p16, and the down-regulation of eNOS from P1 to P4. The increased DHE signal was prevented by apocynin and indometachin, and associated with an increased expression of the NADPH oxidase subunits gp91 phox, p47 phox, p22 phox, COX-1 and COX-2. The Crataegus extract, apocynin and indomethacin prevented the increase in SA-ß-gal activity, the DHE signal, the up-regulation of gp91 phox, p47 phox, p21 phox, COX-1, COX-2, p53, p21 and p16, and the down-regulation of eNOS in cells at P3.In conclusion, the present findings indicate that the Crataegus extract WS1442® delays endothelial cell replicative senescence most likely by preventing oxidative stress, which promotes the down-regulation of eNOS expression and the up-regulation of the p53/p21 and p16 and pathways leading to cell cycle arrest.

0126

Pharmacological activities of CORM-401, a redox sensitive carbon monoxide-releasing molecule, in H9C2 cardiomyocytes

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Carbon monoxide (CO) is an important signaling mediator in living organisms. To exploit the beneficial properties of CO in a therapeutic context, we developed CO-releasing molecules (CO-RMs), a class of metal carbonyls that release controlled amounts of CO in biological systems. CO-RMs elicit distinct pharmacological activities, including vasodilatation and cardioprotection against ischemia-reperfusion injury. In addition, certain CO-RMs stimulate respiration in isolated cardiac mitochondria when used at low micromolar concentrations. Here we investigated the effects of CORM-401, a new manganese-containing CO-RM, against oxidant damage and modulation of respiration in H9C2 cardiomyocytes. Using a new fluorescent probe highly sensitive to CO, we found that CORM-401 delivers higher amounts of CO to cardiomyocytes compared to CORM-3, supporting our previous findings showing that CORM-401 liberates three times more CO than CORM-3. Challenge of H9C2 cells with H_2O_2 induced significant loss of cell viability while co-treatment with CORM-401 rendered cells more resistant to oxidative damage. Inactive CORM-401, which does not release CO, also exerted protection against the damage caused by H_2O_2, suggesting a potential anti-oxidant role for the manganese metal. We measured respiration in intact cardiomyocytes and found that CORM-401 reverses the reduction in O_2 consumption caused by oligomycin (inhibitor of ATP synthase) and diminishes the increase of respiration elicited by FCCP, a mitochondrial uncoupler. These effects suggest potential mitochondrial uncoupling properties of CORM-401. Interestingly, only high CORM-401 concentrations decreased cellular ATP production. In summary, CORM-401 can protect from oxidative damage, although this effect may be partially mediated by the manganese metal contained in the compound. The ability of CORM-401 to modulate cardiomyocytes respiration supports an important role for CO in the control of cellular energy production and metabolism.

0179

Polyphenol-rich blackcurrant juice induces NO-mediated relaxation in porcine coronary artery rings via a copper- and iron-dependent redox-sensitive activation of the Src/PI3-kinase/Akt/eNOS pathway

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The beneficial cardiovascular effect of polyphenol-rich food and beverages has been attributed, at least in part, to the improvement of the vascular function through the induction of two major endothelial vasoprotective mechanisms. The aim of the present study is to determine whether a polyphenol-rich blackcurrant juice (PRBJ, 2.7 g/l), improves the vasoprotective endothelial function, and, if so, to characterize the underlying mechanism. The reactivity of porcine coronary artery rings was assessed in organ chambers, and the expression and phosphorylation levels of proteins in cultured porcine coronary endothelial cells by Western blot analysis. PRBJ caused potent endothelium-dependent relaxations that were significantly reduced by an eNOS inhibitor, not affected by inhibition of endothelium-dependent hyperpolarization, and abolished by both treatments. PRBJ-induced NO-mediated relaxations were significantly reduced by chelators of either copper or iron, membrane permeant analogues of superoxide dismutase and catalase, inhibitors of either Src or PI3-kinase, and by calmidazolium, a calmodulin inhibitor. The NO-mediated relaxation was not affected by inhibitors of either PKC, EGFR, IGFR, or of several endogenous enzymes involved in the formation of ROS (NADPH oxidase, xanthine oxidase, mitochondrial respiration chain, cytochrome P450). In cultured endothelial cells, PRBJ increased the formation of NO as assessed by electron paramagnetic resonance spectroscopy. Moreover, PRBJ induced the phosphorylation of Akt and eNOS on activator sites, which were inhibited membrane permeant analogues of superoxide dismutase and catalase and inhibitors of either Src or PI3-kinase. PRBJ is a potent inducer of endothelium-dependent NO-mediated relaxations in porcine coronary artery rings. The NO-mediated relaxation involves an intracellular copper- and iron-dependent redox-sensitive activation the Src/PI3-kinase/Akt pathway leading to activation of eNOS and subsequent formation of NO.