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A novel antibody specific to full-length stromal derived factor-1 alpha reveals that remote conditioning induces its cleavage by endothelial dipeptidyl peptidase 4

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Background/Introduction: Stromal derived factor-1α (SDF-1α/CXCL12) is a chemokine that has been implicated in both acute and chronic cardioprotection. Remote ischaemic conditioning (RIC), a technique of cyclical, non-injurious ischaemia applied to an organ or tissue remote from the heart, has been shown to increase circulating SDF-1α. Subsequent activation of its receptor, CXCR4, has been proposed as the mechanism by which RIC facilitates acute cardioprotection. However, the mechanism of SDF-1α regulation is poorly understood.

Purpose: We hypothesized that dipeptidyl peptidase 4 (DPP4/CD26), a protease that cleaves and inactivates SDF-1α, may be centrally involved in the mechanism of acute cardioprotection conferred by RIC.

Methods: To elucidate the role of SDF-1α cleavage after RIC we established an ELISA to full-length SDF-1α using a novel recombinant human antibody we developed called HCLSDF1α. RIC was achieved by 3 cycles of 5 min cuff inflation and 5 min deflation on the hind limb of Sprague-Dawley rats or on the arms of healthy human volunteers, and samples were taken for SDF-1α measurement either immediately or 1 h after the protocol. The role of membrane-bound DPP4 was investigated using cultured human umbilical vein endothelial cells (HUVECs) subjected to oxidative stress with 5 min x 1 mM H2O2 to mimic RIC.

In a separate experiment, rats were randomly allocated to receive a sham procedure, RIC or RIC + AMD3100, a specific inhibitor of CXCR4, prior to 30 min reversible occlusion of the left anterior descending artery and 120 min reperfusion.

Results: In vivo infarction experiments confirmed that AMD3100 eliminated protection by RIC (56.2±4.2% vs. 31.3±3.3%, P<0.01). We demonstrate for the first time that HCLSDF1α can be used to specifically quantify full-length SDF-1α in blood. Unexpectedly, despite an increase in total SDF-1α as previously reported, levels of intact SDF-1α declined after RIC in both rats (670 ± 130 pg/ml vs. 1150 ± 160 pg/ml, P<0.05) and humans (329.8 ± 148.7 pg/ml vs. 380.0 ± 154.9 pg/ml, P<0.05). Furthermore, oxidative stress significantly increased DPP4 activity (150 ± 20 vs. 90 ± 20, P<0.05).

Conclusion(s): We report for the first time the application of a novel antibody for full-length, active SDF-1α, which demonstrated a significant decrease in intact SDF-1α in response to RIC. This may be mediated by the activation of membrane-bound DPP4 in response to oxidative stress. These results suggest that RIC is not mediated by an increase in full length SDF-1α, as previously thought. We speculate that altered CXCR4 trafficking or an alternative ligand for CXCR4 may mediate RIC. Further studies will investigate the relation between SDF-1α, acute cardioprotection and the dynamics of bone marrow progenitor cell mobilization.