ROLE OF REACTIVE OXYGEN SPECIES (ROS) IN THE ANTIAPOPTOTIC EFFECT OF OXYSTEROLS ON HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVEC)

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Oxidized low density lipoproteins (oxLDLs) are mainly involved in the pathogenesis of atherosclerosis. The cytotoxicity of oxLDLs has been linked to the formation of oxysterols (1) such as 7-ketocholesterol (7-KC) and 7β-hydroxycholesterol (7β-OHC). In vitro studies in endothelial cells have demonstrated that high concentrations of oxLDLs (40 μg/ml) are cytotoxic but lower concentrations induce cell proliferation. This dual effect is mediated by different levels of reactive oxygen species (ROS) (2). Preliminary studies from our laboratory indicate that 7KC and 7βOHC, like oxLDLs, posses a dual effect on human umbilical vein endothelial cells (HUVEC), inducing apoptosis at high concentrations (>20ug/mL) and protection from apoptotic stimuli at lower concentrations. There is evidence about the role of ROS in endothelial cells as signaling molecules, involved in the regulation of cell survival and NO biodisponibility. NADPH oxidase and mitochondria are the major sources of ROS (3). The aim of this work was to determine the role of ROS in the effect of oxysterols on HUVEC. **Methods.** HUVEC were isolated from human umbilical cord and used from passage two to six. HUVEC apoptosis was induced by growth factor (bFGF)-deprivation. Cell viability was determined by the MTT assay. Apoptosis was detected by the cytofluorimetric analysis of annexin-V and propidium iodide binding. Intracellular ROS was measured in intact cells with the fluorescent probe CM-H_2DCFDA. **Results.** 7-KC and 7β-OHC induced a concentration-dependent increase in intracellular ROS at low concentrations (1, 5, 10, 20 μg/mL) and confirmed their dual effect on cell viability. Increase in intracellular ROS induced by both oxysterols was not inhibited by the mitochondrial complex I inhibitor rotenone (2 μM). Instead, the NADPH oxidase inhibitor hydralazine (25 μM) partially inhibited only 7β-OHC-induced increase in ROS levels. However, the antiapoptotic effect of 7β-OHC was not affected by pre-treatment with hydralazine. Furthermore, eNOS inhibition with L-NAME was not able to block the antiapoptotic action of the two oxysterols. **Conclusions.** The results show that neither NADPH oxidase nor eNOS are involved in the antiapoptotic effect of oxysterols on HUVEC. Further investigations will analyze the involvement of ROS in the antiapoptotic effect of the two oxysterols and their possible sources.

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