EFFECT OF OXYSTEROLS ON CELL SURVIVAL AND PROLIFERATION PATHWAYS IN HUMAN ENDOTHELIAL CELLS

Laura Agnoletto, Chiara Poggiani, Federico Cusinato, Lucia Trevisi and Sisto Luciani

Department of Pharmacology and Anaesthesiology, University of Padova

Oxidized low density lipoproteins (oxLDLs) are involved in the pathogenesis of atherosclerosis because of their cytotoxic and pro-apoptotic effect on vascular cells (1). However it has been shown that oxLDLs induce not only apoptosis but also cell proliferation in endothelial cells (EC), depending on their concentration (1). The cytotoxicity of oxLDLs has been linked to their content in oxysterols, in particular 7-ketocholesterol (7KC) and 7β-hydroxycholesterol (7βOHC) (3). Preliminary studies from our laboratory indicate that 7KC and 7βOHC, like oxLDLs, posses a dual effect on viability of EC, inducing an increase in cell death at high concentrations (>20μg/mL) and protection from apoptotic stimuli at lower concentrations. The aim of this study was to investigate the effects of low concentrations of oxysterols on viability of human umbilical vein endothelial cells (HUVEC) and the pathways involved. **Methods:** HUVEC were isolated from human umbilical cord and used from passage two to six. Cell proliferation was measured by [^{3}H] thymidine incorporation assay. Apoptosis was determined by: a) flow cytometric analysis of annexin V and propidium iodide (PI) binding; b) caspase-3 activity of cell lysates. Cell cycle was measured by flow cytometric analysis of PI binding. **Results:** Low concentrations (1-10 μg/ml) of 7KC and 7βOHC protected HUVEC from apoptosis induced by two different stimuli: growth factor (bFGF)-deprivation and staurosporine (50 nM). This result was confirmed by the decrease in annexin V/PI binding and caspase-3 activation, two hallmarks of apoptosis. Treatment of HUVEC for 24h with cholesterol (1-10 μg/mL) produced no effect on cell viability suggesting that oxidation in C7 is necessary for the antiapoptotic activity. The two oxysterols did not induce cell proliferation as shown by the lack of [^{3}H] thymidine incorporation and the increase in the percentage of cells in the phase G_0/G_1 of the cell cycle. However, treatment of HUVEC with low concentrations (<10 μg/ml) of 7βOHC enhanced cell proliferation induced by three different growth factors: bFGF (1-5 ng/mL), EGF (5-10 ng/mL) and VEGF (5-10 ng/mL). **Conclusions**: In the absence of growth factors, low concentrations of 7βOHC and 7-KC activate cell survival pathways that protect HUVEC from apoptotic stimuli while, in the presence of growth factors, oxysterols enhance growth factor-induced cell proliferation. Further investigations will analyse whether the two effects depend on a common pathway.

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