**ACTIVATION OF AMPK/SIRT-1 AXIS IS REQUIRED FOR PROTECTIVE EFFECTS OF ADIPONECTIN ON MYOCARDIAL ISCHEMIA-REPERFUSION (I/R) INJURY IN RAT HEARTS**

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Adiponectin (AD) is an adipose-derived hormone with metabolic and cardiovascular protective effects whose reduced levels are associated with increased risk of cardiovascular events (Kumada et al., 2003). Administration of exogenous AD reduces myocardial infarct size and improves cardiac function in animal models of ischemia-reperfusion (I/R) injury (Gonon et al., 2008). Metabolic effects of AD are mediated by the AMP-activated protein kinase (AMPK), a key molecule for bioenergetic metabolism and cell survival. Recently, the histone deacetylase sirtuin-1 (SIRT-1) has been proposed to regulate several cellular processes such as cell cycle, apoptosis and aging (Cattelan et al., 2015). There is accumulating evidence that SIRT1 and AMPK control each other's activity, and the SIRT1-AMPK axis has emerged as a potential signaling system for the metabolic effects of AD. However, mechanisms underlining beneficial effects of AD on myocardial ischemia-reperfusion (I/R) injury are still unclear.

We investigated whether activation of both AMPK and SIRT-1 is implicated in the protective effects of AD on myocardial ischemia-reperfusion (I/R) injury. Functional and morphological outcomes, together with activation of AMPK/SIRT-1 axis, were evaluated in rat hearts administered with AD before ischemia.

Hearts isolated from male Sprague-Dawley rats were mounted on a Langendorff system and exposed to I/R injury (30/180 min). All combinations of drugs were infused in volumes of 3 ml for 1 min into the aortic cannula starting at the onset of ischemia. The effects induced by infusion with human recombinant AD (3μg/ml for 1 min; n = 6) were compared with those obtained in hearts infused with SIRT-1 activator resveratrol (RSV, 10 μM for 1 min; n = 4), SIRT-1 inhibitor sirtinol (STN, 10 μM for 1 min; n = 4) or vehicle (I/R, n= 4). Left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (dLVP) and coronary flow (CF) were monitored throughout the experiments. For each heart, left ventricular infarct mass (IM) was quantified together with the activation status of AMPK (WB) and SIRT-1 (fluorimetric assay) in all groups, at the end of reperfusion.

When compared to hearts subjected to I/R alone, RSV significantly reduced total infarct mass (RSV vs. I/R, $p < 0.01$). Similarly, AD slightly but significantly reduced ischemic area (AD vs. I/R, $p < 0.05$) and ameliorated cardiac recovery. Conversely, STN administration was not able to enhance cardioprotection. At the molecular level, both RSV and AD treatments significantly increased AMPK and SIRT-1 phosphorylation over basal levels ($p< 0.05$ vs. I/R, for both), whereas STN treatment did not. Finally, SIRT-1 activity was slightly increased in hearts treated with RSV ($p < 0.05$ vs. I/R), and highly enhanced in hearts treated with AD ($p < 0.001$ vs. I/R); as expected, SIRT-1 activity did not increase in hearts exposed to STN treatment. Parallel experiments performed on human endothelial cells (HAEC) showed that AD was able to increase phosphorylation of both

AMPK and SIRT-1. Pre-treatment with STN abrogated SIRT-1, but not AMPK activation, induced by AD. Conversely, cellular pre-incubation with AMPK inhibitor Compound C, was able to reduce both AMPK and SIRT-1 phosphorylation.

Our results suggest that acute pre-ischemic AD administration is effective in mediating cardioprotection. The infarct mass reduction by AD seems related on its specific ability to activate both AMPK and SIRT-1 signaling in rat hearts, therefore supporting the hypothesis of a reciprocal SIRT-1/AMPK modulation.

Kumada et al. (2003) Arterioscler Thromb Vasc Biol 23, 85-9

Gonon et al. (2008) Cardiovascular Res 78, 116-22

Cattelan et al. (2015) Vasc Pharmacol 70, 35-44