Clinical Benefits of Remote Ischemic Preconditioning
New Insights…and New Questions

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In the 2 decades since the first report of the infarct-sparing effect of remote ischemic preconditioning (RIPC), the concept has evolved from a provocative laboratory observation to the focus of multiple phase II clinical trials. Investigation of RIPC in patient populations has largely centered on the assessment of surrogate biomarkers of cardiac injury (ie, cardiac troponins and creatine kinase) after cardiac surgery, with emerging evidence of improvement in long-term clinical outcome. Moreover, with rare exceptions, previous clinical trials have not been designed to yield insight into the cellular mechanisms underlying the protection afforded by RIPC. Accordingly, the recent publication by Slagsvold et al provides multiple, novel contributions to our understanding of RIPC-induced cardioprotection: focusing on atrial myocardium, the authors report preservation of mitochondrial respiration and modified expression of microRNAs, together with an attenuation in the incidence of postoperative atrial fibrillation in patients randomized to receive RIPC before coronary artery bypass graft surgery.

Atrial Fibrillation: an Unappreciated Clinical End Point for RIPC?

The hallmark of myocardial ischemia–reperfusion injury associated with coronary artery bypass graft surgery is the well-described increase in plasma concentration of cardiac enzymes during the initial 48 to 72 hours after release of the aortic clamp. Plasma levels of troponins or creatine kinase are not, however, the sole markers of cardiac damage. A substantial proportion of patients reportedly develop atrial fibrillation during the first 3 postoperative days, a complication associated with long-term adverse outcomes including increased mortality.

Indeed, in 2010, a phase II multicenter trial was initiated to test the hypothesis that RIPC would reduce the incidence of atrial fibrillation after coronary artery bypass graft surgery. Slagsvold et al, in their single-center study, found that 14% versus 50% of patients in the RIPC versus control groups developed atrial fibrillation during the first 3 days after coronary artery bypass surgery. Although, as emphasized by the authors, confirmation of these data in larger patient populations is required, Slagsvold et al provide the first evidence in support of the concept of RIPC-induced protection against atrial fibrillation.

It is interesting to note that the reduction in the incidence of postoperative atrial fibrillation was achieved despite the use of propofol, an anesthetic that has been demonstrated to render RIPC ineffective in reducing infarct size as defined by cardiac enzyme release. Indeed, Slagsvold et al observed no difference in plasma concentrations of cardiac troponin T or creatine kinase-MB in the RIPC cohort versus controls. The authors propose 2 alternative explanations for the comparable enzyme release in the 2 groups: the shorter cross-clamp times in the current protocol (∼41 versus ∼70 minutes) and the abbreviated postoperative time frame during which blood samples were analyzed (24 versus 72 hours). However, the apparent discrepancy in clinical outcomes may also reflect as-yet unidentified differential effects of propofol on atrial rhythmogenesis versus enzyme release or a dissociation between the effects of RIPC on the 2 end points, 2 potentially important concepts that merit investigation.

RIPC and Mitochondrial Respiration

The overarching question in the field of RIPC is: what are the mechanisms by which brief episodes of ischemia applied at a remote site confer cardioprotection? There is a consensus that the infarct-sparing effect of all forms of ischemic conditioning involves the upregulation of ≥1 signal transduction cascades in ischemic-reperfused cardiomyocytes that, ultimately, serve to stabilize the mitochondria. Overwhelming emphasis has focused on the mitochondrial permeability transition pore, and delay or prevention of pore opening, as the end effector in achieving conditioning-induced cardioprotection. In contrast, Slagsvold et al are among the few who have investigated mitochondrial respiration and components of the electron transport chain.

Mitochondrial respiration was assessed in right atrial biopsy samples obtained at 2 time points: before and after aortic cross-clamping. The control group displayed a significant, global reduction in oxidative phosphorylation after versus before cross-clamping, with no evidence of selective defects in any specific components of the electron transport chain. In contrast, this deficit was not observed in the RIPC-treated group.

Because mitochondrial turnover is slow and the duration of the study is short, Slagsvold et al appropriately conclude that “the alterations in mitochondrial respiration rates are likely to be due to functional status of the mitochondria.” The overall concept of preservation of mitochondrial function with RIPC...
is consistent with data obtained in the isolated buffer-perfused rabbit heart model, where RIPC was achieved by transfer of coronary effluent from preconditioned hearts to naïve recipient hearts. However, in contrast to the results obtained by Slagsvold et al., the rabbit model was characterized by (1) selective deficits in mitochondrial complex I/state 3 and complex IV/state 3 respiration in controls, and (2) attenuation of these defects in mitochondrial respiration with RIPC.

This potentially important incongruity may be a consequence of 2 fundamental differences between the studies. The first issue is the origin of the tissue: Slagsvold et al. assessed mitochondrial respiration in right atrium harvested from surgical patients with underlying coronary disease, experiencing ≥1 comorbidities and receiving multiple standard drug therapies, as opposed to ventricular myocardium harvested from healthy young adult animals. Second, analysis of the right atrial samples was performed after macerating and permeabilizing the tissue, a method that differs from the more traditional approach in which populations of intact mitochondria are isolated. The in situ permeabilization technique has, however, become more commonplace, has the advantage of requiring smaller volumes of tissue (a benefit in clinical protocols), and reportedly yields results equivalent to those obtained with traditional mitochondrial isolation.

In interpreting the data obtained by Slagsvold et al., additional technical issues warrant consideration. The contribution of signal transduction in conditioning-induced cardioprotection may extend beyond the well-described survival kinase pathways; cell signaling also plays an important role in regulating the oxidative phosphorylation machinery within the mitochondria. For complex IV (cytochrome c oxidase: the proposed rate-limiting enzyme of the electron transport chain in vivo), ischemia leads to changes in its phosphorylation signature, and reintroduction of oxygen precipitates changes in cytochrome c oxidase subunit composition with an overall reduction of protein levels. Recent evidence has revealed that conventional preconditioning-induced cardioprotection is accompanied by changes of cytochrome c oxidase phosphorylation and partial inhibition of its activity. Such a partial inhibition of cytochrome c oxidase during reperfusion should be cytoreductive because it would prevent the hyperpolarization of the mitochondrial membrane potential and thus the production of reactive oxygen species. However, to obtain a comprehensive assessment of the effect of any intervention on oxidative phosphorylation, the phosphorylation state of the complexes must be maintained (ie, the use of nonspecific phosphatase inhibitor cocktails during mitochondria isolation is required). Most of the published data, including the results reported by Slagsvold et al., were obtained in the absence of phosphatase inhibitor cocktails, raising the possibility that, had the phosphorylation state of the complexes been preserved, additional or more pronounced effects of RIPC on mitochondrial function may have been unmasked.

A second potentially confounding issue is the authors’ use of N,N,N′,N′-tetramethyl-p-phenylenediamine and ascorbate in the measurement of cytochrome c oxidase activity. N,N,N′,N′-tetramethyl-p-phenylenediamine facilitates electron transfer; as a result, cytochrome c does not have to dissociate from complex IV to be re-reduced, thus rendering the kinetics of electron transfer artificial. If, for example, RIPC leads to changes in the phosphorylation state of cytochrome c or the epitope on cytochrome c oxidase to which it binds, possible effects on respiration might be missed.

**RIPC and microRNA Expression**

The third novel component of the study by Slagsvold et al. is the screening for expression levels of multiple microRNAs. Two group differences were observed: there was a temporal upregulation in expression of microRNA-1 after release of the aortic clamp in control subjects (but not in the RIPC cohort), whereas expression of microRNA-338-3p was increased after cross-clamping in the RIPC group versus controls.

The increase in expression of microRNA-1 after relief of global ischemia is consistent with previous evidence obtained in other model systems of an association between microRNA-1 and mitochondrial dysfunction, cytochrome c release, and apoptosis. Moreover, in a mouse model of ischemia–reperfusion injury, microRNA-1 was implicated to contribute to postischemic myocardial damage. This concept has not, however, been corroborated in all studies. In addition, attempts to identify a relationship between microRNA-1 and ischemic conditioning have yielded contradictory results (ie, upregulation with preconditioning versus downregulation with postconditioning and RIPC). Interpretation of the increase in microRNA-338-3p in response to RIPC is even more challenging: microRNA-338-3p has been implicated to play a role in tumorigenicity and cell growth, with no recognized function in heart.

**The Big Picture: Observations, Associations, or Cause and Effect?**

The novel findings reported by Slagsvold et al. raise multiple, intriguing questions and provide substantial groundwork for future investigation into: (1) the clinical translation and molecular mechanisms of RIPC, and (2) the pathophysiology of ischemia–reperfusion injury per se. There are, however, 2 big picture issues that beg resolution. The first is to establish the nature of the relationships among the study outcomes: do the effects of RIPC on postoperative atrial fibrillation, mitochondrial respiration, and microRNA expression represent discrete and unrelated observations, an association among the end points, or mechanistic insight into the better maintenance of electric stability and suppression of postoperative atrial fibrillation? The second issue will be to reconcile the apparent dissociation between cardiac enzyme release and atrial arrhythmogenesis and identify the best clinical outcomes to establish efficacy. Slagsvold et al. conclude that “RIPC induces myocardial protection of the human atrium even when differences in release of cardiac markers cannot be detected”; if confirmed, this would represent a paradigm shift that could broaden the scope for the successful clinical translation of RIPC.

**Disclosures**

None.
References


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