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INVITED EDITORIAL

'Going out on a limb': SDF-1α/CXCR4 signaling as a mechanism of remote ischemic preconditioning?

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Communication: the cornerstone of RIPC

Remote ischemic preconditioning (RIPC) is the intriguing phenomenon whereby brief, non-lethal episodes of ischemia in one organ or vascular bed render remote tissue resistant to a subsequent, sustained period of ischemia [23, 32]. While initially regarded as a laboratory curiosity [24], interest in RIPC was piqued by the observation that limb ischemia, achieved noninvasively by simple inflation of a blood pressure cuff, significantly reduced myocardial infarct size in the acute swine model of coronary artery occlusion-reperfusion [17]. In the ensuing years, since these first reports, progress has been made in defining the characteristics of RIPC-induced cardioprotection (including as-yet limited insights into cellular mechanisms), expansion of the concept beyond heart to encompass protection of other organs (including brain, kidney, liver and mesentery), and the investigation of RIPC in Phase II and III trials seeking to establish clinical efficacy in patients undergoing cardiac surgery or percutaneous intervention [2, 3, 6, 10, 14, 19, 20, 22, 24, 25, 31]. However, resolution of the distinguishing feature of RIPC has remained elusive [22, 24, 25]: *how is the protective stimulus transferred or communicated from the site of the RIPC stimulus to the heart?*

Among the theories that have been proposed, considerable attention has focused on the concept that: (i) brief ischemia–reperfusion at the remote site triggers the release of one or more protective humoral factors, either directly or as a secondary consequence of neuronal stimulation; and (ii) the humoral factor(s) are then conveyed via the circulation to the myocardium [8, 22–25, 32]. Despite the emerging consensus that the factor(s) of interest are small (~3.5–15 kDa), presumably peptide(s), and hydrophobic [7, 28, 29], precise identification of the endogenous peptides transferred to the heart and capable of conferring cardioprotection has been problematic.

SDF-1α: the sought-after humoral protective factor?

In a recent issue of *Basic Research in Cardiology*, Davidson and colleagues posit that the circulating humoral peptide underlying the infarct-sparing effect of RIPC may be stromal cell derived factor (SDF)-1α, and that SDF-1α may activate cardioprotective signaling pathways by binding to its receptor, CXC chemokine receptor 4 (CXCR4) in heart [5]. To develop and test this novel hypothesis, a rat model was used in which the RIPC stimulus (three 5-min episodes of ischemia) was administered in vivo by tightening a tourniquet on one hindlimb; the heart was then excised and buffer-perfused, and, after 40 min of stabilization, a sustained, 35-min period of coronary artery occlusion was applied in vitro. Support for the authors' hypothesis was provided by three key pieces of evidence: (i) a significant 50 % increase in the plasma concentration of SDF-1α, assessed from samples collected immediately after RIPC, when compared with

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time-matched sham-controls; (ii) documentation of CXCR4 protein expression in rat heart homogenates and isolated adult rat cardiomyocytes, and, of particular importance (iii) inhibition of RIPC-induced reduction of infarct size in rats that received AMD3100, the canonical CXCR4 antagonist, 10 min before imposing the first brief episode of hindlimb ischemia [5].

Strengths and limitations of the hypothesis

SDF-1α contributes to the trafficking, homing and tissue retention of progenitor cells, and has garnered interest as a potential therapeutic strategy to enhance the efficacy of stem cell-based cardiac regenerative therapies [1, 15, 18, 27, 33]. The concept that this chemokine may have an as-yet unappreciated role as the circulating, protective peptide that triggers infarct size reduction by RIPC is, for many reasons, logical and appealing. For example, SDF1-α is a small (10 kDa) molecule that displays an increase in expression under conditions of hypoxia and ischemia [4, 15]. In addition, there is evidence that SDF-1α has a direct, cardioprotective effect when administered before coronary artery occlusion-reperfusion or permanent coronary artery ligation [15, 26], reportedly mediated via SDF-1α/CXCR4 binding and up-regulation of classic 'survival' kinases (including components of the Reperfusion Injury Salvage Kinase [RISK] and Survivor Activating Factor Enhancement [SAFE] pathways) involved in myocardial pre-, post- and remote conditioning [11, 12, 15, 22, 24–26].

However, to definitively establish that release of SDF-1α from the ischemic limb and subsequent SDF-1α/CXCR4 binding in heart plays a mechanistic role in RIPC, compelling evidence of cause-and-effect is required. In this regard, the pivotal experiment, administration of AMD3100 in an effort to block infarct size reduction with RIPC, yielded intermediate results: infarct size averaged 53 ± 3, 27 ± 3 and 40 ± 4 % of the at-risk myocardium in control, RIPC and AMD3100-pretreated RIPC groups, respectively [5]. Indeed, if the sample sizes (and thus the statistical power) were increased from the current value of $n = 6$ to $n = 8$ per group with no change in variance, the intermediate infarct size in the AMD3100-treated RIPC cohort would differ significantly from controls at the $p < 0.05$ level.

This partial inhibition achieved with AMD3100 may, as discussed by Davidson and colleagues, reflect the involvement of multiple circulating factors in RIPC-induced cardioprotection [5]. Alternatively, as only one dose of AMD3100 was evaluated, the outcome may also be explained by a suboptimal dose of the antagonist. There is an additional and potentially confounding issue that also warrants consideration: neither SDF-1α nor AMD3100 bind exclusively to CXCR4. SDF-1α is a ligand for both CXCR4 and CXCR7 [30], while AMD3100 also binds to—and is an *agonist* (rather than antagonist) for—CXCR7 [16]. There is evidence that CXCR7 is expressed in heart [9, 30], but its potential contribution to cardioprotection is unexplored.

Future directions

In addition to resolution of the aforementioned uncertainties and limitations regarding selectivity that plague all studies using pharmacologic antagonists, definitive conclusions regarding the involvement of the SDF-1α/CXCR4 axis in RIPC will require confirmation in multiple models and species, including more 'standard' in vivo protocols with no sustained delay (as in the Davidson study [5]) between the RIPC stimulus and the onset of myocardial ischemia. Of particular importance, clinical evidence of increased plasma concentrations of SDF-1α following brief limb ischemia will be required. Interestingly, in a recent, comprehensive proteomic analysis of human plasma samples, SDF-1α was not among the candidates identified as being up-regulated after an RIPC stimulus [13]. Finally, future studies—and future attempts to exploit SDF-1α as either an 'RIPC-mimetic' or an assay to guide in the optimization of RIPC—must take into consideration the apparent complexities of SDF-1α/CXCR4 signaling, including reports that stimulation of chemokine receptors may up-regulate both pro-survival and pro-apoptotic signaling [18, 21]. Identification of SDF-1α as a protective humoral factor has the potential to represent a breakthrough in our understanding of RIPC, but much work remains before we can conclude with certainty that the SDF-1α/CXCR4 axis plays a mechanistic role in the cardioprotection conferred by remote ischemia.

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