PERSPECTIVE

Remote ischemic conditioning: the brain's endogenous defense against stroke

Introduction to ischemic conditioning: In 1986, Murray built upon a series of accumulated works to demonstrated that brief ischemic "training" episodes fortified cardiac tissue against impending prolonged infarction (Murry et al., 1986). This discovery altered the dogmatic understanding of ischemia, highlighting that time-dependent tissue compromise during infarction was bimodal, not linear, in nature. Instead of being invariably deleterious, an organ's response to ischemia is dependent upon both the duration of the infarction as well as adaptions from previous, transient ischemic episodes.

During prolonged ischemia, ATPase-dependent ion transport is impaired, disrupting cellular homeostasis to incite calcium overload and volume dysregulation. Tissue reperfusion in turn has paradoxically pathological effects, such as the generation of reactive oxygen species and sequestration of proinflammatory immunocytes in ischemic tissue. Any combination of these accumulative insults following ischemic/reperfusion injury results in wide-spread mitochondrial permeability, cell lysis and death (Kalogeris et al., 2016). Transient ischemia, in contrast, confers a conditioning stimulus protecting against subsequent infarction.

Conditioning agents: Ischemic conditioning has been successfully tested in animal models, initially via direct vascular occlusion. The high risk of permanent vascular and tissue damage prompted further research into alternative conditioning agents. Numerous other cellular insults aside from ischemia were discovered to produce conditioning responses, indicating a lack of specificity between ischemic tolerance and ischemia. Likely, these related responses are due to integration into cellular degeneration or defense pathways. The "cross-tolerance" of conditioning to various insults provoked the study of ischemic mimetics. For example, successful cerebral conditioning techniques altered metabolic states (e.g., hypoxia, hypoglycemia, hypothermia) and included some existing pharmaceuticals (e.g., fluranes) (Thushara Vijayakumar et al., 2016). Despite improvements over direct conditioning, ischemic memetics still require an underlying neuronal insult, drastically limiting their clinical application.

Remote ischemic conditioning (RIC): RIC is a novel conditioning method involving application of ischemia in one organ to stimulate ischemic tolerance in another. In clinical settings, this is most frequently accomplished by using a blood pressure cuff to intermittently induce transient ischemia in a peripheral limb, such as an arm or leg. RIC avoids direct insult to cerebral tissue and has been studied in critically ill patients, where no adverse effects were observed following its use (Koch et al., 2011). Following RIC, a systemic messenger transverses to the target organ.

RIC: systemic pathways: Several systemic pathways have been described for RIC: 1) blood-borne factor release, 2) neuronal pathway activation, 3) systemic modification of immune cells, and 4) activation of hypoxia inducible genes (Tapuria et al., 2008; Le Page and Prunier, 2015; Anttila et al., 2016) (**Figure 1A**). While the literature centers around cardioprotective pathways, consistencies between organs systems are apparent. For example, following RIC for cerebral conditioning, a reduction in circulating cerebrocortical leukocytes is observed (Anttila et al., 2016). The numerous pathways converge on the target organ to trigger a protective intracellular signaling response that reduces mitochondrial permeability, conserves ATP levels, and prevents apoptosis (Tapuria et al., 2008).

Cerebral conditioning response: Alterations in the target organ's physiology contribute to the ischemic tolerance. In the cerebrum, some studied mechanisms specifically counteract the tissue's inherit susceptibility to ischemic damage. Adaptations within the cerebrum's neurovascular network, synaptic signaling, and subcellular organelles account for the brain's ischemic tolerance following conditioning (Wang et al., 2015). Damages to the neurovascular network place the brain at high risk for inflammatory damage. This risk is due in part to lower levels of protective antioxidant enzymes, lower levels of cytochrome c (and thus increased superoxide spillover from the mitochondrial transport chain), and higher levels of polyunsaturated fatty acids in its cellular membranes (Kalogeris et al., 2016). Conditioning increases reactive oxygen species scavenging astrocytes that also support the blood brain barrier, stimulates pre-ischemic microvessel formation and post-ischemic vessel dilation, and reduces leukocyte adhesion via intercellular adhesion molecule 1 downregulation (Wang et al., 2015). These changes diminish and disrupt the inflammatory cycle, preserving endothelial function and promoting sustained blood flow to cerebral tissue (Tapuria et al., 2008; Thushara et al., 2016; **Figure 1B**).

Synaptic regulated cytotoxicity also plays a key role in ischemic tolerance. The brain is at risk from damage due to excessive release of glutamate, which normally triggers calcium overload and cellular cytotoxicity (Kalogeris et al., 2016). However, in neurons conditioned with mild N-methyl-D-aspartate receptor activation, glutamate excitotoxicity was diminished by inhibition of stress kinase release and rapid calcium adaptations (Thushara Vijayakumar et al., 2016). Intracellularly, changes in gene expression incite ATP conservation via alterations in the mitochondrial electron transport chain and regulation of calcium via the endoplasmic reticulum and Golgi complex (Wang et al., 2015).

Clinical models: introduction: Integrating RIC into clinical practice remains challenging due to the erratic nature of cerebral infarction. Following a conditioning stimulus, there are two windows of ischemic tolerance. Acute tolerance is developed within minutes and confers short-term benefits lasting a few hours. This effect is thought to be related to post-translational modifications. Delayed tolerance emerges following genetic alterations and de novo protein synthesis. Its effects lasts several days to 1 week (Thushara Vijayakumar et al., 2016). The conditioning response may be delivered prior, during or before the event via ischemic pre/per/postconditioning, respectively. The limited window of neuroprotection provided and mantra of "time is brain" highlight the essentialness of selecting an appropriate conditioning model. Clinical models to be discussed are summarized in **Additional Table 1**.

Clinical models: Remote ischemic preconditioning (RIPC): To study RIPC, Meng et al. (2012) identified a population at high risk for recurrent stroke: patients with intracranial stenosis. Following their first episode of infarction, Meng et al. (2012) applied twice-daily bilateral-limb ischemia for 300 days. They found increased cerebral perfusion, decreased incidence of recurrent stroke, and faster recovery time after primary and recurrent stroke. The success of preconditioning in Meng's study stems from using

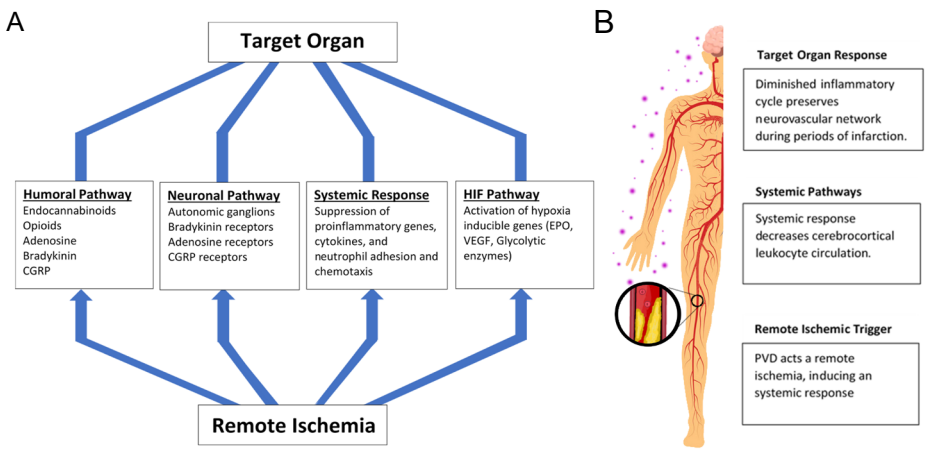

Figure 1 Pathways for remote ischemic conditioning.
(A) Remote ischemia (e.g., blood pressure cuff occlusion of femoral artery) induces a systemic pathway, possibly through humoral, neuronal, systemic, or HIF pathways, that triggers a ischemic conditioning response at the target organ (e.g., brain). (B) PVD causes prolonged hypoperfusion that acts as remote ischemic mimetic, triggering a systemic response and cerebral conditioning. CGRP: Calcitonin gene-related peptide; EPO: erythropoietin; HIF: hypoxia inducible factor; PVD: peripheral vascular disease; VEGF: vscular endothelial growth factor.

a population with a known timetable for recurrent stroke, subverting the disease's unpredictability. Although recurrent conditioning stimuli (2 × 300 days in Meng's study) has been shown to be a graded phenomenon, wherein multiple cycles produce a more robust response, this improvement is isolated to the magnitude of effect and has not proved to be related to its duration (Schulz et al., 1998). Therefore, each of the conditioning stimuli would have provided neuroprotection for 1 week maximum via the delayed conditioning response rooted in de novo protein translation. In general populations without such a defined stroke window, the rigorousness of Meng's conditioning regime makes this methodology unlikely to be suitable for prophylaxis.

Clinical models: remote ischemic perconditioning (RIPerC): Hougaard et al. (2014) utilized RIPerC as an adjunct to thrombolysis. The large, randomized trial applied remote ischemic conditioning during the ambulance ride to the stroke unit. The results showed no conclusive benefit on diffusion weighted imaging infarct salvage/size/progression or functional recovery at 1 and 3 months, respectively. However, when adjusted for baseline severity of hypoperfusion, a voxel-by-voxel analysis at 1-month demonstrated increased tissue survival with perconditioning. Hougaard et al. (2014) supports the acute phase of ischemic conditioning but the lack of continued stimuli limited the expanse of neuroprotective coverage into the patients recovery. Further, unlike Meng et al. (2012) conditioning was not repeated to amplify the magnitude of its effect. However, Hougaard's methodology is highly feasible and applicable to all stroke patients brought to hospitals via emergency services.

Clinical models: remote ischemic postconditioning (RIPostC): While extensively studied in patients with acute myocardial infarction and with clinical success, RIPostC has only been limitedly studied within the realm of neurological disorders (Le Page and Prunier, 2015). Phase 1 trials have demonstrated the safety of RIPostC in populations of patients with subarachnoid hemorrhage (Koch et al., 2011; Gonzalez et al., 2014). Meng et al. (2012) could be viewed as both an analysis of RIPC and RIPostC, as the results showed improved primary stroke recovery regarding modified ranking scale outcomes. As with RIPerC, RIPostC maintains an advantage over RIPC as patients are in a controlled setting under a known window of time. However, further study is required to make definitive claims on RIPostC's role in neuroprotection.

Clinical models: prolonged hypoperfusion: All trials, to date, have utilized transient, intermittent episodes of sub-lethal peripheral occlusion to induce RIC. However, Connolly et al. (2013) described a novel variant of RIC achieved by prolonged hypoperfusion as opposed to transient occlusion. In their retrospective study, where peripheral arterial disease was used as a mechanism for hypoperfusion, they illustrated improved clinical outcomes (lower admission National Institute of Health Stroke Scale and 3-month modified ranking scale), smaller infarct volumes, and lower mortality rates in peripheral arterial disease afflicted patients. A further retrospective analysis demonstrated that increasing degrees of hypoperfusion (i.e., mild, moderate, severe peripheral arterial disease) were positively correlated with improved outcomes, however all degrees of hypoperfusion were associated with neuroprotective effects (Heiberger et al., 2019).

Prolonged hypoperfusion, as a remote ischemic mimetic, would theoretically provide neuroprotective coverage throughout the entire duration of the stimulus. Given that even mild peripheral arterial disease elicited a conditioning response, only a minimal restriction in peripheral blood flow appears necessary to sustain a state of cerebral ischemic tolerance. Incorporating this knowledge into conditioning regimes may improve the patient experience and tolerability, making a clinical model of RIC more feasible. However, the evidence used to speculate these claims is limited to retrospective studies with small sample sizes in a disease population fraught with comorbidities that induce extraneous variables and therefore should be taken only as preliminary results for future study. Further delineating the safety of induced prolonged hypoperfusion as a mechanism for RIC is necessary before its incorporation into clinical models may be seriously considered.

Clinical models: Implementation: Detailed discussion on the technique of RIC (i.e., chosen limb, length of occlusion, number of cycles and time between cycles) is beyond the scope of this article but remains an important consideration. It seems reasonable to implement a clinical regime with aspects containing all forms of conditioning: RIPC, RIPerC and RIPostC, given they promote coverage during different stroke windows. RIPC appears the most challenging to effectively implement, as its target population is rarely in a controlled enough setting to regulate the necessary protocols. However, as it is positioned to not only improve stroke outcomes, but limit its occurrence altogether, applicable methods for RIPC in populations at risk for stroke should be explored.

Concluding thoughts: In the nearly three decades since the term ischemic preconditioning was coined, great strides have been made to translate its impressive potential to a clinical platform. Unfortunately, the envisaged paradigm shift predicted to accompany it has not yet come to fruition. Further, despite being the most susceptible organ to ischemic damage in the human body, evidence supporting ischemic conditioning of the brain lags behind other specialties. Preliminary results remain promising and developing trials will serve to elucidate a model of cerebral conditioning. The reasons for pursuing clinical models of cerebral conditioning are clear: over fifteen million people suffer stroke world-wide per year, inciting massive amounts of personal and economic strife. For these patients, ischemic conditioning holds potential to minimize stroke's damage, improve its recovery, and even prevent its occurrence altogether.

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Additional file:
***Additional Table 1:** Summary of clinical studies.*

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Additional Table 1 Summary of clinical studies

Study	Design	Conditioning	Outcomes
Meng et al., 2012	RCT	RIPC/RIPostC	Following AIS, the treatment group of intracranial stenosis patients had quicker recovery time (RIPostC related) and decreased recurrent stroke (RIPC related)
		(300 days, 2x Daily RIC after first stroke in intracranial stenosis patients followed through recurrent stroke)	
Hougaard et al., 2014	RCT	RIPerC	Although the overall results were neutral, a tissue survival analysis suggests that RIPerC may have immediate neuroprotective effects
		(4 cycles RIC in route to the hospital, suspected AIS patients)	
Koch et al., 2011	Prospective	RIPostC	
Gonzalez et al., 2014		(14 days [Koch] or 4 sessions [Gonzalez] of RIC in hospitalized aSAH patients)	No adverse effects in critically ill-populations and evidence of improved cerebral perfusion with RIPostC
Connolly et al. 2013	Retrospective	Prolonged hypoperfusion	PVD was correlated with lower NIHSS and stroke volume upon admission, improved mRS recovery, and lower mortality
Heiberger et al., 2019		(modeled by PVD in patients prior to AIS)	

AIS: Acute ischemic stroke; aSAH: aneurysmal subarachnoid hemorrhage; mRS: modified ranking scale; NIHSS: National Institute of Health Stroke Scale; PVD: peripheral vascular disease; RCT: randomized controlled trial; RIC: remote ischemic conditioning; RIPC: remote ischemic preconditioning; RIPerC: remote ischemic perconditioning; RIPostC: remote ischemic postconditioning.