[Frontiers in Bioscience 12, 791-799, January 1, 2007]

Activation of the mitochondrial pathway of apoptosis by oxysterols

Gabriella Leonarduzzi, Giuseppe Poli, Barbara Sottero, and Fiorella Biasi

Department of Clinical and Biological Sciences of University of Turin, San Luigi Hospital, 10043 Orbassano, Turin, Italy

TABLE OF CONTENTS

1. ABSTRACT

A significant fraction of cholesterol that accumulates in atherosclerotic lesions is actually oxidized to yield a number of derivatives, named oxysterols, which are provided with much stronger biochemical effects than the parental compound. Of note, an increasing bulk of studies is giving evidence of accumulation of oxysterols in a number of other chronic disease processes including quite common neurodegenerative diseases. In particular, defined cholesterol oxidation products, among those of main interest in pathophysiology, may strongly activate the mitochondrial pathway of apoptotic death. Modulation by oxysterols of various pro- and anti-apoptotic molecules involved in that pathway are hereafter examined under the light of the most recent relevant literature.

2. INTRODUCTION

Oxysterols represent a family of 27-carbon cholesterol derivatives that may be absorbed with the diet or originated endogenously. Food products rich in cholesterol, such as powdered milk, cheese, meats and eggs can be susceptible to form products of cholesterol degradation and oxidation after prolonged storage or cooking (1-2). Exogenous oxysterols are completely absorbed from the bowel, cleared from plasma and taken up in different tissues and organs more rapidly than parental unoxidized cholesterol (3-4). Endogenous production of oxysterols may partly occur within tissues through a non enzymatic oxidation of cholesterol, in which are involved oxygen species reactions, or via enzymatic catalysis, the latter one representing for some oxysterols the only way of formation (5). It is now well accepted that these compounds are able to modulate various signaling pathways, by this way exerting a number of biochemical effects that include promotion of chronic inflammation, fibrosis and programmed cell death. Thus, oxysterols are now definitely considered as potentially involved in the pathogenesis of several chronic diseases occurring in humans (for review see 4-6).

Here we will rapidly comment on the possible pathogenetic implication of oxysterol molecules in neurodegenerative diseases and atherosclerosis, before going to the main aim of the present report, i.e. a review

Table 1. Summary of recent studies that quantified oxysterols in normal and atherosclerotic blood vessels

Sample	7alpha-OH	7beta-OH	7K	27-OH	Cholesterol	Unit	Analytical technique	Reference
Human carotid:								32
normal vessel	n.d.	0.2 ± 0.1	0.8 ± 0.7	n.d.	2200 ± 600	pmol/mg wet weight tissue	GC-MS	
lesion	n.d.	7.7 ± 3.9	35.9 ± 21.5	n.d.	40600 ± 28600			
Human carotid lesion:								33
alpha-Tocopherol supplementation	n.d.	437 ± 707	n.d.	n.d.	125000 ± 82000	pmol/mg wet weight tissue	GC-MS	
placebo	n.d.	420 ± 724	n.d.	n.d.	117000 ± 88000			
Human carotid lesion	n.d.	146 ± 88	322 ± 279	432 ± 323	93328 ± 45009	pmol/mg wet weight tissue	GC-FID	Sottero et al., unpublished data
Cholesterol fed rabbit aorta:								34
vitamin E supplemented diet	0.2 ± 0.04	0.3 ± 0.1	0.3 ± 0.1	n.d.	n.d.	mmol/mol cholesterol	HPLC-UV	
vitamin E normal diet	0.4 ± 0.1	0.6 ± 0.1	1.6 ± 0.5	n.d.	n.d.			
vitamin E deficient diet	1.3 ± 0.2	2.2 ± 0.3	7.2 ± 0.9	n.d.	n.d.			
Human aorta:								35
no lesions	n.d.	n.d.	30	20	10600	pmol/mg protein	HPLC-UV	
fatty streak lesions	n.d.	n.d.	50	290	28600			
fibro-fatty lesions	n.d.	n.d.	90	640	79900			
ulcerated/complicated lesions	n.d.	n.d.	140	960	92500			

7alpha-OH, 7alpha-hydroxycholesterol; 7beta-OH, 7beta-hydroxycholesterol; 7K, 7-ketocholesterol; 27-OH, 27-hydroxycholesterol; n.d., not detected; GC-MS, gas chromatography-mass spectrometry; GC-FID, gas chromatography-flame ionization detector; HPLC-UV, high-performance liquid chromatography-ultraviolet detection.

analysis of the pro-apoptotic effect of this class of compounds.

Indeed, a rapidly growing interest is focusing on the potential toxic role of oxysterols in central nervous system, the tissue with the relatively highest concentration of cholesterol in the body. Perturbation of cholesterol metabolism in central nervous system may be etiologically related to the onset of neurological diseases. Most of the cholesterol present in the brain derives from *de novo* synthesis because of the inability of plasma lipoproteins to cross the blood-brain barrier, and the fact that oxysterols represent the only way of cholesterol efflux to plasma. The major oxysterol involved in the regulation of that process is the 24S-hydroxycholesterol, produced only in the brain by cholesterol 24-hydroxylase. Changes of 24S-hydroxycholesterol plasma levels have been correlated with the progression of dementia in Alzheimer's disease (AD) (7). Recently, other oxysterols were found to be produced *in situ* or even flow from the circulation into the brain (8), and be metabolized in neurons and glial cells with the generation of other reactive hydroxylated cholesterol products (9).

It has been suggested that overproduction of oxysterols in the brain could represent a risk factor for AD development: in this relation, a link between neuronal sites of cholesterol 24- and 27-hydroxylase expression and amyloid beta-peptide (Abeta) formation and accumulation was observed. Furthermore, oxysterols are able to regulate the processing of beta-amyloid protein precursor (APP) (10) and cholesterol accumulates in mature plaques in the brain of AD patients as well as in the brain of APP transgenic mice (11).

A rapidly increasing body of literature deals with the association between oxysterols and the vascular wall impairment of atherosclerosis (12). During atherogenesis, local oxidative degradation of LDL may generate lipid-derived bioactive molecules, such as oxysterols, peroxidized fatty acids and lysophospholipids; the accumulation of these compounds within the vascular intima is considered to be involved in the recruitment of circulating monocytes into the subendothelial space with their transformation into macrophages, and the subsequent uptake of modified lipoprotein particles and formation of foam cells. These lipid-filled cells undergo apoptosis leading to an abnormal accumulation of cell debris in the lesion that contribute to the formation of an unstable plaque and to atherothrombosis (13-14). Among the different lipid oxidation products which are present in the unstable atherosclerotic plaque, there are oxysterols which tend to accumulate in the core region of the lesion (15-16). Of note, the available data on oxysterol content of the plaques are quite inconsistent. This fact may depend on the stage development of the plaque, as well as on the great variability of fat deposition in different lesions even of the same individual, but also on the sensitivity of the analytical technique employed. However, despite quantitative discrepancies, the same trend is observed in all studies, which report 27-hydroxycholesterol, 7-ketocholesterol and 7beta-hydroxycholesterol as the most abundant oxysterols in atherosclerotic lesions. The most recent analyses of oxysterol content in atherosclerotic plaques (17-20) are grouped in Table 1, while for less recent data one should refer to the review of Brown and Jessup (15). Most likely, oxysterols provide a primary contribution to the whole atherogenetic process not only through the activation of pro-inflammatory and pro-fibrogenic pathways, but also by sustaining the overexpression of the apoptotic death of vascular cells, especially in the advanced stages of the atherogenic process (21-26).

Understanding the main mechanisms by which these agents can induce apoptosis in mammalian cells could contribute to modulate cell death and prevent lesion development. Of the two main pathways of programmed cell death we will focus our attention on that involving mitochondria.

3. THE MITOCHONDRIAL PATHWAY OF APOPTOSIS

Programmed cell death occurs during physiological organism development, during maintenance of tissue homeostasis throughout adult life and when cells are severely damaged as in the case of cancer, autoimmune disorders, neurodegenerative and cardiovascular diseases. This process is a genetically controlled cellular self-destruction in response to a wide range of endogenous or exogenous stimuli which lead to cell shrinkage, membrane blebbing and chromatin condensation.

Apoptosis may be generated by signals arising within the cell, or triggered by death activators which bind to surface receptors, as in the case of TNF-alpha, lymphotoxin, Fas ligand (FasL). In response to all these signals, apoptosis proceeds either through the receptor-mediated pathway (extrinsic path) or through the mitochondrial pathway (intrinsic path) centered on the mitochondrial outer membrane permeabilization. The intrinsic pathway of apoptosis is regulated by a number of molecules which are members of Bcl-2 family: the actual balance between anti-apoptotic Bcl-2 and Bcl-x_L, and pro-apoptotic homologues Bax, Bad, Bim, regulates the mitochondrial membrane permeability.

Mitochondria of normal cells mainly express on outer membrane the anti-apoptotic proteins Bcl-2 and Bcl-x_L which inhibit the opening of mitochondrial permeability transition pore which regulates the flux of ions in and out of mitochondria. In the first steps of activation of the intrinsic pathway of apoptosis, pro-apoptotic proteins translocate from the cytoplasm to the mitochondrial membrane where interact with anti-apoptotic Bcl-2 homologues to antagonise their function; insertion of these proteins into the mithocondrial membrane increases its permeability by opening the pore that allows release of proteins of the mitochondrial intermembrane, including cytochrome c into the cytosol. In response to apoptotic stimuli, the cytosolic inactive Bax undergoes conformational changes that trigger its translocation to mitochondria and its interaction with Bcl-2 or Bcl-x_L; several Bax-binding proteins with caspase recruitment domains (CARD) are involved in this process, as Bax inhibitors (*i.e.* ARC: apoptosis repressor with CARD), or activators (*i.e.* ASC: apoptosis-associated speck-like protein containing a CARD); in addition Bax and ASC are both regulated by pro-apoptotic protein p53 (27).

Bad is regulated by maintenance of phosphorylated serine residues (Ser 112, Ser 136, and Ser 155) in which a variety of survival kinases are involved. Dephosphorylation at these sites enables Bad to bind anti-apoptotic Bcl-2 proteins and induce their oligomerization (28).

Bim is sequestered within dynein cytoskeleton motor complex away from the mitochondria. In response to apoptotic stimuli the bond with cytoskeleton is disrupted and Bim becomes free to translocate to Bcl-2 (29). Emerging studies suggest the regulation of Bim activity through different mechanisms: by its release through phosphorylation and proteosomal degradation, or by a rapid *de novo* expression and synthesis. All these pro-apoptotic proteins contribute to the destabilization of mitochondrial membrane integrity that eventually leads to alteration of ion exchange and loss of cytochrome c.

Once released from mitochondria, cytochrome c binds to the protein Apaf-1 (apoptotic protease activating factor-1); the presence of ATP mediates a conformational change of Apaf-1, promoting its oligomerization and formation of the apoptosome. The apoptosome binds to and activates caspase-9, which, in turn, cleaves and activates the executioner caspase-3. The onset of the proteolytic cascade leads to digestion of cytoplasmic structural proteins, degradation of chromosomal DNA and eventually activation of cell phagocytosis.

Of note, especially in the early steps of activation of the intrinsic pathway of apoptosis, modulation of both cytosolic and stored intracellular Ca^{2+} appears central. Under apoptotic stimulus, calcium is released from endoplasmic reticulum (ER) stores and is taken up by mitochondria. Then, rise of mitochondrial Ca^{2+} can directly induce swell and rupture of the subcellular organelle and indirectly stimulate Ca^{2+}-dependent enzymes that engage other apoptotic mechanisms. Bcl-2 is also present in the ER where likely represents one major factor able to maintain ER calcium homeostasis. Conversely, the overexpression of pro-apoptotic Bax and Bak, also localized to the ER, favors endoplasmic Ca^{2+} release which is followed by increase of mitochondrial Ca^{2+} and cytochrome c release (30-31).

4. PRO-APOPTOTIC EFFECT OF OXYSTEROLS OF PATHOPHYSIOLOGICAL RELEVANCE

Over the last decade, reliable *in vitro* studies characterized the potential pro-apoptotic effect of the major oxysterols with regard to vascular cells, namely smooth muscle cells, endothelial cells, fibroblasts, and monocyte-macrophages.

Nishio and Watanabe (32) showed that both 7-ketocholesterol and 25-hydroxycholesterol (30 microM) induce apoptosis in rabbit cultured smooth muscle cells (SMCs). Lizard and colleagues confirmed the effect of 7-ketocholesterol, using cultured SMCs obtained from human artery, and also tested 7beta-hydroxycholesterol. In their experimental model, 7beta-hydroxycholesterol displayed pro-apoptotic effects at final concentrations of 20-30 microM or above, whereas 7-ketocholesterol showed induced apoptosis at 40-60 microM final concentration (33). Similar concentrations of the two oxysterols triggered apoptotic death also in human umbilical vein endothelial cells (HUVECs) (33). With regard to cells of the macrophage lineage, Aupeix and colleagues showed that apoptosis occurred shortly after treatment of human monocytic cell lines with either 25-hydroxycholesterol or 7beta-hydroxycholesterol, at final concentrations of 20 to 30 microM or above (34). Treating U937 human promonocytic cells (35) or murine J774A.1 macrophages with 7-ketocholesterol (25), a significant pro-apoptotic

effect of that cholesterol oxide was evident between 10 and 30 microM.

Therefore, based on the presently available data, oxysterols are able to activate programmed cell death in various vascular cell types. The only apparent exception were the fibroblasts of vascular origin, that underwent necrosis in the presence of the same oxysterol concentrations (20 to 50 microM) able to induce apoptosis in the other cell types of the arterial wall (33). By reviewing the available literature on oxysterol-dependent cytotoxicity we are developing the opinion that, in general terms, defined concentrations of oxysterols lead vascular cells to apoptosis, while relatively higher amounts induce straight necrosis. Our own experience is definitely confirming this point of view. In fact, already at the concentration of 30 microM, 7-ketocholesterol increases the percentage of J774A.1 macrophages which take up Trypan Blue dye, a clear index of necrosis (Biasi et al., unpublished data). Consistently, Lizard and colleagues, treating BAE endothelial cells with 7-ketocholesterol or 7beta-hydroxycholesterol (50 microM and 24.8 microM respectively) showed both apoptosis and necrosis, with the latter becoming predominant and eventually main death pathway at relatively higher concentrations (36).

In a very recent and comprehensive study, the group of Lizard provided clear biochemical and morphological evidence of two different types of cell death being inducible by oxysterols of pathophysiologic interest. Namely, human promonocytic cells U937 were challenged up to 24 hours with relatively high concentrations of 7-ketocholesterol (100 microM) and 7beta-hydroxycholesterol (50 microM) and a strong activation of both extrinsic and intrinsic pathways of apoptosis was demonstrated in terms of net activation of caspases 3, 7, 8 and 9. With regard to the mitochondrial path, both oxysterols significantly induced release of cytochrome c and degradation of cytosolic Bid, being the effect of 7beta -hydroxycholesterol much stronger than that of 7-ketocholesterol. The inhibition of caspase 3-dependent death pathway in U937 cells or, alternatively, the oxysterol challenge of caspase-3 null MCF-7 cells, did not preserve cells from death, but in this case mainly cells with swollen or punctuated nuclei were observed being those with fragmented and/or condensed nuclei less than 1%, to indicate that oxysterols may induce two different types of cell death, a caspase 3-dependent one (apoptosis) and a caspase-3 independent one (oncosis) (37).

Another research group challenged cultivated rabbit aortic smooth muscle cells with 7-ketocholesterol in the 25-50 microM concentration range and showed the oxysterol as able to early up-regulate protein Bax levels and its translocation from cytosol into the mitochondria, to induce marked mitochondrial cytochrome c release and eventually lead to cell loss. Of interest, at least in SMCs, cytochrome c release from mitochondria appeared the consequence of a reversible change of the outer membrane rather than due to a no return rupture of it. In fact, when after 16 hours cultivation in the presence of 25 microM 7-ketocholesterol the sterol oxide was removed and SMCs were incubated in the presence of fetal bovine serum, cytosolic dispersion of cytochrome c was not any more evident and cell loss slowed down significantly (38).

5. PRO- AND ANTI-APOPTOTIC SIGNALING TRIGGERED BY OXYSTEROLS

Still in relation to 7-ketocholesterol-induced apoptosis in cells of the macrophage lineage, the group of Lizard showed how complicated this process is, being simultaneously triggered more than one signaling pathway. This group suggests with reliable experimental data that the very initial event is represented by a significant increase of cytosolic free Ca^{2+} which in turn start up at least three biochemical processes: 1) the activation of calcineurin, a calcium-regulated phosphatase, by translocation of Trpc-1, a component of the store-operated Ca^{2+} entry channel, into lipid raft domains; this event would lead to apoptosis through dephosphorylation of the pro-apoptotic protein Bad (39); 2) the translocation of the pro-apoptotic protein Bim from the microtubule dynein motor complex to mitochondria, and thus its interaction with Bcl-2 complex, inhibition of this anti-apoptotic element and favoring effect on cytochrome c release from mitochondria (40); 3) the involvement of the proline rich tyrosine kinase-2 (PYK-2) which transduces survival signals through the activation of mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) kinase (MEK)/ERK pathway, followed by inactivation of Bad through its phosphorylation in serine 75 and consequent delay of the apoptotic program (40). Maybe of interest, all this findings were obtained by treating the human monocytic line THP-1 with a relatively high concentration of 7-ketocholesterol (100 μM) and dose-dependent data were not provided.

Indeed, an early increase of cytosolic Ca^{2+} as induced by oxysterols was also observed by Ares and colleagues following *in vitro* challenge of human aortic smooth muscle cells with 7beta-hydroxycholesterol 24.8 microM (41). The downregulation of the anti-apoptotic protein Bcl-2 that is induced by 7beta-hydroxycholesterol, 25-hydroxycholesterol, and 7-ketocholesterol in different cell models, with consequent activation of the mitochondrial pathway of programmed cell death, has been reported (32,42-43). Now, on the basis of the results obtained by Berthier and colleagues, one should definitely consider the oxysterol-induced interaction of Bim with Bcl-2 as a/the mechanism of Bcl-2 inactivation by cholesterol oxidation products in pro-apoptotic concentrations. But the most striking finding obtained by Berthier et al. (40) is, in our opinion, the unexpected activation of a survival pathway by 7-ketocholesterol, besides the two, actually prevailing, pro-apoptotic pathways. Indeed, the activation of MEK/ERK pathway by oxysterols could be crucial in providing the cells with a possibly valid anti-apoptotic mechanism, whose presently elucidated aspects are depicted in Figure 1. A more valid activation of ERK-dependent survival mechanism, or better apoptosis-delay mechanism, likely occurs in cells of the macrophage lineage (U937 and J774A.1) treated with a pathophysiologically relevant oxysterol mixture instead of 7-ketocholesterol alone.

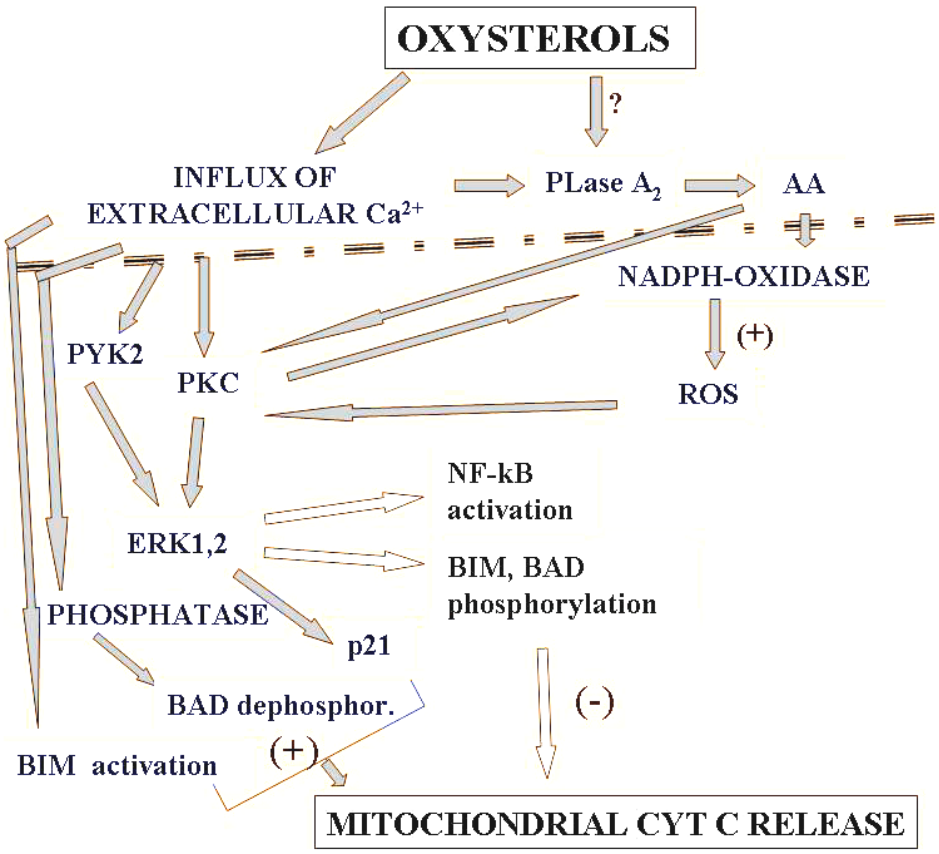

Figure 1. Modulation of cytochrome c release by oxysterols: main signaling pathways likely involved in the process. White arrows: survival signaling. Grey arrows: pro-apoptotic signaling.

In this relation, a very interesting point has recently been stressed, namely the relatively lower cytotoxicity of oxysterols when given to cell models as a mixture rather than as individual compounds. Oxysterols are always present as a mixture, in foods, oxidized LDL, or in the core region of atherosclerotic plaque, and molecular interactions often occur among mixed compounds. Starting from this evidence, we analyzed the effect of 7-ketocholesterol on murine J774A.1 macrophages, in the presence or in the absence of equimolar concentrations of 7beta-hydroxycholesterol, in terms of reactive oxygen species (ROS) generation, cytochrome c morphological appearance of condensed nuclei and apoptotic bodies. All events along the mitochondrial apoptotic pathway triggered by 7-ketocholesterol were significantly quenched when cells were co-treated with identical amounts of the second sterol oxide (25). In our opinion, the quenching effect on 7-ketocholesterol-induced apoptosis that we observed occurred chiefly at the level of ROS production. Indeed, the concomitant addition of 7beta-hydroxycholesterol strongly inhibited a pathobiological rise of intracellular ROS that had been induced by 7-ketocholesterol through a marked upregulation of constitutive NADPH oxidase activity (25,44). We hypothesized that the concomitant addition of 7 beta-hydroxycholesterol, which also binds to NADPH oxidase but apparently less efficiently, likely reduced the concentration of free enzyme available for 7-ketocholesterol binding. Now, on the basis of the clear findings by Berthier and colleagues about oxysterol-inducible survival pathway involving ERK activation (40), another mechanism by which oxy-mixtures would be able to delay programmed cell death would actually imply a strong upregulation of ERK. This particular aspect is presently under investigation together with the possible involvement in the survival pathway also of NF-kB, whose nuclear binding was actually up-regulated by oxysterol mixture (45) (Figure 1).

Thus, besides modulation of intracellular Ca^{2+} another early biochemical event consistently observed in monocytic cells challenged with various oxysterols was a significant upregulation of NADPH oxidase enzyme and, as a direct consequence, upregulation of intracellular steady-state levels of ROS (25,46-47). Upregulation of NADPH oxidase in oxysterol-treated monocytic cells is likely the consequence of Ca^{2+}-activated phospholipase A_2 followed by arachidonic acid release (46-47). Further, indirect confirmation of a primary role of oxidative imbalance of the cell redox equilibrium in oxysterol-induced apoptosis comes from the significant protection against this event that occurs when cells are pretreated with antioxidants or with selective NADPH oxidase inhibitors (44). With regard to the steps between oxysterol-induced ROS overproduction and changes in the expression of genes related to apoptosis,

a very recent literature is providing some important evidence. Overproduction of ROS induced by 1-(2-hydroxy-5-methylphenyl)-3-phenyl-1,3-propanedione (HMDB) in human A431 cancer cells triggered marked apoptosis apparently through the induction of DNA damage followed by activation of growth arrest DNA damage153 gene (GADD153), then by upregulation of Bad and p21 and downregulation of Bcl-2 (48). Treating neuroblastoma cells with the synthetic retinoid fenretinide, Lovat and colleagues showed that the oxidative stress-dependent induction of the transcription factor GADD153 was leading to apoptosis through the upregulation of p21 gene and protein (49). However, no literature is yet available about possible effect of cholesterol oxidation products on GADD153, while net upregulation of p21 by 7-ketocholesterol was already observed in J774A.1 cells (25). Another way, not necessarily alternative, of p21 upregulation by ROS overproduction was described as involving ERK cascade, a pathway actually modulated by oxysterols. Oxidative stress induced in human monocytic THP-1 cells by challenge with phorbol myristate acetate was able to trigger programmed death through PKCs (protein kinase C) and MAPKs (mitogen-activated protein kinases) activation up to p21 upregulation (50). Consequent PKCs and ERK activation, p21 induction, cell growth arrest was also observed in IEC-18 non transformed intestinal crypt cells (51). The requirement of enhanced ERK phosphorylation for p21 induction and consequent apoptosis was finally suggested by a very recent study of TGFbeta1 upregulation of p21 in human keratinocytes (52).

This last series of papers supporting the involvement of ERK activation in ROS-triggered mitochondrial pathway of apoptosis did not consider oxysterols, but actually contribute to make the overall picture quite complicated. In fact, 7-ketocholesterol at least is able to induce intracellular rise of both free Ca^{2+} and ROS steady-state levels, early after cell challenge. The signaling pathways thus activated likely involve modulation of PKCs, MAPKs, and key molecules of the Bcl family.

6. CONCLUSIONS AND PERSPECTIVES

The actual modulation of the balance between anti-apoptotic and pro-apoptotic Bcl family components by defined oxysterols may vary with cell type, oxide concentration and mixture, time, etc., so that it seems impossible but also illogical trying to make unique statements and definition, at least before more comprehensive information and evidence will become available.

Presently, we may conclude that certain oxysterols of primary interest in pathophysiology are certainly able, at concentrations recovered both in plasma of hypercholesterolemic patients and in atherosclerotic lesions, to trigger and sustain the mitochondrial pathway of apoptosis through a complex induction of pro-apoptotic signaling pathways. However, both intensity and duration of programmed cell death inducible by oxysterols appears easily modulated by the simultaneous property that these compounds retain to regulate and possibly activate also anti-apoptotic biochemical processes.

7. ACKNOWLEDGMENTS

The research from our laboratory, quoted and/or discussed in the present review, has been supported by grants from the Italian Ministry of University, Rome, Italy (PRIN 2003, 2004; FIRB 2002); Region of Piedmont, Italy; University of Turin, Italy; Fondazione Monzino, Milano, Italy; Compagnia di San Paolo, Turin, Italy.

8. REFERENCES

1. A. I. Rey, J. P. Kerry, P. B. Lynch, C. J. Lopez-Bote, D. J. Buckley and P. A. Morrissey: Effect of dietary oils and alpha-tocopheryl acetate supplementation on lipid (TBARS) and cholesterol oxidation in cooked pork. *J Anim Sci* 79, 1201–1208 (2001)

2. J.T. Chien, D.Y. Huang and B.H. Chen: Kinetic studies of cholesterol oxidation as inhibited by stearylamine during heating. *J Agric Food Chem* 52, 7132-7138 (2004)

3. L.H. Krut, W. Joseph, J.W. Yang, G. Schonfeld and R.E. Ostlund Jr.: The effect of oxidizing cholesterol on gastrointestinal absorption, plasma clearance, tissue distribution, and processing by endothelial cells. *Arterioscler Thromb Vasc Biol* 17, 778-785 (1997)

4. G. J. Schroepfer Jr.: Oxysterols: modulators of cholesterol metabolism and other processes. *Physiol Rev* 80, 361-554 (2000)

5. G. Leonarduzzi, B. Sottero and G. Poli: Oxidized products of cholesterol: dietary and metabolic origin, and proatherosclerotic effects. *J Nutr Biochem* 13, 700-710 (2002)

6. G. Leonarduzzi, B. Sottero, V. Verde and G. Poli: Oxidized products of cholesterol: toxic effect. In: Reviews in food and nutrition toxicity. Eds. Preedy VR, Watson RR, *CRC Press, Taylor & Francis Group*, Boca Raton, FL 3, 129-164 (2005)

7. I. Björkhem and S. Meaney: Brain cholesterol: long secret life behind a barrier. *Arterioscler Thromb Vasc Biol* 24, 806-815 (2004)

8. M. Heverin, S. Meaney, D. Lütjohann, U. Diczfalusy, J. Wahren and I. Björkhem: Crossing the barrier: net flux of 27-hydroxycholesterol into the human brain. *J Lipid Res* 46, 1047–1052 (2005)

9. J. Zhang, Y. Akwa, M. El-Etr, E.-E. Baulieu and J.Sjövall: Metabolism of 27-, 25- and 24-hydroxycholesterol in rat glial cells and neurons. *Biochem J* 322, 175-184 (1997)

10. J. Brown III, C. Theisler, S. Silberman, D. Magnuson, N. Gottardi-Littell, J.M. Lee, D. Yager, J. Crowley, K. Sambamurti, M.M. Rahman, A.B. Reiss, C.B. Eckman and

B. Wolozin: Differential expression of cholesterol hydroxylases in Alzheimer's disease. *J Biol Chem* 279, 34674–34681 (2004)

11. T. Mori, D. Paris, T. Town, A.M. Rojiani, D.L. Sparks, A. Delledonne, F.Crawford, L.I. Abdullah, J.A. Humphrey, D.W. Dickson and M. Mullan: Cholesterol accumulates in senile plaques of Alzheimer disease patients and in transgenic APP(SW) mice. *J Neuropathol Exp Neurol* 60, 778-785 (2001)

12. G. Poli, G. Leonarduzzi, F. Biasi and E. Chiarpotto: Oxidative Stress and Cell Signalling *Curr Med Chem* 11, 1023-1042 (2004)

13. R. Salvayre, N. Auge, H. Benoist and A. Negre-Salvayre: Oxidized low-density lipoprotein-induced apoptosis. *Biochim Biophys Acta* 1585, 213-221 (2002.

14. Z. Mallat and A. Tedgui: Apoptosis in the vasculature: mechanisms and functional importance. *Br J Pharmacol* 130, 947-962 (2000)

15. A.J. Brown and W. Jessup: Oxysterols and atherosclerosis. *Atherosclerosis* 142, 1–28 (1999)

16. S. Garcia-Cruset, K.L. Carpenter, F. Guardiola and M.J. Mitchinson: Oxysterols in cap and core of human advanced atherosclerotic lesions. *Free Radic Res* 30, 341-350 (1999)

17. J.M. Upston, P.K. Witting, A.J. Brown, R. Stocker and J.F. Keaney Jr.: Effect of vitamin E on aortic lipid oxidation and intimal proliferation after arterial injury in cholesterol-fed rabbits. *Free Radic Biol Med* 31, 1245-1255 (2001)

18. J.M. Upston, X. Niu, A.J. Brown, R. Mashima, H. Wang, R. Senthilmohan, A.J. Kettle, R.T. Dean and R. Stocker: Disease stage-dependent accumulation of lipid and protein oxidation products in human atherosclerosis. *Am J Path* 160, 701-710 (2002)

19. K.H.L. Carpenter, P.J. Kirkpatrick, P.L. Weissberg, I.R. Challis, I.F. Dennis, M.A. Freeman and M.J. Mitchinson: Oral alpha-tocopherol supplementation inhibits lipid oxidation in established human atherosclerotic lesions. *Free Radic Res* 37, 1235-1244 (2003)

20. M. Iuliano, F. Micheletta, S. Natoli, S. Ginanni Corradini, M. Iappelli, W. Elisei, L. Giovannelli, F. Violi and U. Diczfalusy: Measurement of oxysterols and alpha-tocopherol in plasma and tissue samples as indices of oxidant stress status. *Anal Biochem* 312, 217-223 (2003)

21. Y. J. Geng and P. Libby: Progression of atheroma. A struggle between death and procreation. *Arterioscler Thromb Vasc Biol* 22, 1370-1380 (2002)

22. A. Meynier, A. Andre, J. Lherminier, A. Grandgirard and L. Demaison: Dietary oxysterols induce in vivo toxicity of coronary endothelial and smooth muscle cells. *Eur J Nutr* 44, 393-405 (2005)

23. C.J. Choy, J. David, J. Granville, D.W.C. Hunt and B.M. McManus: Endothelial cell apoptosis: biochemical characteristics and potential implications for atherosclerosis. *J Mol Cell Cardiol* 33, 1673–1690 (2001)

24. S.R. Panini, L. Yang, A.E. Rusinol, M.S. Sinensky, J.V. Bonventre and C.C. Leslie: Arachidonate metabolism and the signaling pathway of induction of apoptosis by oxidized LDL/oxysterol. *J Lipid Res* 42, 1678-1686 (2001)

25. F. Biasi, G. Leonarduzzi, B. Vizio, D. Zanetti, A. Sevanian, B. Sottero, V. Verde, B. Zingaro, E. Chiarpotto and G. Poli: Oxysterol mixtures prevent pro-apoptotic effects of 7-ketocholesterol in macrophages: implications for proatherogenic gene modulation. *FASEB J* 18, 693-695 (2004)

26. A.E. Rusiñol, D. Thewke, J. Liu, N. Freeman, S.R. Panini and M.S. Sinensky: AKT/protein kinase B regulation of BCL family members during oxysterol-induced apoptosis. *J Biol Chem* 279, 1392–1399 (2004)

27. T. Ohtsuka, H. Ryu, Y.A. Minamishima, S. Macip, J. Sagara, K.I. Nakayama, S. A. Aaronson and S.W. Lee: ASC is a Bax adaptor and regulates the p53-Bax mitochondrial apoptosis pathway. *Nat Cell Biol* 6, 121-128 (2004)

28. S.R. Datta, A.M. Ranger, M.Z. Lin, J.F. Sturgill, Y.C. Ma, C.W. Cowan, P. Dikkes, S.J. Korsmeyer and M.E. Greenberg: Survival factor-mediated BAD phosphorylation raises the mitochondrial threshold for apoptosis. *Dev Cell* 3, 631-643 (2002)

29. H. Puthalakath, D.C. Huang, L.A. O'Reilly, S.M. King and A. Strasser: The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex. *Mol Cell* 3, 287-296 (1999)

30. S.A. Oakes, J.T. Opferman, T. Pozzan, S.J. Korsmeyer and L. Scorrano: Regulation of endoplasmic reticulum Ca^{2+} dynamics by proapoptotic BCL-2 family members. *Biochem Pharmacol* 66, 1335-1340 (2003)

31. C. W. Distelhorst and G.C. Shore: Bcl-2 and calcium: controversy beneath the surface. *Oncogene* 23, 2875-2880 (2004)

32. E. Nishio and Y. Watanabe: Oxysterols induced apoptosis in cultured smooth muscle cells through CPP32 protease activation and bcl-2 protein downregulation. *Biochem Biophys Res Commun* 226, 928–934 (1996)

33. G. Lizard, S. Monier, C. Cordelet, L. Gesquiere, V. Deckert, S. Gueldry, L. Lagrost, P. Gambert: Characterization and comparison of the mode of cell death, apoptosis versus necrosis, induced by 7beta-hydroxycholesterol and 7-ketocholesterol in the cells of the vascular wall. *Arterioscler Thromb Vasc Biol* 19, 1190–1200 (1999)

34. K. Aupeix, D. Weltin, J.E. Mejia, M. Christ, J. Marchal, J.M. Freyssinet, and P. Bischoff: Oxysterol-induced apoptosis in human monocytic cell lines. *Immunobiol* 194, 415–428 (1995)

35. G. Leonarduzzi, A. Sevanian, B. Sottero, M.C. Arkan, F. Biasi, E. Chiarpotto, H. Basaga and G. Poli: Up-regulation of the fibrogenic cytokine TGF-beta1 by oxysterols: a mechanistic link between cholesterol and atherosclerosis. *FASEB J* 15, 1619–1621 (2001)

36. G. Lizard, V. Deckert, L. Dubrez, M. Moisant, P. Gambert and L. Lagrost: Induction of apoptosis in endothelial cells treated with cholesterol oxides. *Am J Pathol* 148, 1625–1638 (1996)

37. C. Prunet, S. Lemaire-Ewing, F. Menetrie, D. Neel and G. Lizard: Activation of caspase-3-dependent and –independent pathways during 7-ketocholesterol- and 7beta-hydroxycholesterol-induced cell death: a morphological and biochemical study. *J Biochem Mol Toxicol* 19, 311–326 (2005)

38. C.I. Seye, M.W.M. Knaapen, D. Daret, C. Desgranges, A.G. Herman, M.M. Kockx and H. Bult: 7-Ketocholesterol induces reversible cytochrome c release in smooth muscle cells in absence of mitochondrial swelling. *Cardiovasc Res* 64, 144-153 (2004)

39. A. Berthier, S. Lemaire-Ewing, C. Prunet, S. Monier, A. Athias, G. Bessede, J.P. Pais De Barros, A. Laubriet, P. Gambert, G. Lizard and D. Néel: Involvement of a calcium-dependent dephosphorylation of BAD associated with the localization of Trpc-1 within lipid rafts in 7-ketocholesterol-induced THP-1 cell apoptosis. *Cell Death Differ* 11, 897–905 (2004)

40. A. Berthier, S. Lemaire-Ewing, C. Prunet, T. Montange, A. Vejux, J.P. Pais de Barros, S. Monier, P. Gambert, G. Lizard and D. Néel : 7-Ketocholesterol-induced apoptosis. Involvement of several pro-apoptotic but also anti-apoptotic calcium-dependent transduction pathways. *FEBS J* 272, 3093–3104 (2005)

41. M.P. Ares, M.I. Porn-Ares, S. Moses, J. Thyberg, L. Juntti-Berggren, P. Berggren, A. Hultgardh-Nilsson, B. Kallin and J. Nilsson: 7beta-hydroxycholesterol induces Ca^{2+} oscillations, MAP kinase activation and apoptosis in human aortic smooth muscle cells. *Atherosclerosis* 153, 23–35 (2000)

42. G. Lizard, S. Lemaire, S. Monier, S. Gueldry, D. Neel and P. Gambert: Induction_of apoptosis and of interleukin-1beta secretion by 7beta- hydroxycholesterol and 7-ketocholesterol: partial inhibition by Bcl-2 overexpression. *FEBS Lett* 419, 276-280 (1997)

43. M. Harada-Shiba, M. Kinoshita, H. Kamido and K. Shimokado: Oxidized low density lipoprotein induces apoptosis in cultured human umbilical vein endothelial cells by common and unique mechanisms. *J Biol Chem* 273, 9681-9687 (1998)

44. G. Leonarduzzi, B. Vizio, B. Sottero, V. Verde, P. Gamba, C. Mascia, E. Chiarpotto, G. Poli and F. Biasi: Early involvement of ROS overproduction in apoptosis induced by 7-ketocholesterol. *Antioxid Redox Signal* 8, 375-380 (2006)

45. G. Leonarduzzi, P. Gamba, B.Sottero, A. Kadl, F. Robbesyn, R.A. Calogero, F. Biasi, E. Chiarpotto, N. Leitinger, A. Sevanian and G. Poli: Oxysterol-induced up-regulation of MCP-1 expression and synthesis in macrophage cells. *Free Radic Biol Med* 39, 1152-1161 (2005)

46. M. Rosenblat, P. Belinky, J. Vaya, R. Levy, T. Hayek, R. Coleman, S. Merchav and M. Aviram: Macrophage enrichment with the isoflavan glabridin inhibits NADPH oxidase-induced cell-mediated oxidation of low density lipoprotein. A possible role for protein kinase C. *J Biol Chem* 274, 13790–13799 (1999)

47. M. Rosenblat and M. Aviram: Oxysterol-induced activation of macrophage NADPH oxidase enhances cell-mediated oxidation of LDL in the atherosclerotic apolipoprotein E deficient mouse: inhibitory role for vitamin E. *Atherosclerosis* 160, 69–80 (2002)

48. M.H. Pan, Y.H. Sin, C.S. Lai, Y.J. Wang, J.K. Lin, M. Wang and C.T. Ho: Induction of apoptosis by 1-(2-hydroxy-5-methylphenyl)-3-phenyl-1,3-propanedione through reactive oxygen species production, GADD153 expression, and caspases activation in human epidermoid carcinoma cells. *J Agric Food Chem* 53, 9039-9049 (2005)

49. P.E. Lovat, M. Corazzari, B. Goranov, M. Piacentini and C.P. Redfern: Molecular mechanisms of fenretinide-induced apoptosis of neuroblastoma cells. *Ann N Y Acad Sci* 1028, 81-89 (2004)

50. K. Traore, M.A. Trush, M. Jr. George, E.W. Spannhake, W. Anderson and A. Asseffa: Signal transduction of phorbol-12-myristate 13-acetate (PMA)-induced growth inhibition of human monocytic leukaemia THP-1 cells is reactive oxygen dependent. *Leuk Res* 29, 863-829 (2005)

51. J.A. Clark, A.R. Black, O.V. Leontieva, M.R. Frey, M.A. Pysz, L. Kunneva, A. Woloszynska-Read, D. Roy and J.D. Black: Involvement of ERK signalling cascade in protein kinase C-mediated cell cycle arrest in intestinal epithelial cells. *J Biol Chem* 279, 9233-9247 (2004)

52. Y.K. Kim, G.U. Bae, J.K. Kang, J.W. Park, E.K. Lee, H.Y. Lee, W.S. Choi, H.W. Lee and J.W. Han: Cooperation of H_2O_2-mediated ERK activation with Smad pathway in TGF-beta1 induction of $p21^{WAF1/Cip1}$. *Cell Signal* 18, 236-243 (2006)

Key Words: Oxysterols, Mitochondria, Cell Signaling, Apoptosis, Cholesterol, Review

Send correspondence to: Professor Giuseppe Poli, Dept. of Clinical and Biological Sciences, University of Torino, San Luigi Hospital,10043 Orbassano, Torino, Italy, Tel: 39-011-6705401, Fax: 39-011-2365401, E-mail: giuseppe.poli@unito.it

http://www.bioscience.org/current/vol12.htm