Influence of intracellular calcium on apoptosis in differentiated U937 cells following long-term exposure to oxysterols

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Oxysterols, or cholesterol oxidation products, are formed endogenously and can also be absorbed from the diet. Certain foods, in particular milk powders, meat and meat products, contain significant amounts of oxysterols1–2. Oxysterols may be involved in the pathogenesis of atherosclerosis and neurodegenerative diseases and this role has been attributed to their ability to induce apoptosis3–9. The oxysterols 7β-hydroxycholesterol (7β-OH) and cholesterol-5β,6β-epoxide (β-epoxide) have previously been shown to induce apoptosis in the U937 human monocytic cell line9–6. Alterations in intracellular Ca2+ homeostasis have been implicated in the initiation of apoptotic cell death in many experimental systems7; however, little is known about the role, if any, of Ca2+ in oxysterol-induced apoptosis. Thus, the objective of the present study was to examine the changes in intracellular Ca2+ levels following chronic exposure to 7β-OH and β-epoxide.

Phorbol 12-myristate 13-acetate-differentiated U937 cells were exposed to 30 μM-7β-OH or 30 μM-β-epoxide in the absence or presence of the Ca2+ channel blocker nifedipine. Lactate dehydrogenase (LDH) release was determined as an index of cell viability and apoptotic nuclei were quantified after staining with Hoechst 33342. Ca2+ responses in the cells were assessed by epifluorescence videomicroscopy using the ratiometric dye fura-2, loaded as an acetoxymethyl ester.

After 72 h the treatment with the oxysterols resulted in a significant (P<0.05) increase in LDH release relative to the untreated control. This increase was not observed following pretreatment with 0.1 mM-nifedipine, indicating that the Ca2+ channel blocker may protect against oxysterol-induced toxicity. A significant (P<0.05) increase in apoptotic nuclei was detected over 72 h following exposure to both oxysterols. At the 24 h time point nifedipine reduced apoptosis in 7β-OH-treated cells; however, this effect was not observed after 48 h and 72 h. In the experiments involving fura-2 the cytosolic Ca2+ levels in 7β-OH-treated cells were significantly (P<0.05) higher at 24 h and were significantly (P<0.05) lower after 72 h compared with untreated control cells. In addition, pretreatment with nifedipine blocked the increase in intracellular Ca2+ but had no effect at 48 h or 72 h. These results indicate that the 7β-OH-induced influx of Ca2+ may occur through voltage-dependent Ca2+ channels. Chronic exposure to β-epoxide did not alter the Ca2+ levels of the cells and nifedipine did not inhibit apoptosis induced by this oxysterol.

Overall, following long-term exposure to 7β-OH increased levels of cytosolic Ca2+ were not maintained and nifedipine did not protect against apoptotic cell death. In conclusion, the increase in intracellular Ca2+ may be an initial trigger of 7β-OH-induced apoptosis, but following chronic exposure to the oxysterol the influence of Ca2+ appears to be less significant. Moreover, Ca2+ does not appear to play a role in β-epoxide-induced apoptotic cell death.

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