45	1: Sham operation 2: Control 3: rIPostC	MCA occlusion (90 min.)	Right hind limb 3x5 min.	At the beginning of reperfusion	↓ infarct size	No improvement	Randomized	Related to neuronal apoptosis and inflammation.
Su et al. 2014.[62]	SD Rats Male 28-320 g. N=168	Seven experimental groups	MCA occlusion (120 min.)	Bilateral femoral artery 4x10 min.	At the beginning of MCA occlusion.	↓ infarct size ↓ edema	↓ NDS	Randomized Blinded	Through the autophagy-lysosome pathway
Khan et al. 2015.[63]	C57BL/6J mice Male, 10 weeks old N=20	1: Sham group 2: Control group 3: rIPostC	BCAS induced by microcoils around both CCA's.	Hind limb 4x10 min.	1 week after induction of BCAS. Daily for 2 weeks.		↑ Cognitive function	Randomized Blinded Sample size estimation	Increased cerebral perfusion.
Li et al. 2015. [64]	SD rats Male 220-280 g. 8-10 weeks old	1: Sham surgery 2: Control 3: rIPostC	MCA occlusion (120 min.)	Bilateral femoral artery 3x10 min.	Immediately after reperfusion.		↓ NDS	Randomized Blinded	Attenuation of neuronal apoptosis and suppression of p38 MAPk-AFT2 pathway.
Ren et al. 2015.[30]	Adult SD rats Male 280-320 g.	1: Single rIPerC 2: rIPerC + repeated rIPostC 3: Sham stroke 4: Ischemic control	MCA occlusion (90 min.)	Bilateral hind limb 3x10 min.	1: Single RIC: Immediately after stroke 2: Repeated RIC: Immediately after stroke + daily repeated RIC during 14 days	1: ↓ infarct size after 7 days 2: ↓ infarct size after 7 and 14 days	↑ neurological outcome	Blinded	Increased expression of neuroglobin.
Li et al. 2015.[65]	Adult SD rats Male 250-280 g. N=185	1: Sham group 2: Control group 3: rIPostC	MCA occlusion (60 min.)	Bilateral hind limb. 3x10 min.	During reperfusion.	↓ infarct volume ↓ edema	↑ neurological function	Randomized Blinded	Elevation of the integrity of blood-brain barrier.

Table 2. Continued.

Study	Animals	Randomization groups	Stroke model	RIC location and cycles	Time of RIC	Infarct size	Neurological outcomes	Quality*	Physiological mechanism
Li et al. 2015.[66]	CD1 mice Male 25-30 g. N=18	1: Sham group 2: Control group 3: rIPostC	MCA occlusion (60 min.)	Bilateral femoral artery.	Immediately after reperfusion	↓ infarct volume ↓ edema	↑ neurological outcome	Randomized Blinded	Reduction of oxidative stress.
Zong et al. 2015[67]	SD rats Male 250-280 g.	1: Sham 2: Control 3: rIPostC	MCA occlusion (60 min.)	Bilateral hind limb.	At the beginning of reperfusion	↓ infarct volume ↓ edema	↓ NDS	Randomized Blinded	Inhibition of HIF-1α.
Chen et al. 2016.[11]	SD rats Male 250-280 g.	1: rIPostC 2: Sham conditioning	MCA occlusion (90 min.)	Left femoral artery.	1: Immediately after reperfusion 2: 1 hour after reperfusion 3: 3 hours after reperfusion	1: ↓ infarct volume 2: No effect 3: No effect	1: ↑ Neurobehavioral scores 2: No effect 3: No effect	Randomized Blinded	Downregulation of the activation of NADPH oxidase in neutrophils.
Wang et al. 2016.[68]	Adult SD rats Male 250-280 g.	1: Sham 2: Control 3: rIPerC 4: IPOC 5: rIPerC +IPOC	MCA occlusion (120 min.)	rIPerc: left hind limb IPOC: MCA	rIPerC: 40 min prior to reperfusion IPOC: At the beginning of reperfusion	rIPerC + IPOC: ↓ infarct volume by >50% rIPerC alone: ↓ infarct volume by 25%	↓ NDS	Blinded	Inhibition of autophagy
Zhang et al. 2017.[69]	SD rats Male 300-320 g.	1: Sham 2: Control 3: rIPostC	MCA occlusion (120 min.)	Bilateral femoral artery	At the beginning of reperfusion	↓ infarct volume	↑ Neurobehavioral scores	Blinded	Suppression of blood brain barrier leakage.
Li et al. 2018.[70]	SD rats Female 250-280 g. 15-16 weeks N=81	1: rIPostC 2: Sham-stroke 3: ischemic control	MCA occlusion (60 min.)	Bilateral hind limb	Immediately after reperfusion	↓ infarct size by 41.9% ↓ edema by 27.6%	↓ NDS	Randomized Blinded	Reduction of blood-brain barrier injury and leakage.
Doeppner et al. 2018.[31]	C57BL6 mice Male 24-28 g.	1: rIPostC 2: Control	MCA occlusion (60 min.)	Bilateral hind limb	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	1: ↓39.8% 2: ↓26% 3: ↑ neuronal density by 60.1%	1: Transient improvement 2: transient improvement 3: Sustained improvement	Randomized Blinded	Mediated via HSP-70.

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

Study	Patients	Randomization groups	Location of RIC	Cycles (occlusion/reperfusion)	Time of RIC	Effect on infarct size	Effect on neurological outcomes	Physiological mechanism
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. ↓ Tissue risk of infarction	No effect (NIHSS and mRS)	
Meng et al. 2015.[71]	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 No effect on number of lacunar infarcts	↓ Dizziness handicap inventory	Accelerated flow velocity in MCA.
England et al. 2017.[32]	Patients with acute ischemic stroke (N=26).	1: rIPostC (N=13) 2: Sham (N=13)	Upper arm	4x5 min.	Within 24 hours after onset of symptoms.		↓ NIHSS	Augmentation of plasma HSP-27.

Table 3. Continued.

Study	Patients	Randomization groups	Location of RIC	Cycles (occlusion/reperfusion)	Time of RIC	Effect on infarct size	Effect on neurological outcomes	Physiological mechanism
Wang et al. 2017.[38]	Patients with cerebral small vessel disease-related mild cognitive impairment (N=30).	1: RIC (N=14) 2: Sham (N=16)	Bilateral upper arm	5x5 min.	Twice daily for 1 year	↓ White matter hyperintensities	↑ visuospatial and executive abilities	Effect on triglycerides, cholesterol and homocysteine
Zhao et al. 2017.[33]	Patients undergoing carotid artery stenting (N=189).	1: rIPreC (N=63) 2: Sham (N=63) 3: No intervention (N=63)	Bilateral upper arm	5x5 min.	Twice daily during two weeks before carotid artery stenting.	↓ new DWI lesions ↓ DWI lesions volume	No effect on clinical ischemic events	No changes in Enolase or S-100B levels.

Table 4. Risk of bias assessment of clinical studies into the effect of remote ischemic conditioning

Study	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other sources of bias
Meng et al. 2012.[36]	Low	Unclear	High	Low	High	Low	High
Hougaard et al. 2014.[35]	Low	Unclear	High	Low	High	Low	High
Meng et al. 2015.[71]	Low	Low	Low	Low	High	Low	High
Mi et al. 2016.[39]	Low	Unclear	Low	Low	Low	Low	High
England et al. 2017.[32]	Low	Low	Low	Low	Low	Low	Low
Wang et al. 2017.[38]	Unclear	Unclear	Low	Low	High	Low	High
Zhao et al. 2017.[33]	Low	Low	High	Low	High	Low	High