

Translational challenges of remote ischemic conditioning in ischemic stroke – a systematic review

Line Fuglsang Hansen^{1,2}, Nicholine S. K. Nielsen¹, Laura Cathrine Christoffersen¹ & Christina Kruuse^{1,3} 

¹Department of Neurology, Neurovascular Research Unit, Herlev Gentofte Hospital, Copenhagen, Denmark

²Department of Anesthesiology and Intensive Care, Holbaek Hospital, Holbaek, Denmark

³Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

Correspondence

Christina Kruuse, Department of Neurology, Neurovascular Research Unit, University of Copenhagen, Borgmester Ib Juuls Vej 1, DK-2730 Herlev, Herlev og Gentofte Hospital, Copenhagen, Denmark. Tel: +4538681233; E-mail: ckruuse@dadlnet.dk

Funding Information

CK was supported by a grant from Novo Nordisk Foundation (Borregaard Stipend, NNF18OC0031840), LC was supported by a research scholarship from Lundbeck Foundation.

Received: 11 February 2021; Revised: 20 April 2021; Accepted: 19 May 2021

Annals of Clinical and Translational Neurology 2021; 8(8): 1720–1729

doi: 10.1002/acn3.51405

Abstract

Remote ischemic conditioning (RIC) has well-established cardioprotective effects in preclinical studies and promising results in preclinical stroke research. Effective translation from preclinical studies to clinical trials has yet to be accomplished, perhaps because of the use of multiple applications of RIC (e.g., pre-, per-, or post-conditioning) in preclinical studies by both invasive and non-invasive protocols, some of which not clinically applicable. Our systematic review conformed to PRISMA guidelines and addressed differences in clinically relevant RIC applications and outcomes between preclinical and clinical studies. We retrieved a total of 30 studies (8 human; 22 animal) that met the inclusion criteria of testing clinically relevant procedures; namely, non-invasive and per- or post-conditioning protocols. Per-conditioning was applied in 6 animal and 3 human studies, post-conditioning was applied in 16 animal and 5 human studies, and both conditioning methods were applied in 2 animal studies. Application of RIC varied between human and animal studies regarding initiation, duration, repetition, and number of limbs included. Study designs did not systematically apply blinding, randomization, or placebo controls. On only a few occasions did preclinical studies include animals with clinically relevant comorbidities. Clinical trials were challenged by not completing the intended number of RIC cycles or addressing this deficit in the data analysis. Consistency and transferability of methods used for positive animal studies and subsequent human studies are essential for the optimal translation of results. Consensus on preclinical and clinical RIC procedures should be reached for a full understanding of the possible beneficial effects of RIC treatment in stroke.

Introduction

Remote ischemic conditioning (RIC), performed by applying non-fatal, short, intermittent ischemia on a limb, could be a promising new add-on treatment of cardio- and cerebrovascular ischemic diseases. In experiments using animals with induced ischemic stroke, RIC reduces ischemic lesions, improves neurological outcome, and can be applied before (remote ischemic pre-conditioning; RIPreC), during (remote ischemic per-conditioning; RIPerC) the ischemic event or after reperfusion (remote ischemic post-conditioning; RIPostC).^{1–3}

Notwithstanding new advances in reperfusion therapy, cerebrovascular disease is a major cause of disability and death in the Western world.⁴ Reperfusion therapy is

only available for the smaller number of patients who receive timely medical care, so new treatments are highly warranted. RIC treatment shows positive effects in animal stroke models, but effects do not translate well into clinical results, as noted in a recent meta-analysis of seven clinical trials that found no consistent effect on stroke outcome.⁵ Such data call for a review of methods applied in preclinical studies to address pitfalls in procedures that challenge the translation of preclinical results to human studies and to improve preclinical data prior to clinical trials.

In general, the challenge of translating treatment effects on stroke from animal studies to human trials is often ascribed to non-physiological experimental conditions in animals that do not reflect human conditions.⁶

Most animal experiments apply RIC in pre-conditioning protocols, which is highly relevant in situations where ischemia may be anticipated, such as during cardiac or vascular surgery.^{7,8} Valuable results on the effects of pre-conditioning have been achieved and tested in humans.⁹ However, in medical conditions with unpredictable onset, such as acute stroke, results from the pre-conditioning studies may be less applicable, and results from per-or post-conditioning more likely to mimic the clinical condition. Tests of pre-, per-, and post-conditioning regimens of RIC show that each application elicits contradictory findings or varying levels of effectiveness.^{3,10,11} Invasive and non-invasive methods are applied in administering RIC, which may impact elicited physiological responses differently.

Though lacking comparability, results from these very methodically diverse studies have led to conclusions on the efficacy of RIC also in humans.^{12,13} Non-invasive per-or postconditioning RIC protocol would be the most relevant application in human acute stroke, and results from applying these conditions in animals would be most relevant to translate to human use.

In this review, we focused on methods reported in positive preclinical studies that test clinically relevant applications of RIC for acute stroke. We aimed to determine the comparability of trial procedures to those of human trials to further optimize both preclinical and human studies and disclose possible beneficial effects of RIC in stroke treatment (Fig. 1).

Methods

Electronic search

The electronic search followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines in MEDLINE and Embase.¹⁴ A search string was developed and revised by three reviewers (LH, LC, and CK). Reference lists of included papers were searched for other relevant studies. The search strategy combined terms for stroke and ischemic conditioning, including relevant MeSH terms (MEDLINE) or Map terms (Embase) (Fig. S1). Only studies on focal ischemic stroke were included in the final data processing. The final electronic search was performed on March 30, 2020 (LH and NN).

Study selection

Inclusion criteria consisted of studies investigating RPerC or RPostC in animal stroke models or patients with acute focal ischemic stroke. Exclusion criteria included review studies, studies that were written in languages other than Danish or English, investigated only RPreC, used an invasive RIC protocol or global ischemia/asphyxia models, and studies investigating hemorrhagic stroke or subarachnoid hemorrhage. The electronic data search generated 1811 hits (Fig. S2). All papers were transferred into the web-based review-screening tool Covidence (Covidence, Melbourne, Australia). After the electronic removal

Remote ischemic conditioning in stroke - aim and hypothesis

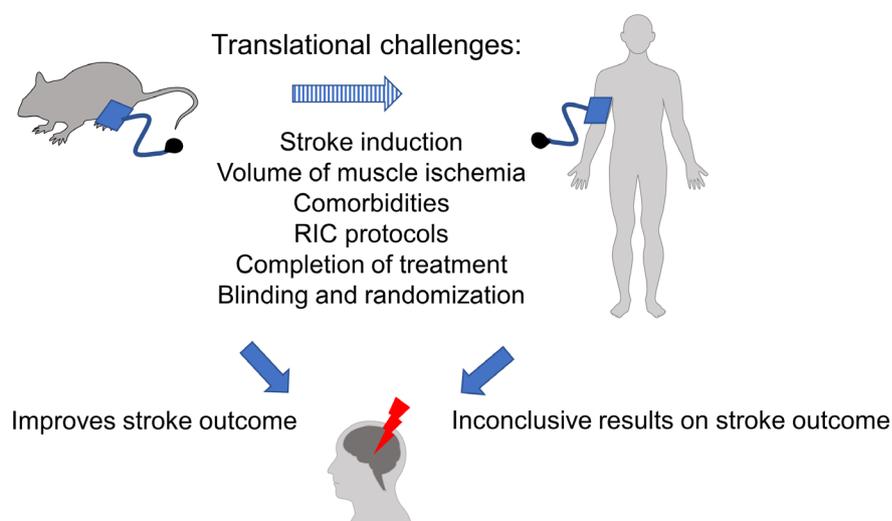


Figure 1. Graphic presentation of aim and hypothesis. Translation of preclinical positive data may be challenged by a multitude of factors accounting for unresolved effects in clinical trials.

of duplicates, two reviewers (LH and NN) screened titles and abstracts and read the full text to assess the eligibility of papers. Disagreements were resolved by consensus and a third reviewer (CK).

One of the 10 studies lacking details of the RIC procedure was included in our analysis after the authors provided the details. The most frequent cause of exclusion was the use of an invasive RIC protocol. Regular meta-analysis was not possible because data from the selected papers had highly variable outcome measures and result reporting.

Results

Full-text screening resulted in 30 papers comprising 22 animal experiments and 8 clinical trials (Fig. 2).

Methods applied in animal experiments

Methodology varied between studies (Tables 1–3 and Table S1).

Focal cerebral ischemia was induced by the unilateral filament middle cerebral artery occlusion (MCAO) model in the majority of studies (Table 1).^{1–3,10,11,15–29} The duration of MCAO (and thus size of induced lesions) varied from 30 to 120 min, with 60^{2,18,23,24,26–28} or 90^{1,15,16,20,21,25,29} min of occlusion being the most common duration. Only two studies used the MCAO embolic model (embolic stroke is induced by the injection of a homologous blood clot into the middle cerebral artery).^{30,31} Anesthetics were used during MCAO in all studies; most used chloral hydrate,^{17–21,25–28} eight used

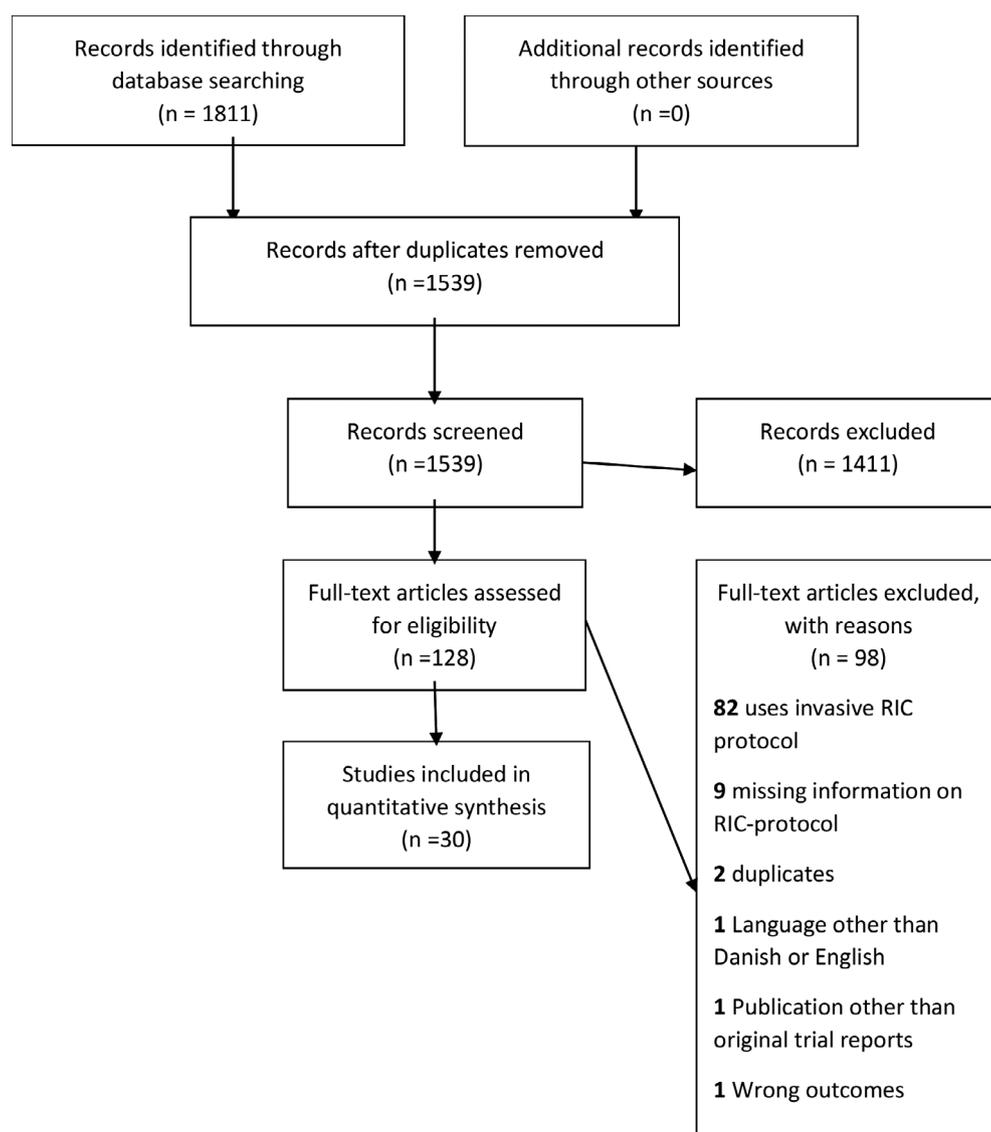


Figure 2. PRISMA flow chart on the selection process in the systematic review.

Table 1. Methods of stroke induction in preclinical RIC studies.

Method	Details of method		Number of studies (N) and references
Middle cerebral artery occlusion (MCAO)	Type of MCAO	Filament MCAO	(20) ^{1-3,10,11,15-29}
		Embolic MCAO	(2) ^{30,31}
	Side	Right	(16) ^{1-3,10,15-20,25-29,31}
		Left	(3) ^{11,21,23}
	Duration	Not published	(3) ^{22,24,30}
		30 min	(1) ²²
		45 min	(2) ^{10,11}
		60 min	(7) ^{2,18,23,24,26-28}
		90 min	(7) ^{1,15,16,20,21,25,29}
		120 min	(3) ^{3,17,19}
		Blood clot – dissolve time unknown	(2) ^{30,31}
	Anesthesia under MCAO	Isoflurane/enflurane	(8) ^{1,3,10,11,22-24,30}
		Enflurane	(2) ^{2,29}
		Halothane	(1) ²⁴
		Chloral hydrate	(9) ^{17-21,25-28}
Ketamine + propofol		(1) ³¹	
	Not published	(2) ^{15,16}	

isoflurane,^{1,3,10,11,22-24,30} and two used the structural isomer enflurane.^{2,29} One study used isoflurane and halothane during the MCAO to determine if either had additional effects on the outcomes of RPostC.²⁴ Sodium pentobarbital was used prior to inducing RPostC/sham

procedures in two studies, but the type of anesthesia used was not reported.^{15,16} During post-conditioning procedures, three studies reported the use of ketamine+xylazine and either propofol,³¹ isoflurane,²² or isoflurane, respectively.²⁴ The remaining 13 studies using RPostC did not disclose whether RIC procedures were carried out, while animals were under additional use of anesthesia or if the control groups were exposed to similar doses and durations of anesthesia.^{1,2,10,11,18-21,23,25-28}

Of 22 animal studies, six applied RPerC, 16 applied RPostC, and two used a combination of RPerC and RPostC (Table 2). RPerC studies were performed during MCAO^{3,11,15-17,29} or immediately following the introduction of a fibrinogen clot.³⁰ The latter study applied RIC for 2 h, making it a borderline RPostC-study experiment. Likewise, one study claimed to investigate RPostC but initiated RIC just before reperfusion, thereby almost resembling RPerC.¹⁹ Almost all the 16 RPostC protocols initiated RIC immediately after reperfusion^{2,10,11,18-20,25-28,31}; only five protocols delayed the RPostC.^{1,21-24} Variations in the protocol of delayed RPostC studies disallowed a direct comparison. Two studies initiated single-session RPostC at 30¹ and 120²² min, respectively, after the completion of MCAO. One study initiated RPostC at 12 h, 24 h, and 5 days after MCAO, and applied it up to three times with variable time intervals to investigate very delayed post-conditioning.²³ Two studies tested consecutive RPostC; one initiated the protocol for 90 min and applied RIC for a total of four sessions during the

Table 2. Procedures for RIC intervention in preclinical studies.

Details of Method		Number of studies (N) and references
Intervention	Type of RIC tested	RPerC (6) ^{3,11,16,17,29,30}
		RPostC (16) ^{1,2,10,11,18-28,31}
		Combination of RPerC and RPostC (2) ^{15,16}
Equipment	Tourniquet	(13) ^{1,3,11,15-17,22-25,29-31}
	Rubber bands	(7) ^{2,18,19,21,26-28}
	Gauze ropes	(1) ¹⁰
	Undisclosed, but non-invasive	(1) ²⁰
		Unilateral hindlimb (8) ^{1,3,11,17,19,22,25,30}
Limb	Bilateral hindlimb	(12) ^{2,10,15,16,18,20,23,24,26-29}
	Tested both unilateral, bilateral, and multiple limbs	(1) ³¹
	Not indicated	(1) ²¹
Initiation of RPostC protocols*	Immediately after MCAO	(11) ^{2,10,11,18-20,25-28,31}
	30/90/120 min after MCAO	(1) ¹ /(1) ²⁴ /(1) ²²
	12/24/48/120 h after MCAO	(1), ²³ for 48 h (1) ²¹
Evaluation of appropriate ischemia by...	Pulse monitoring/Doppler	(7) ^{1,2,25-29}
	Clinical evaluation	(7) ^{3,11,17-19,21,30}
	Not published	(8) ^{10,15,16,20,22-24,31}

*Describes only RPostC protocols. RPerC protocols are all initiated under MCAO,^{3,11,15-17,29} except one study using a fibrinogen clot; here RPerC is initiated 2 h after clot induction.³⁰ The combinatory (RPerC + RPostC) protocols all initiated RIC after 24 h.^{15,16}

Table 3. RIC protocols used in the included preclinical studies.

Protocol	Number of cycles	Inflation/deflation (minutes)	Number of studies (N) and references
Single session	3	5/5 10/10	(2) ^{1,25} (8) ^{2,10,18,20,26–29}
RIPerC/RIPostC	4	15/15 5/5 10/10	(1) ¹⁹ (3) ^{3,11,17} (1) ³⁰
	5	5/5	(1) ²²
	10	5/5	(1) ³¹
Repeated	3	5/5	(2) ^{21,24}
RIPostC	3	10/10	(1) ²³
Combination	3	10/10	(2) ^{15,16}

following 72 h²⁴ and another initiated RIC at 48 h after reperfusion and applied it daily for 21 consecutive days.²¹ In two studies testing the combination of RIPerC and RIPostC for 7 or 14 days, the RIPostC regime started 24 h after the MCAO.^{15,16}

Most RIPostC protocols investigated immediate RIPostC, but the application and number of cycles varied so the extent of the ischemic response elicited varied across the experiments.

RIC was mainly applied on hindlimbs, either on one limb^{1,3,11,17,19,22,25,30} or more commonly bilaterally.^{2,10,15,16,18,20,23,24,26–29} One study did not reveal the number of limbs subjected to RIC.²¹ Effects of RIC applied on unilateral, bilateral, and multiple limbs were also compared to explore differences in the efficacy of RICs based on the number of RIC-affected limbs.³¹

RIC was accomplished using custom-made tourniquets,^{1,3,11,15–17,22–25,29–31} rubber bands,^{2,18,19,21,26–28} and gauze ropes.¹⁰ In one study, the authors only stated that RIC was performed non-invasively.²⁰ Complete limb ischemia was confirmed using monitoring or Doppler ultrasound equipment to detect the loss of pulse or flow^{1,2,25–29} or clinical evaluation.^{3,11,17–19,21,30} More than one-third of the studies omitted how adequate ischemia was ensured.^{10,15,16,20,22–24,31}

The number of cycles and the occlusion/reperfusion time in RIC sessions differed between studies (Table 3). The most commonly applied protocol ($N = 9$) comprised three cycles of 10 min of occlusion interspersed with 10 min of deflation.^{2,10,18,20,23,26–29} Most studies administered RIC as a single session; only five studies applied multiple RIC sessions.^{15,16,21,23,24} Studies testing multiple RIC applications or combinations of RIPerC and RIPostC differed in RIC application protocol and follow-up periods.

Most studies were performed in rats,^{1,3,15–21,25–29} seven studies used mice^{2,10,11,22–24,30} and one study included

primates³¹ (Table S1). Most studies used males,^{2,3,10,11,15–17,19–21,23–25,29,31} four studies used females,^{26–28,30} and one study included both sexes.²² Two studies did not disclose the sex of the investigated animals.^{1,18} Only two studies investigated RIC in non-healthy animals, one in ovariectomized female mice,³⁰ and one in diabetic rats.¹⁰

Trial designs were challenged by several important issues such as information on randomization; only one of the 22 studies described randomization procedures,²⁹ some claimed randomization but gave no further details,^{3,18,19,22–26,28} and others described randomization as groups formed by randomly selecting animals from cages.^{2,27,30} Nine studies did not inform on randomization to groups.^{1,10,11,15–17,20,21,31} Most studies reported blinding was applied for parts of the data analysis.^{1,3,10,11,15–24,26–31}

Methods applied in human trials

Eight stroke patient trials were eligible for this review. In four studies, the effect of RIC was investigated in patients who were not eligible for revascularization therapies.^{32–35} In three studies, RIC was applied as an add-on for treatments of thrombolysis by intravenous alteplase^{36,37} or thrombectomy.³⁸ One study included patients receiving additional medical treatment and patients not eligible for thrombolysis or thrombectomy.³⁹ None of the included studies used a comparable study protocol and/or follow-up period, so the results of the individual studies remain unconfirmed.

Of three studies including patients who received revascularization therapy, only one tested single-session RIC on patients during transport to the stroke unit for thrombolysis.³⁷ The second study administered RIPerC within 2 h after the administration of thrombolysis, followed by twice-a-day RIC sessions for a total of 7 days.³⁶ The third study combined RIPerC followed by RIPostC for 7 days to patients who received endovascular thrombectomy treatment within 6 h of the onset of stroke.³⁸

Studies of patients who did not undergo additional revascularization initiated RIC within 24 h from stroke onset³² or 24–72 h (with subsequent daily sessions up to 14 days³³). Two studies did not indicate when RIC was initiated,^{34,35} revealing only that one inclusion criterion was a symptom onset between 4.5 h and 14 days prior to the initiation of RIC.³⁵ The study that included patients with and without additional medical revascularization included patients within 6 h from disease onset and had three groups. Each group received one RIC session, two RIC sessions 1 h apart, or RIC sessions twice daily for 4 days from ictus.³⁹

In two studies, protocols comprised five cycles of 5 min of inflation and 5 min of deflation of a tourniquet,

5 days a week for 8 weeks in total,³⁴ or one daily RIC for 6 months.³⁵ These protocols are in contrast to remaining studies in which each RIC session comprised only four cycles with 5 min of inflation and 5 min of deflation.^{32,33,36–39}

The clinical trials mainly applied RIC to a single upper extremity; five studies performed RIC on the non-paretic arm^{32–35,39} and two studies did not specify on which arm the intervention occurred.^{37,38} One study performed RIC bilaterally on upper limbs but due to the administration of intravenous fluids or blood pressure cuffs, not all cycles were performed bilaterally. No information was disclosed regarding the number of patients who did not receive all cycles of bilateral RIC or the impact on data analysis.³⁶

Overall, RIC protocols of clinical studies differed in the type of RIC, number of affected limbs, initiation, repetition, time periods, and completing all intended cycles. Studies offering data on completion showed rate ranges of 40%–97%.^{32,36–39} One study described that not all cycles were completed, but did not include data on the number of completed cycles.³³ Two studies also failed to report the number of completed cycles, but had the most extensive testing and follow-up periods of 8 weeks³⁵ and 6 months,³⁴ respectively. The RPerC study performed in the ambulance discontinued the intervention upon arrival to the stroke unit, leading to the lowest published completion rate of included studies, with only 33 of 81 patients receiving the intended number of cycles.³⁷ None of the studies reported a possible bias of non-completed cycles in their data analysis.^{32–39}

There was a male preponderance of 52%–80% in seven studies.^{32,34–39} One study did not disclose the gender distribution.³³ Unlike preclinical studies, clinical studies commonly had an intervention group with multiple known stroke risk factors like smoking, hypertension, and obesity.^{32–39}

Randomization was applied in seven of the eight clinical trials,^{32–37,39} three of which were placebo controlled.^{32,33,39} All studies were open-labeled, but five studies used outcome observer blinding.^{32–34,36,37,39} One study did not inform on blinding,³⁵ and the remaining study did not include controls, randomization, or blinding.³⁸

Discussion

Based on the preclinical data in animals and clinical data in humans identified in our review, we found multiple important discrepancies in the design and performance of the RIC protocols that could be barriers in the translation of results. Important differences were (1) preclinical studies applied RIC on hindlimbs representing a larger muscle

mass than that of forearm occlusion applied in humans, (2) the need for the use of anesthetics in animal studies, (3) lack of relevant comorbidities in animals used for testing, and (4) no homogeneity of timing, number, duration, or repetition of RIC in animal or human studies. These significant differences in methodology may account for the poor translation of otherwise promising preclinical data on using RIC in acute stroke into positive results in clinical trials of which four are now completed.

Failing to translate positive results from animals to human clinical trials is a well-known phenomenon. To reduce the risk of pitfalls in the process of translation, the Stroke Treatment Academic Industry Roundtable (STAIR) recommendations⁶ on preclinical trials were formulated. STAIR recommendations describe the importance of considering the choice of species and gender, proposed mechanism of action of treatment, and how to incorporate human comorbid conditions into preclinical trials. In research on cardioprotection, a four-step process was identified by Rossello and Yellon to improve the translation of data.⁴⁰ Similar steps could be applicable in neuroscience translational research to help identify gaps in current knowledge, which may improve outcomes in future clinical trials (Fig. 3).

Studies included in this review suffer from pitfalls addressed in both the STAIR recommendations and in identified steps on translational studies in cardioprotection.

In preclinical studies, transient filament MCAO with variable occlusion time was the most frequently applied method for stroke induction, with only two exceptions that applied the embolic MCAO model.^{30,31} In contrast to clinical stroke, the MCAO filament model used in animal experiments offers immediate brain ischemia and complete reperfusion of blood flow when terminated.^{15,16,29} This fast return of perfusion resembles the clinical situation of patients receiving endovascular thrombectomy treatment or thrombolysis. However, the method bears little resemblance to the clinical situation of patients who experience ischemic stroke with spontaneous dissolving clots and reperfusion. Furthermore, introducing the filament through the arteries may induce significant mechanical endothelial damage not equivalent to human stroke. An animal model that mimics focal cerebral ischemia with gradual reperfusion, as is expected to occur in human ischemic stroke, has yet to be found. Although normal filament MCAO may be the best preclinical method to investigate conditions resembling those in patients receiving reperfusion therapy, animal models that apply a modified blood clot^{30,31} may be preferred when exploring conditions similar to stroke patients not eligible for revascularization procedures, but not to those with minor strokes.

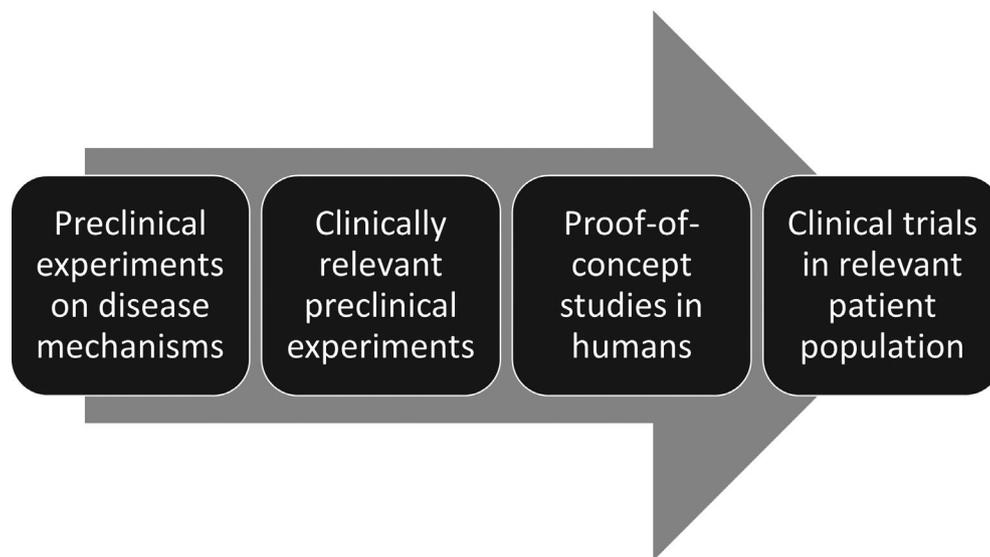


Figure 3. Investigations of RIC effects in stroke partly omit the essential intermediate steps 2 and 3 in the process of translation suggested by Rossello and Yellon.⁴⁰

Most preclinical trials administered RPostC immediately after reperfusion, primarily mimicking the small group of patients undergoing abrupt reperfusion therapies in acute stroke. Testing effects of delayed conditioning is perhaps more interesting for future treatment because not all arrive in a timely fashion or are eligible for revascularization therapy. Unfortunately, the few studies that tested a delayed application of RIC all applied different RIC protocols, leaving their conclusions unconfirmed.^{1,15,16,21–24} Studies testing and confirming the effect of delayed RIC from animal studies, could have huge translational potential and become important in clinical treatment.

Another important challenge of the animal models is using isoflurane or enflurane as anesthetics during MCAO and while administering RPostC. Isoflurane is a potential confounder as decreases excitotoxicity and improves functional and histological outcomes^{41–43} in stroke. One study investigated a possible difference in outcome after using RIC on animals subjected to isoflurane anesthesia under MCAO and under RIC compared to halothane MCAO and ketamine-xylazine RIC and concluded that both anesthetics might have influenced the results.²⁴ Thus, even though volatile anesthetics are easy to handle and have rapid effects, they should be avoided in experimental stroke research. An alternative could be the use of choral hydrate.^{17–21,25–28}

Common to many studies is a discrepancy in the extent of remote ischemia induced by the RIC protocol in terms of the amount of afflicted tissue subjected to ischemia and length of RIC number of repeated cycles. All human studies performed RIC on upper limbs and, with only

one exception,³¹ all animal trials applied RIC unilaterally or bilaterally on hindlimbs. The hindlimb of any animal (mouse, rat, or primate), represents a larger muscle mass than that of an arm in a human, thereby increasing the relative amount of induced ischemia. In primates, the optimal number of limbs subjected to RIC was a two-limb protocol, which elicited a greater effect than a one-limb protocol; a four-limb protocol had no additional effect.³¹ This is an interesting and important finding, but all human trials except one conducted RIC on only one upper extremity. The study that intended to provide RIC bilaterally failed and did not disclose the level of fulfillment or how it may have affected the results.

The number and length of RIC cycles varied greatly in animal studies (Table 3), whereas the preferred protocol in humans consisted of four cycles of 5 min of occlusion interspersed by 5 min of reperfusion. None of the human trials succeeded in applying the intended RIC protocol; trials had low completion rates that were not taken into account in the data analysis and interpretation of results.^{32,33,37,38} A recent study published following the completion of our final search reported to complete all intended cycles but reported no clinical effect of RIC.⁴⁴

There are currently no animal or human studies that have aimed to define the optimal number of cycles of occlusion/deflation time and still have a translatable RIC protocol. Although RIC was achieved non-invasively by tightening a rubber band, gauze rope, or a tourniquet, only a few preclinical trials assured complete ischemia of the limb (e.g., by using pulse monitoring) to confirm a uniform ischemic response in the subjects.

It is evident that the ischemic response induced in pre-clinical studies is larger than those induced in human trials. This finding warrants new experiments in which the amount of administered RIC is more comparable. This approach could be achieved if RIC was induced on the upper limbs in preclinical trials or if RIC was administered on legs or bilaterally on arms in clinical trials, and by considering the number and length of obtained RIC sessions in data analysis.

Another pitfall was to include animals that are not comparable to relevant patient populations. Studies are needed that test RIC in animals with known risk factors that are characteristic for stroke patients (i.e., hypertension, diabetes, atrial fibrillation) to properly estimate the effect of RIC in stroke. Few studies tested non-invasive RIC in animals with risk factors,^{10,30} but data from both pre-conditioning protocols and invasive RIC experiments suggest that risk factors impact the effectiveness of RIC.⁴⁵ This translational gap underscores the need for animal studies to investigate possible confounders when evaluating a beneficial effect of RIC in stroke patients. Preclinical RIC experiments should also include multiple species, including gyrencephalic species in which anatomy and physiology show stronger resemblance to humans, which has only been performed in one study.³¹

Rigor, reproducibility, and clinical feasibility, the main pillars of translational research, decrease the risk of bias and confounders and increase translational potential. In the animal experiments reviewed, the use of randomization and a thorough description of how randomization was performed was only included in one of 22 studies.²⁹ The inconsistency in using true randomization greatly increases the risk of bias and false-positive results, decreasing the rigor and reproducibility of the studies. Similar pitfalls apply in human studies reviewed, in which only three of the eight studies used placebo in the control group and all were open-labeled. Importantly, both randomization and blinding of evaluators were common in the human trials.

In addition to the RIC methods applied, which is the scope of the current review, major pitfalls may also lie in the selection of endpoints, like infarct size and neurological scores. Both infarct size and behavioral scoring were often evaluated only 24 h from stroke induction; thus, long-term effects could not be determined. More work needs to be performed to harmonize the use of preclinical and clinical outcome measures.

Conclusion

A translational gap exists in understanding the effects of RIC in stroke. Most preclinical studies use methods that

are not applicable in acute stroke treatment in humans. We found only 22 studies that investigated RIC for acute treatment. Major methodological differences that decrease the translational potential of preclinical studies (e.g., use of healthy male rats and undergoing MCAO under the influence of confounding anesthesia) were revealed in diverse RIC protocols. There were no studies that determined optimal RIC protocol on the length, number of cycles, repetition, or time of initiation. Human trials were challenged by the non-completion of intended protocols and by not addressing this problem in data analysis.

Future animal studies on RIC should incorporate STAIR recommendations when planning and executing preclinical trials and determine clinically relevant endpoints. Human trials should strive to apply intended cycles of RIC and consider optimal RIC protocols and applications resembling those performed in positive pre-clinical trials.

Acknowledgment

CK was supported by a grant from Novo Nordisk Foundation (Borregaard Stipend, NNF18OC0031840), LC was supported by a research scholarship from Lundbeck Foundation.

Conflict of Interest

The authors have no conflict of interest except for grants mentioned in acknowledgments.

References

1. Bonova P, Gottlieb M. Blood as the carrier of ischemic tolerance in rat brain. *J Neurosci Res* 2015;93:1250–1257.
2. Cheng X, Zhao H, Yan F, et al. Limb remote ischemic post-conditioning mitigates brain recovery in a mouse model of ischemic stroke by regulating reactive astrocytic plasticity. *Brain Res* 2018;1686:94–100.
3. Hahn CD, Manliot C, Schmidt MR, et al. Remote ischemic per-conditioning: a novel therapy for acute stroke? *Stroke* 2011;42:2960–2962.
4. World Health Organization. WHO - The top 10 causes of death [Internet]. 24 Maggio 2018;1–7. [cited 2020 Apr 23]. Available from: <http://www.who.int/en/news-room/factsheets/detail/the-top-10-causes-of-death>
5. Zhao W, Zhang J, Sadowsky MG, et al. Remote ischaemic conditioning for preventing and treating ischaemic stroke. *Cochrane Database Syst Rev* 2018;7:CD012503.
6. Kahle MP, Bix GJ. Successfully climbing the “STAIRs”: surmounting failed translation of experimental ischemic stroke treatments. *Stroke Res Treat* 2012;2012:8.
7. Healy DA, Clarke Moloney M, McHugh SM, et al. Remote ischaemic preconditioning as a method for perioperative

- cardioprotection: concepts, applications and future directions. *Int J Surg* 2014;12:1093–1099.
8. Sprick JD, Mallet RT, Przyklen K, Rickards CA. Ischaemic and hypoxic conditioning: potential for protection of vital organs. *Exp Physiol* 2019;104:278–294.
 9. Hausenloy DJ, Yellon DM. Ischaemic conditioning and reperfusion injury. *Nat Rev Cardiol* 2016;13:193–209.
 10. Liu C, Yang J, Zhang C, et al. Remote ischemic conditioning reduced cerebral ischemic injury by modulating inflammatory responses and ERK activity in type 2 diabetic mice. *Neurochem Int* 2020;135:104690.
 11. Kitagawa K, Saitoh M, Ishizuka K, Shimizu S. Remote limb ischemic conditioning during cerebral ischemia reduces infarct size through enhanced collateral circulation in murine focal cerebral ischemia. *J Stroke Cerebrovasc Dis* 2018;27:831–838.
 12. Ma D, Feng L, Deng F, Feng J. Overview of experimental and clinical findings regarding the neuroprotective effects of cerebral ischemic postconditioning. *Biomed Res Int* 2017;2017:6891645.
 13. Hess DC, Blauenfeldt RA, Andersen G, et al. Remote ischaemic conditioning—a new paradigm of self-protection in the brain. *Nat Rev Neurol* 2015;11:698–710.
 14. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009;6:e1000097.
 15. Ren C, Li S, Wang B, et al. Limb remote ischemic conditioning increases Notch signaling activity and promotes arteriogenesis in the ischemic rat brain. *Behav Brain Res* 2018;340:87–93.
 16. Ren C, Wang P, Wang B, 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;33:369–379.
 17. Wang J, Han D, Sun M, Fen J. A combination of remote ischemic preconditioning and cerebral ischemic postconditioning inhibits autophagy to attenuate plasma HMGB1 and induce neuroprotection against stroke in rat. *J Mol Neurosci* 2016;58:424–431.
 18. Zong Y, Jiang L, Zhang M, et al. Limb remote ischemic postconditioning protects cerebral ischemia from injury associated with expression of HIF-1 α in rats. *BMC Neurosci* 2015;16:1.
 19. Chen G, Shan X, Li X, Tao H. Remote ischemic postconditioning protects the brain from focal ischemia/reperfusion injury by inhibiting autophagy through the mTOR/p70S6K pathway. *Neurol Res* 2018;40:182–188.
 20. Huang D, Liu H, Qu Y, Wang P. Non-invasive remote ischemic postconditioning stimulates neurogenesis during the recovery phase after cerebral ischemia. *Metab Brain Dis* 2017;32:1805–1818.
 21. Liang D, He XB, Wang Z, et al. Remote limb ischemic postconditioning promotes motor function recovery in a rat model of ischemic stroke via the up-regulation of endogenous tissue kallikrein. *CNS Neurosci Ther* 2018;24:519–527.
 22. Yang J, Balkaya M, Beltran C, et al. Remote postischemic conditioning promotes stroke recovery by shifting circulating monocytes to CCR2(+) proinflammatory subset. *J Neurosci* 2019;39:7778–7789.
 23. Doeppner TR, Zechmeister B, Kaltwasser B, et al. Very delayed remote ischemic post-conditioning induces sustained neurological recovery by mechanisms involving enhanced angiogenesis and peripheral immunosuppression reversal. *Front Cell Neurosci* 2018;12:383.
 24. Chen G, Kamat PK, Ahmad AS, Dore S. Distinctive effect of anesthetics on the effect of limb remote ischemic postconditioning following ischemic stroke. *PLoS One* 2020;15:e0227624.
 25. Cheng Z, Li L, Mo X, 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;34:957–966.
 26. Li J, Hu XS, Zhou FF, et al. Limb remote ischemic postconditioning protects integrity of the blood-brain barrier after stroke. *Neural Regen Res* 2018;13:1585–1593.
 27. Li S, Hu X, Zhang M, et al. Remote ischemic post-conditioning improves neurological function by AQP4 down-regulation in astrocytes. *Behav Brain Res* 2015;289:1–8.
 28. Qi W, Zhou F, Li S, et al. Remote ischemic postconditioning protects ischemic brain from injury in rats with focal cerebral ischemia/reperfusion associated with suppression of TLR4 and NF- κ B expression. *NeuroReport* 2016;27:469–475.
 29. Ren C, Li N, Wang B, et al. Limb ischemic preconditioning attenuates blood-brain barrier disruption by inhibiting activity of MMP-9 and occludin degradation after focal cerebral ischemia. *Aging Dis* 2015;6:406–417.
 30. Hoda MN, Bhatia K, Hafez SS, et al. Remote ischemic preconditioning is effective after embolic stroke in ovariectomized female mice. *Transl Stroke Res* 2014;5:484–490.
 31. Guo L, Zhou DA, Wu DI, et al. Short-term remote ischemic conditioning may protect monkeys after ischemic stroke. *Ann Clin Transl Neurol* 2019;6:310–323.
 32. England TJ, Hedstrom A, O'Sullivan S, et al. RECAST (remote ischemic conditioning after stroke trial): a pilot randomized placebo controlled phase II trial in acute ischemic stroke. *Stroke* 2017;48:1412–1415.
 33. Li Y, Liang K, Zhang L, et al. Upper limb ischemic postconditioning as adjunct therapy in acute stroke patients: a randomized pilot. *J Stroke Cerebrovasc Dis* 2018;27:3328–3335.
 34. Ji Z, Yu L, Wu W, Fang Q. Remote ischemic postconditioning promotes collateral circulation and

- down-regulates TLR4/NF-kappaB signaling pathway in patients with acute ischemic stroke. *Int J Clin Exp Med* 2020;13(1 PG-246–252):246–252.
35. Feng X, Huang L, Wang Z, et al. Efficacy of remote limb ischemic conditioning on poststroke cognitive impairment. *J Integr Neurosci* 2019;18(4 PG-377–385):377–385.
 36. Che R, Zhao W, Ma Q, et al. rt-PA with remote ischemic postconditioning for acute ischemic stroke. *Ann Clin Transl Neurol* 2019;6:364–372.
 37. Hougaard KD, Hjort N, Zeidler D, et al. Remote ischemic preconditioning as an adjunct therapy to thrombolysis in patients with acute ischemic stroke: a randomized trial. *Stroke* 2014;45:159–167.
 38. Zhao W, Che R, Li S, et al. Remote ischemic conditioning for acute stroke patients treated with thrombectomy. *Ann Clin Transl Neurol* 2018;5:850–856.
 39. England TJ, Hedstrom A, O'Sullivan SE, et al. Remote ischemic conditioning after stroke trial 2: a phase IIb randomized controlled trial in hyperacute stroke. *J Am Heart Assoc* 2019;8:e013572.
 40. Rossello X, Yellon DM. Cardioprotection: the disconnect between bench and bedside. *Circulation* 2016;134:574–575.
 41. Hoffmann U, Sheng H, Ayata C, Warner DS. Anesthesia in experimental stroke research. *Transl Stroke Res* 2016;7:358–367.
 42. Li H, Yin J, Li L, et al. Isoflurane postconditioning reduces ischemia-induced nuclear factor-kappaB activation and interleukin 1beta production to provide neuroprotection in rats and mice. *Neurobiol Dis* 2013;54:216–224.
 43. Taheri S, Shunmugavel A, Clark D, Honglian S. Isoflurane reduces the ischemia reperfusion injury surge: a longitudinal study with MRI. *Brain Res* 2014;1586:173–183.
 44. He Y, Guo Z, Qin C, et al. Remote ischemic conditioning combined with intravenous thrombolysis for acute ischemic stroke. *Ann Clin Transl Neurol* 2020;7:972–979.
 45. McCafferty K, Forbes S, Thiernermann C, Yaqoob MM. The challenge of translating ischemic conditioning from animal models to humans: the role of comorbidities. *Dis Model Mech* 2014;7:1321–1333.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

- Figure S1.** Schematic illustration of search string.
Figure S2. Detailed MEDLINE and Embase search string.
Table S1. Species and trial design in animal studies.
Table S2. Overview of the included 22 animal studies.
Table S3. Overview of the included eight human trials.