JACC: BASIC TO TRANSLATIONAL SCIENCE
VOL. 1, NO. 1-2, 2016
ISSN 2452-302X
http://dx.doi.org/10.1016/j.jacbts.2016.01.007

CLINICAL RESEARCH

Kinetics and Signal Activation Properties of Circulating Factor(s) From Healthy Volunteers Undergoing Remote Ischemic Pre-Conditioning

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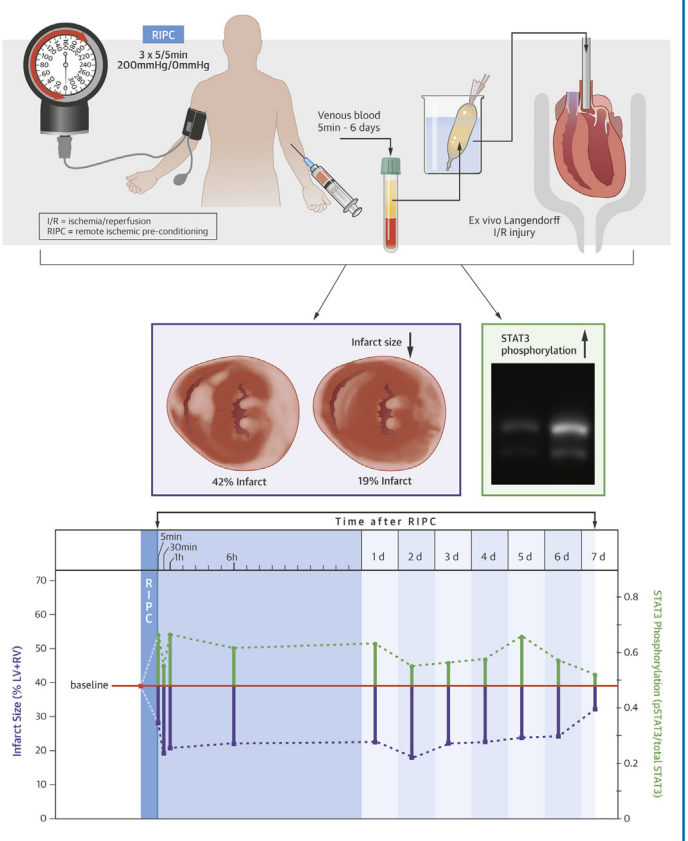
VISUAL ABSTRACT



Hildebrandt, H.A. et al. J Am Coll Cardiol Basic Trans Sci. 2016; 1(1-2):3-13.

HIGHLIGHTS

- Pre-clinical and early phase clinical studies with remote ischemic preconditioning (RIPC) appeared promising; however, RIPC was not effective in phase III clinical trials.
- To improve the translation of RIPC into clinical practice, the kinetic properties and functional effects of humoral factors released after RIPC in humans were characterized ex vivo.
- Venous blood from 20 healthy volunteers was collected at baseline and 5 min, 30 min, 1 h, 6 h and daily from 1 to 7 days after RIPC. Plasma dialysates were infused into Langendorff-perfused mouse hearts subjected to 20/120 min global ischemia/reperfusion.
- Perfusion with dialysates obtained 5 min to 6 days after RIPC significantly reduced infarct size by ~50% when compared to perfusion with dialysates obtained at baseline prior to RIPC, and increased STAT3 phosphorylation beyond values obtained with baseline-dialysate.

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Manuscript received December 3, 2015; revised manuscript received January 13, 2016, accepted January 14, 2016.

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 1, NO. 1-2, 2016
JANUARY/FEBRUARY 2016:3–13

SUMMARY

Although remote ischemic pre-conditioning (RIPC) reduced infarct size in animal experiments and proof-of-concept clinical trials, recent phase III trials failed to confirm cardioprotection during cardiac surgery. Here, we characterized the kinetic properties of humoral factors that are released after RIPC, as well as the signal transduction pathways that were responsible for cardioprotection in an ex vivo model of global ischemia reperfusion injury. Venous blood from 20 healthy volunteers was collected at baseline and 5 min, 30 min, 1 h, 6 h, and daily from 1 to 7 days after RIPC (3 × 5/5 min upper-limb ischemia/reperfusion). Plasma-dialysates (cut-off: 12 to 14 kDa; dilution: 1:20) were infused into Langendorff-perfused mouse hearts subjected to 20/120 min global ischemia/reperfusion. Infarct size and phosphorylation of signal transducer and activator of transcription (STAT)3, STAT5, extracellular-regulated kinase 1/2 and protein kinase B were determined. In a subgroup of plasma-dialysates, an inhibitor of STAT3 (Stattic) was used in mouse hearts. Perfusion with baseline-dialysate resulted in an infarct size of 39% of ventricular mass (interquartile range: 36% to 42%). Perfusion with dialysates obtained 5 min to 6 days after RIPC significantly reduced infarct size by ∼50% and increased STAT3 phosphorylation beyond that with baseline-dialysate. Inhibition of STAT3 abrogated these effects. These results suggest that RIPC induces the release of cardioprotective, dialyzable factor(s) within 5 min, and that circulate for up to 6 days. STAT3 is activated in murine myocardium by RIPC-induced human humoral factors and is causally involved in cardioprotection. (J Am Coll Cardiol Basic Trans Sci 2016;1:3–13)

Remote ischemic conditioning (RIC) with transient limb ischemia/reperfusion is a noninvasive method to protect the myocardium and other parenchymal organs from ischemia/reperfusion injury. Cardioprotection is achieved by RIC before (pre-conditioning; RIPC), during (per-conditioning) or after myocardial ischemia (post-conditioning) (1). RIC has been demonstrated in many experimental studies and also attenuates myocardial ischemia/reperfusion injury in patients undergoing elective interventional (2) or surgical coronary revascularization (3–5) as well as in patients with acute myocardial infarction (6–10). The efficacy of RIC was evidenced by reduced cardiac biomarker release (2–5,9) and reduced infarct size on magnetic resonance imaging (6,8,10); some smaller studies also reported improved short- (5,8) and long-term clinical outcome (2,4,7). However, the recent large-scaled, randomized ERICCA (Effect of Remote Ischaemic Preconditioning on Clinical Outcomes in CABG Surgery) trial and RIPHeart (Remote Ischaemic Preconditioning for Heart Surgery) study in patients undergoing cardiac surgery and ischemic cardioplegic arrest failed to confirm reduced biomarker release and improved clinical outcome with RIPC (11,12). Reasons for the failure of these trials to confirm protection by RIPC have been discussed in detail and related to confounding variables (13–15), notably the use of propofol in the majority of all patients in both trials, which might have abrogated the cardioprotective effect (16).

For more successful translation of experimental animal studies and smaller proof-of-concept trials to clinical reality a better understanding of RIC's signal transduction is mandatory. In particular, the transfer signal from the remote peripheral organ where the RIC maneuver is performed to the heart is still enigmatic. Both neuronal and humoral transfer as well as a neurohumoral interaction have been proposed. Humoral transfer by nitrite (17), stromal cell-derived factor-1α (18), and microRNA-144 (19) has been reported. More systematic proteome analyses of plasma taken after RIPC have not yet identified a specific protein (20,21).

We therefore used another strategy to identify the potential humoral transfer factor(s) by characterizing their properties in kinetic terms and by the signal activation which they elicit in the heart to effect cardioprotection. Prior studies have reported a time delay between the RIPC stimulus and the injurious event from 5 to 10 min (22,23) to more than 24 h (24–27), suggesting that RIPC is a fast acting as well as a long-lasting phenomenon. Supporting the notion of a long-lasting effect, flow-mediated forearm vasodilation in patients with reperfused acute myocardial infarction was improved by RIPC for 1 week (28). However, this particular study could not distinguish whether there was a long-lasting circulation of transfer factor(s) or a long-lasting effect in the target organ.

ABBREVIATIONS AND ACRONYMS

AKT = protein kinase B

ERK = extracellular-regulated kinase

IQR = interquartile range

LV+RV = left and right ventricular

LVDP = left ventricular developed pressure

RIC = remote ischemic conditioning

RIPC = remote ischemic pre-conditioning

SAFE = survival activating factor enhancement

STAT = signal transducer and activator of transcription

TTC = 2,3,5-triphenyltetrazolium chloride