Eur J Nutr 38:255–262 (1999)

ORIGINAL CONTRIBUTION

Y.C. O'Callaghan
J.A. Woods
N.M. O'Brien

Oxysterol-induced cell death in U937 and HepG2 cells at reduced and normal serum concentrations

Received: 3 September 1999
Accepted: 21 December 1999

Y.C. O'Callaghan · J.A. Woods
Dr. N.M. O'Brien (✉)
Nutritional Sciences
Department of Food Science
and Technology
University College
Cork, Ireland
Phone: +353 21 902884
Fax: +353 21 270244, email: nob@ucc.ie

Summary *Background and aims*: Cholesterol oxidation products (oxysterols) are commonly found in foods of animal origin and are also produced endogenously in the body. Oxysterols are cytotoxic to certain cell lines and in some cases have been shown to induce apoptosis. The aim of this study was to investigate the effects of 7β-hydroxy-cholesterol (7β-OHC) and 25-hydroxycholesterol (25-OHC) on cytotoxicity and induction of apoptosis in U937 and HepG2 cells, treated in media containing either 2.5% foetal calf serum (FCS) or 10% FCS to examine the effect of increasing the cholesterol level.
Methods: The cells were incubated for 24 and 48 h with 30μM oxysterol. Viability was assessed by fluorescein diacetate/ethidium bromide staining and cell proliferation was determined by haemocytometer counting. Apoptosis was monitored by detection of DNA fragments (laddering) in 1.5% agarose gels. Cells with condensed or fragmented nuclei were identified by Hoechst 33342 staining. The percentage of cells with sub-G1 levels of DNA was measured by flow cytometry.
Results: Treatment of U937 cells with 7β-OHC, in contrast to 25-OHC, resulted in a decrease in cell viability and proliferation at 24 and 48 h (P <.01). 25-OHC and 7β-OHC were both equally cytotoxic to the HepG2 cell line. 7β-OHC induced DNA laddering and an increase in the percentage of condensed or fragmented nuclei at both time points and at both serum concentrations in the U937 cell line. 25-OHC induced faint laddering in the U937 cells after 48 h in reduced serum media and resulted in a small increase in percentage condensed or fragmented nuclei which was independent of time of oxysterol exposure and serum concentration. The percentage of condensed or fragmented nuclei was low in the HepG2 cell line and no laddering was observed under any of the conditions studied. Flow cytometric analysis showed that only 7β-OHC treated U937 cells had an increased level of hypodiploid cells.
Conclusion: Both oxysterols appear to be equally cytotoxic to the HepG2 cell line. In U937 cells, 25-OHC is much less cytotoxic than 7β-OHC. In addition, we have shown that 7β-OHC induces apoptosis in U937 cells. 10% FCS displays a protective effect on cytotoxicity (as well as on 7β-OHC induced apoptosis in U937 cells), although the data did not reach statistical significance.

Key words Oxysterol – cell death – apoptosis – U937 cells – HepG2 cells

European Journal of Nutrition, Vol. 38, Number 6 (1999)

Introduction

Cholesterol oxidation products (oxysterols) arise from the enzymatic or non-enzymatic oxidation of cholesterol (1, 2). Oxysterols have been identified in foods with a high cholesterol content such as meat, egg yolk and dairy products, and once ingested are rapidly absorbed from the gastrointestinal tract (3, 4). Levels in food increase dramatically on processing with powdered egg yolks one of the highest dietary sources of oxysterols (5). Deep frying also dramatically increases oxysterol content, especially in meat products (6). Oxysterols are also produced endogenously in human tissues as a result of the breakdown of cholesterol by free radical attack or by the activity of microsomal P450 enzyme systems in the liver (1, 7, 8).

Although cholesterol itself has not been shown to be cytotoxic, a number of potential biological activities of oxysterols have been reported. Oxysterols have been detected in oxidised LDL (2, 9) a key substance in atherogenesis. Mixed oxysterol preparations, but not individual oxysterols, have been shown to induce mutagenicity in certain strains of *Salmonella typhimurium* (10). However, a number of oxysterols exhibited a lack of genotoxicity as assessed by the frequency of DNA strand breaks (comet assay) and sister chromatid exchanges in cells in culture (11).

Oxysterols have been shown to induce cytotoxicity and inhibit cell proliferation in various cell lines. One potential mechanism by which oxysterols may inhibit cell proliferation *in vitro* is through the inhibition of the enzyme hydroxymethyl-glutaryl-coenzyme A reductase (HMG-CoA reductase), the regulatory enzyme of the cholesterol biosynthetic pathway. Inhibition of HMG-CoA reductase results in a decrease in endogenous cholesterol, and since cholesterol is required for the formation of cell membranes, the cell is unable to divide (12). In addition, cytotoxicity may be induced due to the introduction of oxysterols into the cell membrane. These oxysterols displace cholesterol and cause irreparable damage to the structure and function of the cell membrane (2). Oxysterols may also modulate cytotoxicity by exerting effects on the induction of apoptosis. Due to the fundamental importance of apoptosis in pathological processes (13), the identification of substances capable of triggering or modulating this form of cell death is now actively researched.

In this study we have focused on the oxysterols 7β-hydroxycholesterol (7β-OHC) and 25-hydroxycholesterol (25-OHC), both of which are commonly found in foods. These oxysterols were investigated for their ability to induce apoptosis in a human monocytic cell line (U937), and a human hepatocellular carcinoma cell line (HepG2). The ability of oxysterols to induce apoptosis in HepG2 cells has not been reported. The effects of varying the culture media serum concentration on cytotoxicity and ability of oxysterols to induce apoptosis in the two cell types was also investigated. Standard culture media containing 10% foetal calf serum provides the cells with cholesterol in large excess (14) and earlier studies report that cytotoxic effects of 7β-OHC vary with culture media serum concentrations (16). Our results suggest that while both 7β-OHC and 25-OHC were more cytotoxic to HepG2 cells they did not induce cell death by apoptosis in this cell line. In contrast, 7β-OHC induced apoptosis in U937 cells and effects were slightly more pronounced under low serum conditions.

Materials and methods

All chemicals and cell culture reagents were obtained from the Sigma Chemical Co. (Poole, UK) unless otherwise stated. Tissue culture plastics were supplied by Costar (Cambridge, UK). Information on the purity of the oxysterols (purity > 95%) was obtained from Sigma. Cell lines were obtained from the European Collection of Animal Cell Cultures (Salisbury, UK).

Maintenance of cell lines

Human monocytic U937 cells were grown in suspension in RPMI-1640 medium supplemented with 2mM L-glutamine and 10% (v/v) foetal calf serum (FCS). Human hepatoma HepG2 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) FCS, 2mM L-glutamine and 1% non-essential amino acids. The cells were grown at 37 °C/5% CO_2 in a humidified incubator. HepG2 cells were subcultured every 8–10 days. Both cell lines were screened for mycoplasma contamination by the Hoechst staining method (17) and were cultured in the absence of antibiotics. Exponentially growing cells were used throughout.

Treatment of cells with oxysterols

HepG2 cells and U937 cells were adjusted to a density of 1 x 10^5 cells/ml and oxysterols were added to the tissue culture medium to give a final concentration of 30 μM. Oxysterols were dissolved in ethanol for delivery to cells and the final concentration of ethanol in the cultures did not exceed 0.3% (v/v). Cells were seeded into either six well culture dishes for morphological analysis of nuclei or 10 cm dishes (HepG2 cells) and 25 cm^2 flasks (U937 cells) for DNA fragmentation analysis by gel electrophoresis. Equivalent quantities of ethanol were added to control cells and samples were incubated for 24 h and 48 h at 37 °C/5% CO_2. In the case of HepG2 cells, both floating and attached cells were collected for analysis. To assess the effects of serum, cells were treated with oxysterols in the presence of either 2.5% (v/v) or 10% (v/v) FCS.

Cell viability

At the indicated time points 25 µl of cells were removed for assessment of cell viability. Viability was monitored using a modification of the fluorochrome-mediated viability assay as described by Strauss (18). Briefly, cells were mixed 1:1 (v/v) with a solution of fluorescein diacetate (FDA) and ethidium bromide (EtBr), then incubated at 37 °C for 2–5 min before being layered onto a microscope slide. Under these conditions, live cells fluoresce green, whereas dead cells fluoresce red. Dying cells have a green cytoplasm and red nucleus. Samples were examined at 200x magnification on a Nikon fluorescence microscope using blue light (450–490nm). 200 cells were scored from each slide and cell viability was expressed as the percentage of viable (green) cells relative to the control.

Cell counting

Cell proliferation was also monitored by haemocytometer counting under an inverted phase contrast microscope (Wilovert) and the population doubling times (PDT) calculated according to the equation (Log cell density-Log seeding cell density) x 3.32.

Morphological analysis of cell nuclei

Nuclear morphology of control and oxysterol-treated cells was assessed by fluorescence microscopy after staining with Hoechst 33342. Approximately 4 x 10^5 cells were centrifuged at 200 x g for 10 min to form a pellet. Hoechst 33342 stain (200 µl, 5 µg/ml) was added and the samples incubated at 37 °C/5% CO_2 for 1 h. Stained samples (25 µl) were placed on a microscope slide and examined under UV light (Nikon Labophot fluorescence microscope 400x magnification). A total of 300 cells per sample were analysed and the percentage of fragmented and condensed nuclei was calculated. Apoptotic cells were characterised by nuclear condensation of chromatin and/or nuclear fragmentation (19).

DNA fragmentation assay

Detection of small DNA fragments was done according to the method of Swat *et al*. (20) with some modifications. Briefly 2 x 10^6 cells were harvested at 200 x g for 10 min (4 °C). The pellets were lysed with 20 µl of a solution containing 50mM Tris, 10mM EDTA and 0.5% (w/v) sodium lauryl sarcosinate. RNAse A (0.25 mg/ml) was added and the samples incubated at 50 °C for 1 h. The condensate was spun down and proteinase K (5mg/ml) added. The samples were incubated at 50 °C for a further hour before being loaded into the wells of a 1.5% agarose gel. A 100–1500bp DNA standard (Promega) was used to assess DNA fragmentation. Electrophoresis was carried out in 1.5% agarose gels prepared in TBE buffer (0.45M Tris, 0.45M boric acid and 2mM EDTA, pH 8), at 3V/cm. DNA was visualised under UV light on a transilluminator (312nm) after ethidium bromide staining and photographed using a digital camera (Kodak).

DNA labelling and flow cytometric analysis

Cells (2 x 10^6) were harvested (200 x g) and the pellet was fixed in 70% (v/v) ethanol overnight (-20 °C). The cells were then centrifuged and re-suspended in 200 µl of PBS containing 50 µg/ml propidium iodide and 5Kunitz RNAse at 4 °C for 60 min. The sheath fluid was ISOTON II balanced electrolyte solution (Coulter). Data acquisition and analysis (10,000 cells) were performed with an Epics Elite Cell Sorter (Coulter) using doublet discrimination (8).

Statistics

All data points are the mean values (± SE) of at least three independent experiments. Where appropriate, data were analysed by one way analysis of variance (ANOVA) followed by Dunnett's test.

Results

Cytotoxicity of 7β-OHC and 25-OHC in U937 and HepG2 cells

Cytotoxicity of the oxysterols in the two cell lines was determined by assessing both cell membrane integrity using the FDA/EtBr method and cell proliferation by haemocytometer counting. Treatment of U937 cells with 30µM 7β-OHC resulted in a significant decrease (P <0.01) in cell viability (EtBr positive cells) relative to the control with increasing time of exposure. In contrast, treatment of U937 cells with 30 µM 25-OHC did not produce significant levels of EtBr permeable cells (Fig. 1A). Treatment with 7β-OHC caused a significant decrease in cell proliferation relative to the control as assessed by cell counting. Cell proliferation was also lower in the presence of 25-OHC but was not significantly different from the control samples over the time points measured (Fig. 1B & C).

The effect of serum concentration on oxysterol-induced cytotoxicity was also examined. Although treatment with both oxysterols in the presence of 10% FCS resulted in less cell lysis this protective effect was only seen after 48 h (Fig. 1A). The proliferation rate of U937 cells was faster in the presence of 10% FCS (PDT = 24.12 h) compared to 2.5% FCS (PDT = 35 h). Increasing the serum concentration from 2.5% to 10% increased the growth rate of both control samples and of 25-OHC-treated U937 cells, but had no effect on cells treated with 7β-OHC (Fig. 1B & C).

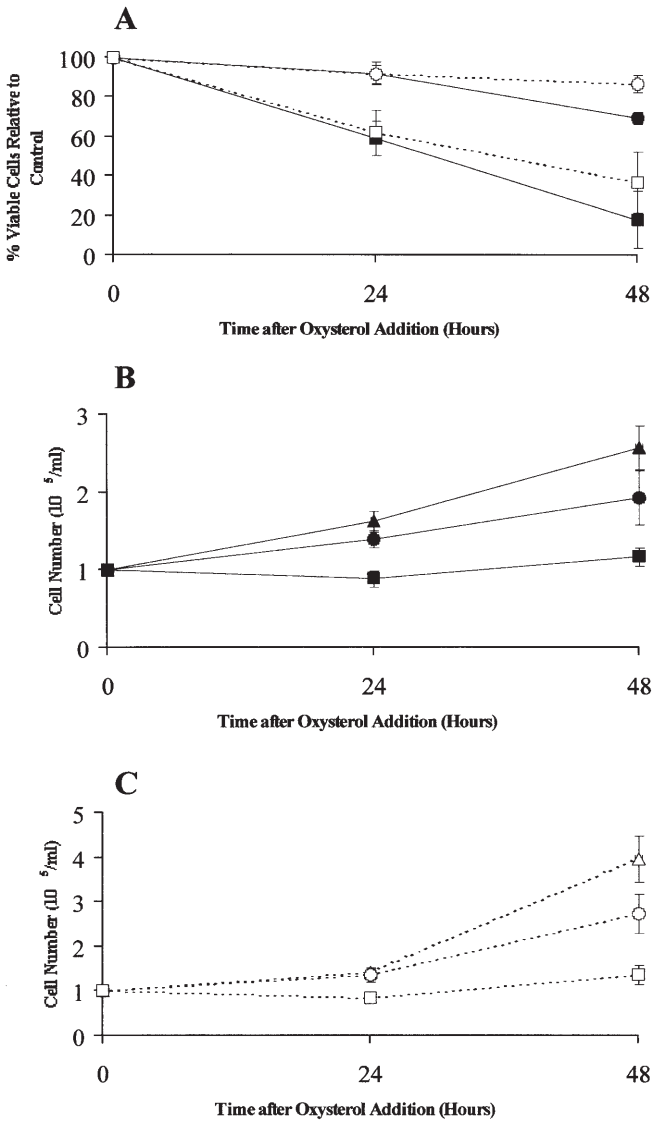

Fig. 1 Oxysterol induced cytotoxicity in U937 cells.
The effect of either 30μM 25-OHC (circles) or 30μM 7β-OHC (squares) on U937 cell membrane integrity was measured using the FDA/EtBr method (A). Cells (1 x 10^5/ml) were incubated for either 24 h or 48 h in the presence of 2.5% serum (closed symbols) or 10% serum (open symbols, dotted lines). 200 cells/sample were analysed by fluorescence microscopy (200x magnification) and results were expressed as a percentage of the control cells. Cell counts were also determined using a haemocytometer. Cells were grown in either 2.5% serum (B) or 10% serum (C) and growth curves for control cells (0.3% (v/v) ethanol, triangles) and oxysterol treated cells were plotted.

The results represent the mean values (± SE) for at least four independent experiments and data were analysed by ANOVA followed by Dunnett's test (**P< 0.01).

In contrast to U937 cells, both 25-OHC and 7β-OHC were almost equally as effective in decreasing viability of HepG2 cells over the 48 h incubation period particularly in the presence of 2.5% serum (Fig. 2A). Slight protection against these toxic effects was afforded when the cells were incubated with the oxysterols in the presence of 10% FCS.

HepG2 control cells divided faster when cultured in the presence of 10% compared to 2.5% FCS (Fig. 2B & C). The delay in cellular growth caused by the oxysterols was evident only after 48 h of treatment and was not seen at all in the samples grown in the presence of 2.5% FCS (Fig. 2B & C). This may have been due to the fact that HepG2 cells have a much longer population doubling time compared to U937 cells. The experimental time period of 48 h would have allowed U937 cells to double their population approximately twice whereas the HepG2 cells would have had time for only one population doubling.

DNA integrity alterations by 25-OHC and 7β-OHC in U937 and HepG2 cells

Morphological examination of cell nuclei was accomplished by staining cells with Hoechst 33342 and examination by fluorescence microscopy. Nuclei that had undergone blebbing, fragmentation, chromatin marginalisation and condensation were identified as cells that were most likely to be apoptotic. The level of these nuclei did not exceed 10% in untreated samples of U937 cells and 4% in untreated samples of HepG2 cells. Treatment of U937 cells with 30 μM 25-OHC resulted in a small increase in the numbers of apoptotic nuclei (not exceeding 14%) which did not increase with time of oxysterol exposure and was unaffected by serum concentration. In contrast, a greater percentage of apoptotic nuclei was observed in U937 cells treated with 30 μM 7β-OHC, particularly after 48 h (Fig. 3A) in both the 2.5 and 10% serum treatments. Analysis of non-random DNA fragmentation on 1.5% agarose gels showed that 7β-OHC resulted in extensive DNA laddering at both 24 and 48 h time points regardless of the serum concentration. Faint DNA ladders were seen in samples treated with 25-OHC after 48 h treatment in the presence of either 2.5% or 10% serum, this suggests at least some of the U937 cells treated with 25-OHC died, possibly by apoptosis (Fig. 4A & B). From the gels, the extent of DNA fragmentation in the 25-OHC treated samples appeared to be more extensive in the presence of 2.5% serum, which correlates with the results for U937 cell viability shown in Fig. 1A.

The overall level of apoptotic nuclei was much lower in HepG2 cells than in U937 cells. Treatment with both oxysterols resulted in a time-dependent increase in the percentage of apoptotic nuclei which seemed to be independent of serum concentration (Fig. 3B). No extensive DNA fragmentation either random or non-random was

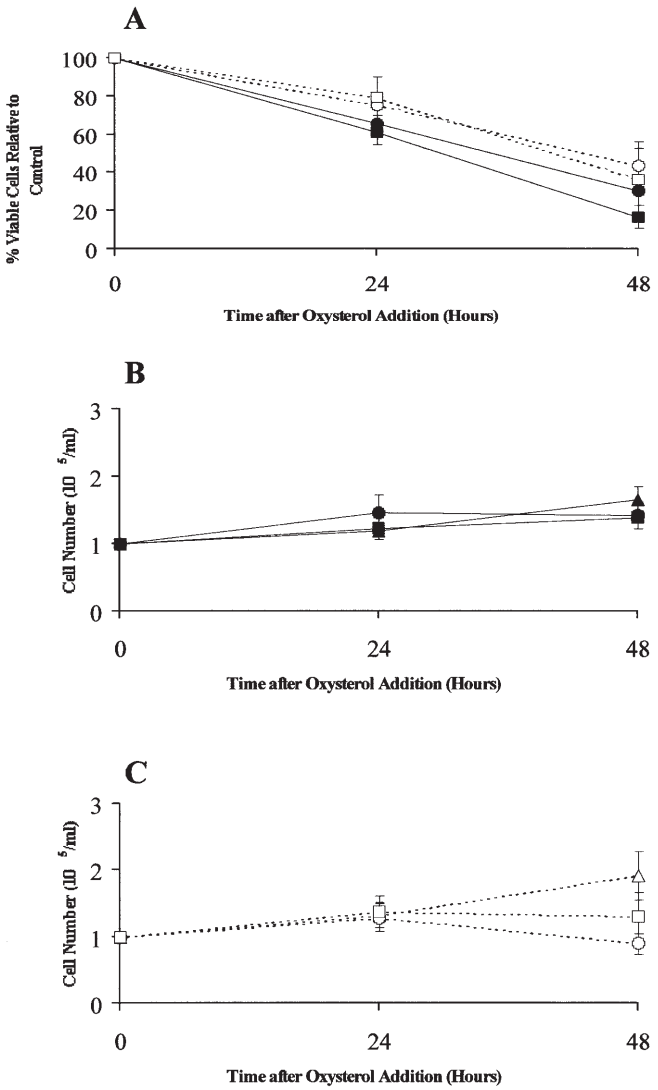

Fig. 2 Oxysterol induced cytotoxicity in HepG2 cells.
The effect of either 30 μM 25-OHC (circles) or 30 μM 7β-OHC (squares) on HepG2 cell membrane integrity was measured using the FDA/EtBr method (A). Cells (1 x 10^5/ml) were incubated for either 24 h or 48 h in the presence of 2.5% serum (closed symbols) or 10% serum (open symbols, dotted lines). 200 cells/sample were analysed by fluorescence microscopy (200x magnification) and results were expressed as a percentage of the control cells. Cell counts were also determined using a haemocytometer. Cells were grown in either 2.5% serum (B) or 10% serum (C) and growth curves for control cells (0.3% (v/v) ethanol, triangles) and oxysterol treated cells were plotted.

The results represent the mean values (± SE) for at least four independent experiments and data were analysed by ANOVA followed by Dunnett's test (**P< 0.01).

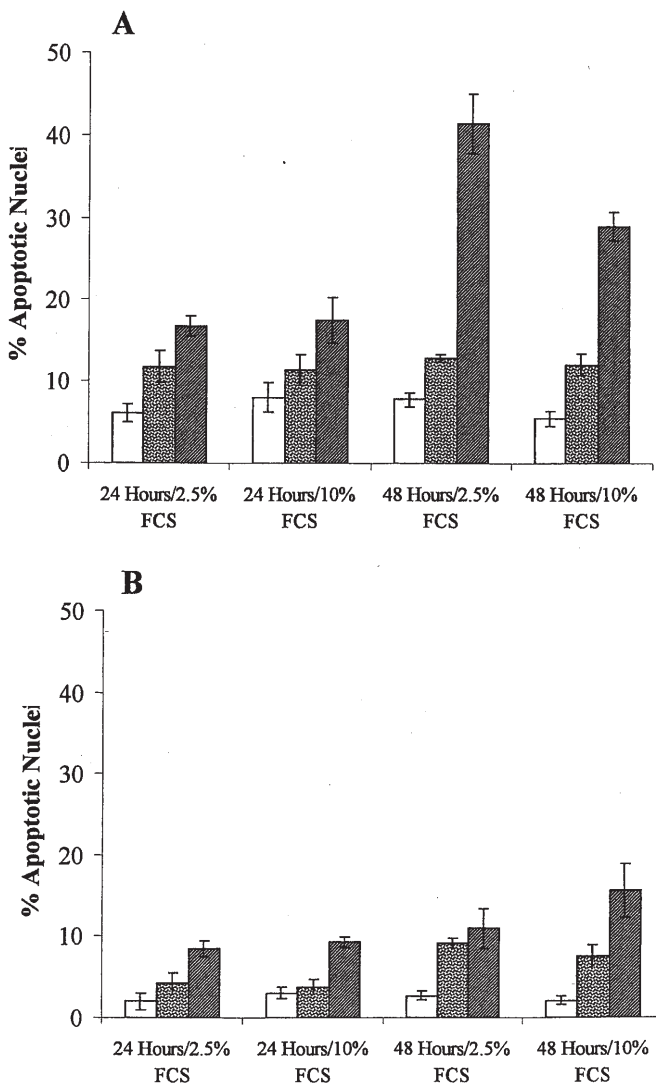

Fig. 3 Ability of oxysterols to induce apoptosis in U937 and HepG2 cells.
U937 cells (A) and HepG2 cells (B) were incubated in the absence (open bars, 0.3% (v/v) ethanol) or presence of either 30 μM 25-OHC (stippled bars) or 30 μM 7β-OHC (hatched bars). Samples were taken after either 24 h or 48 h oxysterol treatment and nuclear morphology assessed by fluorescence microscopy (400x magnification) following staining with Hoechst 33342. Nuclei that had undergone fragmentation, chromatin marginalisation and condensation were identified as cells that were most likely to be apoptotic. 300 cells/sample were analysed and each data point represents the mean value (± SE) for at least three independent experiments.

seen when samples were analysed on 1.5% agarose gels (Fig. 4C & D).

As cell death was more extensive in the two cell lines following a 48 h treatment with oxysterols in the presence of 2.5% FCS, flow cytometric analysis of propidium iodide labelled U937 and HepG2 cells was performed using these experimental conditions in order to detect the presence of a hypodiploid peak. Only U937 cells treated with 7β-OHC showed a significant increase in the level of hypodiploid cells (Table 1, P <0.01).

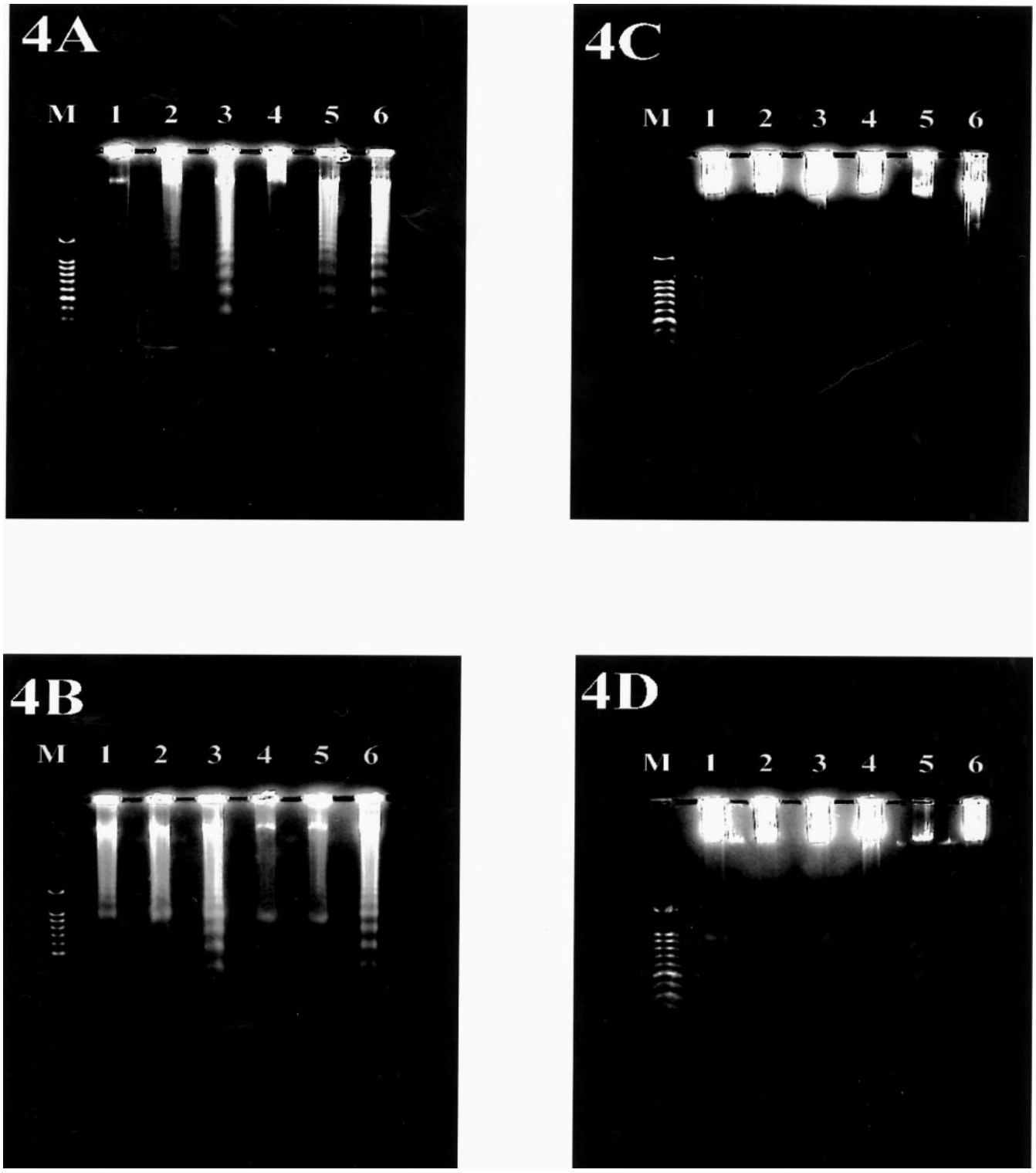

Fig. 4 Oxysterol induced DNA fragmentation in U937 and HepG2 cells.
U937 cells (A & B) and HepG2 cells (C & D) were incubated in the absence or presence of oxysterols. Samples were taken after either 24 h or 48 h oxysterol treatment and DNA was isolated and electrophoresed in 1.5% agarose gels (3V/cm). Samples were incubated in the presence of either 2.5% FCS (A & C) or 10% FCS (B & D). Gels were visualised under UV light on a transilluminator (312nm) after staining with ethidium bromide and photographed using a digital camera (Kodak). Lane M: molecular weight markers (1500bp-100bp); Lane 1: control cells, 24 h; Lane 2: 30μM 25-OHC, 24 h; Lane 3: 30μM 7ß-OHC, 24 h; Lane 4: control cells, 48 h; Lane 5: 30μM 25-OHC, 48 h; Lane 6: 30μM 7β-OHC, 48 h.

Table 1 Percentage of hypodiploid DNA in U937 and HepG2 cells following incubation with oxysterols for 48 h in the presence of 2.5% FCS

	HepG2	U937
Control	11.2 ± 2.2	2.7 ± 0.2
30μM 25-OHC	14.9 ± 3.5	8.4 ± 0.5
30μM 7β-OHC	4.7 ± 1.3	39.0 ± 2.5*

Flow cytometry analysis was performed after staining 2 x 10^6 cells with propidium iodide.
The results represent the mean values ± SE for three determinations. Data were analysed by ANOVA followed by Dunnett's test (*P< 0.01).

Discussion

Diverse biological activities have been ascribed to oxysterols including cytotoxicity, atherogenicity, carcinogenicity and mutagenicity. Recently it has been shown that cell death induced by oxysterols *in vitro* has many common features of apoptosis. The data presented in the present report confirm that 7β-OHC is a potent inducer of apoptosis in human monocytic U937 cells. In addition, we have shown that the mechanism of cell death induced by these oxysterols differs between U937 and HepG2 cells.

7β-OHC was more cytotoxic than 25-OHC in U937 cells as measured by the FDA/EtBr membrane integrity assay. Treatment with 7β-OHC results in a reduction of cell number due to cell lysis. In contrast 25-OHC may have a cytostatic effect in these cells as proliferation was delayed with no accompanying increase in EtBr permeable cells. 7β-OHC was more efficient than 25-OHC at inducing apoptosis in U937 cells as measured by non-random DNA fragmentation, condensed and fragmented nuclei, and the generation of hypodiploid cells. However, DNA fragmentation was evident following treatment with 25-OHC after 48 h incubation in the presence of 2.5% FCS indicating that at least some of these cells may have died by apoptosis. These results are consistent with those obtained in U937 cells by Aupiex *et al.* (14).

Both 7β-OHC and 25-OHC resulted in significant cell lysis in HepG2 cells. The absence of DNA laddering and significant levels of cells with hypodiploid DNA indicates that the oxysterols may induce cell death by a different mechanism in the hepatoma cells, possibly by necrosis (4). Nordmann *et al.* (21) reported that non-proliferating primary rat hepatocytes were resistant to the toxic effects of 7β-OHC. However, our data in human HepG2 cells are in line with the work of Hietter *et al.* (16) who demonstrated cytotoxicity of 7β-OHC in proliferating rat hepatoma HTC cells.

One mechanism proposed for the toxicity of oxysterols has been the inhibition of cholesterol synthesis by suppression of HMG-CoA reductase activity. This mechanism would explain the enhanced toxicity of these compounds to proliferating and neoplastic cells (2). However, treatment of cells with either 2.5% or 10% FCS, which provides exogenous cholesterol in large excess had only small effects on the cytotoxic properties of the oxysterols. Therefore, our results would seem to support the suggestion put forward by Aupiex *et al.* (14) that inhibition of this enzyme may not play a major role in 7β-OHC--induced cell death (14).

The sensitivity of different cell types to oxysterols is believed to be dependent on the ability of oxysterols to bind to intracellular receptors such as oxysterol binding protein which is a high affinity receptor for 25-OHC (8, 22), and regulation of cellular nucleic acid binding protein (23, 24). Moreover, an oxysterol signalling pathway has recently been proposed (25). Work by Lizard *et al.* (15) indicates the involvement of reactive oxygen species (ROS) during 7-ketocholesterol-induced apoptosis in U937 cells. Unpublished data from our own laboratory also support the participation of ROS in 7β-OHC induced apoptosis of U937 cells. Moreover Bcl-2 protein has been shown to partially inhibit oxysterol induced apoptosis in murine P388-D1 cells and transfected U937 cells, in the former instance by suppressing a CPP32 mediated pathway which implies that ROS may play some role in oxysterol induced apoptosis. This caspase has also been shown to be involved in the regulation of sterol metabolism (26, 27). Future work in our laboratory will investigate the involvement of a signalling pathway in 7β-OHC induced apoptosis of U937 cells.

In conclusion, we have shown that varying the serum concentration of the culture media between 2.5% and 10% had no statistically significant effect on cytotoxicity of 7β-OHC and 25-OHC in either U937 or HepG2 cells as measured by the FDA/EtBr assay. In addition, in U937 cells our findings confirm a previous report (14) that the two oxysterols under investigation differ in their potential to induce apoptosis and cytotoxicity. Furthermore, we have shown that 25-OHC is more toxic towards HepG2 in comparison to U937 cells. Finally, the oxysterols induce cell death by different mechanisms in the two cell types.

Acknowledgment This project was part-funded by the Department of Agriculture, Food and Forestry, Dublin under the Food Industry Sub-Programme of EU Structural Funds, 1994-9. The authors wish to thank Mr. Maurice O'Donoghue for performing the FACS analysis.

References

1. Smith LL, Johnson BH (1989) Biological activities of oxysterols. Free Radical Bio Med 7:285–332
2. Guardiola F, Codony R, Addis PB, Rafecas M, Boatella J (1996) Biological effects of oxysterols: current status. Food Chem Toxicol 34:193–211
3. Paniangvait P, King AJ, Jones AD, German BG (1995) Cholesterol oxides in foods of animal origin. J Food Sci 60:1159–1174
4. Lizard G, Deckert V, Dubrez L, Moisant M, Gambert P, Lagrost L (1996) Induction of apoptosis in endothelial cells treated with cholesterol oxides. Am J Pathol 148:1625-1638
5. Tsai LS, Hudson CA (1985) Cholesterol oxides in commercial dry egg products: quantification. J Food Sci 50:229–237
6. Zhang WB, Addis PB, Krick TP (1991) Quantification of 5α-cholestane-3β,5,6β-triol and other cholesterol oxidation products in fast food french fried potatoes. J Food Sci 56:716–718
7. Saucier SE, Kandutsch AA, Gayen AK, Swahn DK, Spencer TA (1989) Oxysterol regulators of 3-hydroxy-3-methylglutaryl-CoA reductase in liver. J Biol Chem 264:6863–6869
8. Christ M, Luu B, Mejia JE, Moosbrugger I, Bischoff P (1993) Apoptosis induced by oxysterols in murine lymphoma cells and in normal thymocytes. Immunology 78:455–460
9. Linseisen J, Wolfram G (1998) Origin, metabolism and adverse health effects of cholesterol oxidation products. Fett/Lipid 100:211–218
10. Smith LL, Smart VB, Made Gowda NM (1986) Mutagenic sterol hydroperoxides. Mutat Res 161:39–48
11. Woods JA, O'Brien NM (1998) Investigation of the potential genotoxicity of cholesterol oxidation products in two mammalian fibroblast cell lines. Nutr Cancer 31:192–198
12. Hwang PL (1991) Biological activities of oxygenated sterols: physiological and pathological implications. Bioessays 13:583–589
13. Thompson CB (1995) Apoptosis in the pathogenesis and treatment of disease. Science 267:1456–1462
14. Aupeix K, Weltin D, Mejia JE, Christ M, Marchal J, Freyssinet J, Bischoff P (1995) Oxysterol induced apoptosis in human monocytic cell lines. Immunobiology 194:415–428
15. Lizard G, Gueldry S, Sordet O, Monier S, Athias A, Miguet C, Bessede G, Lemaire S, Solary E, Gambert P (1998) Glutathione is implied in the control of 7-ketocholesterol-induced apoptosis, which is associated with radical oxygen species production. FASEB J. 12: 1651–1663
16. Hietter H, Trifilieff E, Richert L, Beck J, Luu B, Ourisson G (1984) Antagonistic action of cholesterol towards the toxicity of hydroxysterols on cultured hepatoma cells. Biochem Biophys Res Comm 120:657–664
17. Mowles JM (1990) Mycoplasma Detection. In: Pollard JW, Walker JM (eds) Methods in Molecular Biology Vol. V: Animal Cell Culture, Humana Press, Clifton, NJ, pp 65–74
18. Strauss GHS (1991) Non-random cell killing in cryopreservation: implications for performance of the battery of leukocyte tests (BLT). I. Toxic and immunotoxic effects. Mutat Res 252:1–15
19. Dubrez L, Savoy I, Hamman A, Solary E (1996) Pivotal role of a DEVD-sensitive step in etoposide-induced and Fas-mediated apoptotic pathways. EMBO J 15:5504–5512
20. Swat W, Ignatowicz L, Kisielow P (1991) Detection of apoptosis of immature CD4+8+ thymocytes by flow cytometry. J Immunol Methods 137: 79–87
21. Nordmann P, Diez-Ibanez M, Chessebeuf-Padieu M, Luu B, Mack G, Mersels M (1989) Toxic effects of 7β-hydroxycholesterol on rat liver primary cultures, epithelial lines and cocultures. Cell Biol Toxicol 5:261–270
22. Bakos JT, Johnson BH, Thompson EB (1993) Oxysterol-induced cell death in human leukemic T-cells correlates with oxysterol binding protein occupancy and is independent of glucocorticoid-induced apoptosis. J Steroid Biochem 46:415–426
23. Ayala-Torres S, Johnson BH, Thompson BE (1994) Oxysterol sensitive and resistant lymphoid cells: correlation with regulation of cellular nucleic acid binding protein mRNA. J Steroid Biochem 48:307–315
24. Lund EG, Kerr TA, Sakai J, Li W-P, Russell DW (1998) cDNA cloning of mouse and human cholesterol 25-hydroxylases, polytopic membrane proteins that synthesise a potent oxysterol regulator of lipid metabolism. J Biol Chem 273:34316–34327
25. Janowski BA, Willy PJ, Devi TR, Falck JR Mangelsdorf DJ (1996) An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. Nature 383:728–731
26. Harada K, Ishibashi S, Miyashita T, Osuga J, Yagyu H, Ohashi K, Yazaki Y, Yamada N (1997) Bcl-2 protein inhibits oxysterol-induced apoptosis through suppressing CPP32-mediated pathway. FEBS Letters 411:63–66
27. Lizard G, Lemarie S, Monier S, Gueldry S, Néel D, Gambert P (1997) Induction of apoptosis and of interleukin-1β secretion by 7β-hydroxycholesterol and 7-ketocholesterol: Partial inhibition by Bcl-2 overexpression. FEBS Letters 419:276–280