Remote preconditioning by transfer of coronary effluent from ischemic preconditioned rat heart: identification of cardioprotective humoral factors by mass spectrometry technology

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Introduction: Ischemic preconditioning (IPC) is a well known strategy to induce cardioprotection against ischemia/reperfusion injuries. The IPC can be induced by regional ischemia in the heart or " at distance" in non-cardiac tissues, suggesting the release of an unknown humoral activator. **Aims:** To characterize identify and the cardioprotective humoral factors released during IPC. **Methods:** Isolated rat hearts were submitted to IPC (3 cycles of 5 min ischemia and 5 min reperfusion) before global ischemia (30 min) followed by reperfusion (60 min). The preconditioned coronary effluent (Efl_IPC) collected during IPC was fractionated by molecular weight (<3, 3-5, 5-10, 10-30, 30-50, and >50 kDa) and evaluated for cardioprotective effect by perfusion before ischemia/reperfusion in naive hearts. The cardioprotective fraction was evaluated for concentration-dependent, and purified for hydrophobic or hydrophilic components, and tested in precence of cardioprotective inhibitors pathways. We also analysed the humoral factors by in solution digestion and LC-MS/MS, using a ESI-Q-Tof mass spectrometer **Results and Discussion:** Only the fractions <3kDa and 5-10kDa were able to reduce the infarct area (IA) and to improve the postischemic recovery of left ventricular developed pressure. The cardioprotective effect of 5-10 kDa fraction was concentration-dependent and blunted by glibenclamide and 5HD (ATP-sensitive K+ channels blockers) and chelerythrine (protein kinase C inhibitor). Proteomic analysis of Efl_IPC revealead the presence of 60 cytoprotection-related proteins being 14 of them in the range of 4 to 12 kDa. **Conclusion:** The cardioprotection induced by transfer of preconditioned coronary effluent is afforded by humoral factors with molecular weight <3 kDa and 4 to 12 kDa. The 4-12 kDa humoral factors are hidrophobic and activate cardioprotective pathways dependent of PKC and KATP, similar to those of total Efl_IPC.

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