

THE EFFECT OF REMOTE ISCHAEMIC PRECONDITIONING (RIPC) ON CARDIAC PHOSPHOPROTEINS PROFILING USING TANDEM MASS TAGS AND LC-MS/MS

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doi:10.1136/heartjnl-2013-304019.257

Introduction Remote ischaemic preconditioning (RIPC) is a powerful protective phenomenon in which brief ischaemia of one organ or tissue confers protection of another organ or tissue against a sustained ischaemia-reperfusion insult. The nature of the signal released from the remote organ and the timing of the triggered changes in the target organ (the myocardium) remains controversial. However, several studies have implicated changes in survival signalling seen after ischemia and reperfusion. In this study we characterised a mouse model of RIPC and investigated changes in the expression of phosphoproteins in the myocardium prior to sustained ischaemia.

Methods RIPC was induced in anaesthetised male C57/Bl6 mice using four cycles of 5 min of ischaemia and 5 min of reperfusion of the hindlimb using blood pressure cuff inflated at 200mmHg. The effect of this applied pressure on blood flow was measured using Laser doppler Flowmetry. Ischaemia/reperfusion (I/R) injury was determined by measuring infarct size at the end of reperfusion using a Langendorff-perfused mouse heart model. Reperfusion injury was also determined by creatine kinase release and changes in diastolic tension. To monitor changes in phosphoproteins, hearts were excised either at the end of RIPC or from sham mice. Proteins were extracted for phospho-proteomic analysis using the TMT isobaric mass tagging for LTQ Orbitrap velos mass spectrometer.

Results RIPC significantly reduces infarct size, CK release and diastolic tension following I/R. Phospho-proteomic analysis showed that RIPC increases the expression of several phosph-proteins localized in the Z-disk region of the sarcomere. These include Myozenin 2 (Calsarcin1), a cardiac Calcineurin-adaptor protein localized at Z disks.

Conclusion Our mouse model of RIPC is associated with significant cardioprotection against I/R. RIPC triggers changes in several cardiac phospho-proteins prior to sustained ischemia some of which may be involved in calcineurin signalling.