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MODULATION OF OXIDATIVE STRESS INDUCED APOPTOSIS AND AUTOPHAGY IN CARDIOMYOCYTES BY INTERMEDIN

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The heart is subjected to oxidative stress in conditions of increased reactive oxygen species (ROS) production, such as doxorubicin (DOX) chemotherapy. A major cause of associated ventricular dysfunction is cardiomyocyte loss through apoptosis, while autophagic processes can also be detrimental. Intermedin (IMD) has emerged as a major counter-regulatory peptide with cytoprotective properties. Here we examined its potential to be upregulated and

attenuate indices of cardiomyocyte death by a mechanism involving NADPH oxidase-derived superoxide.

In isolated adult C57BL/6J wild type (WT) mouse ventricular cardiomyocytes, DOX (5×10^{-7} M) increased preproIMD mRNA expression 3.5-fold, which was significantly decreased in NOX2-deficient cells. Similarly, IMD mRNA was upregulated by the direct pro-oxidant, H_2O_2 (10^{-7} M) in both WT and HL-1 cardiomyocytes, as was NOX2; DOX increased IMD protein (immunocytochemistry). Superoxide production stimulated by DOX or H_2O_2 (by ~35%, lucigenin-enhanced chemiluminescence), was abolished by IMD (10^{-10} M– 10^{-8} M) in both WT and HL-1 cardiomyocytes. Apoptosis in HL-1 cells, shown by DOX-induced

increases in caspase 3/7 activity (3.5-fold), was decreased significantly by IMD (10^{-9} M), which also increased cell viability. Increased autophagosome formation after serum starvation or DOX treatment (by ~50%, Cyto-ID fusion with LC3 protein), was decreased to control values by IMD (10^{-9} M). Confocal imaging showed numerous cytoplasmic punctate structures with DOX, whereas addition of IMD showed a diffuse LC-3 staining pattern similar to control.

These findings indicate that a NOX2-mediated increase in IMD in cardiomyocytes could have a potential autocrine effect, acting at nM concentration to reduce levels of superoxide and so limit cell death by apoptotic and autophagic mechanisms.