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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 oxysterols[(1–2)]. Oxysterols may be involved in the pathogenesis of atherosclerosis and neurodegenerative diseases and this role has been attributed to their ability to induce apoptosis[(3)]. 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 line[(4–6)]. Alterations in intracellular Ca^{2+} homeostasis have been implicated in the initiation of apoptotic cell death in many experimental systems[(7)]; however, little is known about the role, if any, of Ca^{2+} in oxysterol-induced apoptosis. Thus, the objective of the present study was to examine the changes in intracellular Ca^{2+} 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 Ca^{2+} 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. Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} but had no effect at 48 h or 72 h. These results indicate that the 7β-OH-induced influx of Ca^{2+} may occur through voltage-dependent Ca^{2+} channels. Chronic exposure to β-epoxide did not alter the Ca^{2+} 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 Ca^{2+} were not maintained and nifedipine did not protect against apoptotic cell death. In conclusion, the increase in intracellular Ca^{2+} may be an initial trigger of 7β-OH-induced apoptosis, but following chronic exposure to the oxysterol the influence of Ca^{2+} appears to be less significant. Moreover, Ca^{2+} does not appear to play a role in β-epoxide-induced apoptotic cell death.

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1. Brown AJ & Jessup W (1999) *Atherosclerosis* **142**, 1–28.
2. Valenzuela A, Sanhueza J & Nieto S (2003) *Biol Res* **36**, 291–302.
3. Panini SR & Sinensky MS (2001) *Curr Opin Lipidol* **12**, 529–533.
4. Ryan L, O'Callaghan YC & O'Brien NM (2004) *Cell Biol Toxicol* **20**, 313–323.
5. Ryan L, O'Callaghan YC & O'Brien NM (2005) *Br J Nutr* **94**, 519–525.
6. Lordan S, O'Callaghan YC & O'Brien NM (2007) *J Biochem Mol Toxicol* **21**, 362–372.
7. Orrenius S, Zhivotovsky B & Nicotera P (2003) *Nat Rev Mol Cell Biol* **4**, 552–565.