Biochemical and Biophysical Research Communications 446 (2014) 709–713

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Review

Oxysterols and symptomatic versus asymptomatic human atherosclerotic plaque

Soliman Khatib, Jacob Vaya *

Department of Oxidative Stress and Human Diseases, MIGAL-Galilee Research Institute, P.O. Box 831, Kiryat Shmona 11016, Israel
Tel-Hai College, Upper Galilee 12210, Israel

ARTICLE INFO

Article history:
Received 19 December 2013
Available online 3 January 2014

Keywords:
Asymptomatic
Atherosclerosis
Oxysterols
Plaque
Symptomatic

ABSTRACT

Atherosclerosis is the most common cause of mortality in the Western world, contributing to about 50% of all deaths. Atherosclerosis is characterized by deposition of lipids onto the coronary or carotid arterial wall and formation of an atherosclerotic plaque. Atherosclerotic plaques are categorized into two groups: symptomatic and asymptomatic. The symptomatic plaques tend to be unstable and prone to rupture, and are associated with an increase in ischemic events. Oxysterols, products of cholesterol oxidation, are cytotoxic materials. Their level and type may be associated with plaque formation, development and stability. Oxysterols stimulate the formation of foam cells, advance atherosclerotic plaque progression, and contribute to plaque vulnerability and instability due to their cytotoxicity and their ability to induce cell apoptosis. Studies indicate that plasma 7β-OH CH level can be used as a biomarker for detecting carotid and coronary artery disease. Further clinical studies are needed to evaluate the potential of oxysterols for use as biomarkers for plaque vulnerability and instability. The identification of biomarkers in the blood that can distinguish between symptomatic and asymptomatic plaques remains an unresolved issue.

Contents

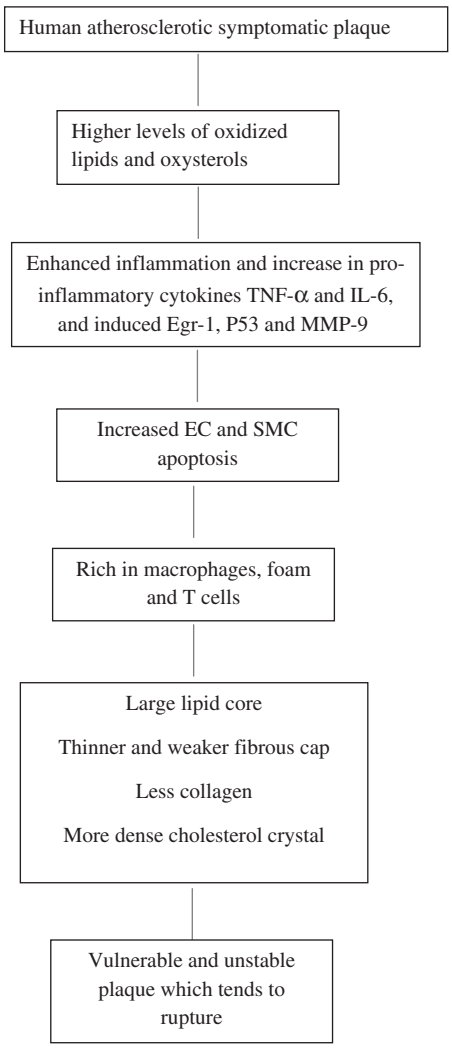
1. Introduction

Atherosclerosis is a multifactorial disease and a major cause of morbidity and mortality in the Western world. It is a pathological process characterized by the deposition of lipids and compounds on the inner arterial wall, forming plaques [1–5]. Oxidative stress (OS) is believed to play a significant role in the initiation and progression of atherosclerosis [6]. It is postulated that this is primarily mediated through the oxidation of low-density lipoprotein (Ox-LDL) and other molecules, such as lipids, proteins, and DNA [5,7–9]. The human atherosclerotic plaque is characterized by increased levels of oxidized lipoproteins, such as LDL, HDL, phospholipids, triglycerides [10,11], oxidized cholesterol products (oxysterols) [12], free fatty acids, and fatty acid derivatives [13], as well as proteins such as fibrinogen, apolipoprotein A-I, clusterin, and paraoxonases (PONs) [14,15]. Atherosclerotic plaques can be categorized as stable (asymptomatic) or unstable (symptomatic, vulnerable), the latter being more prone to rupture, characterized by a large lipid core and a thin fibrous cap containing less collagen, and associated with an increase in ischemic events. Lipid-rich plaques are more often associated with symptomatic plaques (Scheme 1) [16,17]. Identifying biomarkers which can differentiate between asymptomatic and symptomatic patients is important for selecting the appropriate treatment.

Oxysterols are present in human and animal tissues, in the blood, and in coronary and carotid plaques [12,18]. They stimulate the formation of foam cells, advance atherosclerotic plaque formation, and contribute to plaque vulnerability [19,20]. Here we review recent findings related to the differences between symptomatic and asymptomatic plaques, the link between

* Corresponding author at: MIGAL-Galilee Research Institute, P.O. Box 831, Kiryat Shmona 11016, Israel. Fax: +972 4 6944980.
E-mail address: vayaj46@gmail.com (J. Vaya).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.12.116



Scheme 1. Properties of atherosclerotic human symptomatic plaque.

oxysterols and plaque vulnerability and stability, and oxysterol content in symptomatic versus asymptomatic patients.

2. Symptomatic and asymptomatic plaques

Atherosclerotic plaques are categorized as either unstable—designated vulnerable to rupture, or stable. Golledge et al. suggested that stable carotid artery plaques are unlikely to produce symptomatic embolization, whereas unstable plaques are at high risk of producing symptomatic embolization and carotid occlusion [17]. Histological studies have shown that carotid plaques taken from symptomatic vs. asymptomatic patients are more inflamed, are rich in macrophages, foam and T cells, and have a thinner and weaker fibrous cap which tends to rupture [17,21]. Similar findings have been obtained in samples of coronary plaques taken postmortem and postatherectomy [22–24]. Noninvasive techniques, such as cardiovascular magnetic resonance (CMR) with 3 T scanners, have also been used to compare symptomatic and asymptomatic atherosclerotic carotid plaques. Grimm et al. found that symptomatic plaques have a higher prevalence of American Heart Association type 6 lesions (AHA-LT6) compared to asymptomatic plaques, in which AHA-LT3 and AHA-LT7 are more frequent. In the symptomatic group, a ruptured fibrous cap and necrotic core were found more frequently and the prevalence of hemorrhage (intraplaque hemorrhage and combined juxtaluminal hemorrhage/thrombus) was significantly higher than among the asymptomatic group [25]. Moreover, Mughal et al. reported that patients with more frequent neurological symptoms have more dense cholesterol-crystal formations within the necrotic core of the plaque. Cholesterol saturation has been shown to be a potentially major trigger for cholesterol crystallization, which can lead to volume expansion and plaque rupture [26].

Despite the differences between symptomatic and asymptomatic plaques, there is significant overlap among some of these plaques' features which limits the use of solely the CMR variable to completely separate between symptomatic and asymptomatic subjects. Identification of new reliable biomarkers in the blood which can provide information on plaque stability is important and could complement information collected from the various imaging techniques [27]. To date, there are no blood biomarkers available for clinical use to assess stability and status of carotid and coronary plaques.

Hermus et al. published a comprehensive review of serum biomarkers associated with plaque stability. Those shown to be highly associated with the presence of vulnerable carotid artery plaques are mainly markers of inflammation and proteolysis, such as the highly sensitive C-reactive protein, interleukin (IL) 6, matrix metalloproteinases (MMPs) 9 and 2, and tissue inhibitors of metalloproteinases (TIMPs) 1 and 2 [28]. This noninvasive method of identifying high-risk patients might serve as a promising tool in the future to select patients for carotid surgery but to date, none of these serum biomarkers have been designated for routine clinical use.

Serum markers of lipid metabolism and abnormal lipoprotein profiles are important predictors of atherosclerosis. For example, circulating levels of Ox-LDL have become a useful biochemical risk marker for coronary heart disease [29].

Tavori et al. extracted a lipid fraction of human carotid plaque which had the capacity to facilitate atherogenesis by enhancing LDL and macrophage oxidation and inhibiting HDL-mediated cholesterol efflux from macrophages, inducing macrophage foam-cell formation, and inhibiting the HDL-associated antioxidant enzyme PON1. Structural elucidation revealed the major atherogenic element in the plaque extract to be linoleic acid hydroperoxide (LA-OOH), which is derived from lipid peroxidation of linoleic acid [30]. Elad et al. showed significantly higher LA-OOH levels in plaques of symptomatic versus asymptomatic patients; in addition, LA-OOH level in the plaque was inversely correlated with HDL level in the circulation and HDL PON1 activity, and directly correlated to hemoglobin A1c level. Thus, based on HDL level and PON1 activity in the blood, it might be possible to predict the level of LA-OOH in the plaque and consequently, the plaque's status [31].

3. Oxysterols in symptomatic versus asymptomatic human plaques

Oxysterols are products of cholesterol oxidation that are present in human tissue and fluids, including plasma, lipoproteins and coronary and carotid plaques [12,18]. Oxysterols can be synthesized endogenously via enzymatic or radical-mediated oxidation, or they can be derived from food [19,32]. Enzymatic oxidation occurs mainly in the liver and steroidogenic tissues. 7α-Hydroxylated cholesterol (7α-OH CH), synthesized in the liver by the microsomal enzyme cholesterol 7α-hydroxylase, is traditionally considered the first and rate-limiting step in bile acid synthesis. 27-Hydroxylated cholesterol (27-OH CH) is synthesized by sterol 27-hydroxylase in the liver; in addition to its role in hepatic bile acid synthesis, sterol 27-hydroxylase is involved in the elimination of cholesterol from extrahepatic cells [19,33,34].

Table 1
Summary of the effects of oxysterols on endothelial cells, smooth muscle cells and macrophages and their contribution to symptomatic plaque development.

Oxysterol	Biological effect	References
27-OH CH	1 – Increases the synthesis as well as secretion of TNF-α from macrophages	[36]
	2 – Increases the number of adherent cells and induces expression of CD40, CD80, CD83, and CD88 in THP1 cells	[49]
	3 – Affects MMP-9 overexpression	[53]
25-OH CH	Induces smooth muscle cell apoptosis	[47]
7-Keto CH	1 – Induces apoptosis of endothelial cells	[46]
	2 – Induces of smooth muscle cell apoptosis	[47]
	3 – Increases the production of the proinflammatory cytokines TNF-α and IL-6	[48]
	4 – Mediates expression of early growth response protein 1 (Egr1) and apoptosis	[50]
	5 – Induces up regulation of p53, and enhances p53-induced apoptotic cell death	[51]
7α-OH CH	1– Increases the synthesis as well as secretion of TNF-α from macrophages	[36]
	2 – Increases the number of adherent cells and induces expression of CD40, CD80, CD83, and CD88 in THP1 cells	[49]
	3 – Affects MMP-9 overexpression	[52]
7β-OH CH	1 – Induces apoptosis of endothelial cells	[46]
	2 – Mediates expression of early growth response protein 1 (Egr1) and apoptosis	[50]
	3 – Induces up-regulation of p53, and enhances p53-induced apoptotic cell death	[51]
	4 – Increases the level of plasma 7β-OH CH associated with a high risk of developing cardiovascular disease and coronary and carotid atherosclerotic plaques	[54–57]

7-Hydroperoxycholesterol (7-OOH CH), 7-OH CH α and β, 7-ketocholesterol (7-keto CH) and 5,6-epoxy cholesterol (α and β-epoxy CH) are the major oxysterols formed via cholesterol autoxidation, which can occur in a variety of tissues, induced by reactive oxygen and nitrogen species (ROS and RNS, respectively) [27]. Many studies have detected oxysterols in human coronary and carotid atherosclerotic plaques. 27-OH CH is the major oxysterol found in advanced atherosclerotic lesions; its level is approximately proportional to cholesterol levels and increases with increasing severity of atherosclerosis [13,35]. 7-Keto CH is the next most abundant oxysterol, followed by 7β-OH CH and 7α-OH CH. These oxysterols comprise 75–85% of all oxysterols detected in plaques from different sites [36,37].

The oxysterols' potential to trigger prooxidative, proinflammatory and cytotoxic reactions has been widely documented in a number of cells of the vascular wall, including endothelial cells (ECs; [38,39]) and human artery smooth muscle cells [40,41], as well as immunocompetent cells such as monocytes and macrophages (Table 1) [42–45].

EC apoptosis has been implicated in advanced atherosclerosis. Increased levels of EC apoptosis were reported in an atherosclerotic internal carotid artery plaque and even in symptomatic high-grade carotid artery sclerotic patients. Li et al. reported that both 7β-OH CH and 7-keto CH cause apoptosis of ECs associated with early accumulation of cellular lipids and activation of the lysosomal apoptotic pathway, followed by cellular OS, mitochondrial damage, and upregulation and release of von Willebrand factor (VWF) or lysosomal cathepsin; 7β-OH CH was found to be more apoptogenic than 7-keto CH [46].

Apoptosis of vascular smooth muscle cells (VSMCs) is important for the stability of advanced symptomatic plaques. Indeed, symptomatic plaques exhibit higher levels of apoptotic VSMCs than stable lesions, and inhibition of VSMC apoptosis stabilizes atherosclerotic plaques in vivo. Oxysterols are important inducers of smooth muscle cell apoptosis. Appukuttan et al. reported that oxysterol-induced apoptosis of VSMCs via activation of the cytosolic soluble adenylyl cyclase (sAC). Treatment of VSMCs derived from rat aorta with either 25-OH CH or 7-keto CH for 24 h led to activation of the mitochondrial apoptotic pathway (cytochrome c release and caspase-9 cleavage) and mitochondrial ROS formation, which were suppressed by the pharmacological inhibition or knockdown of cytosolic sAC. These authors suggested that strategies directed to suppressing cytosolic sAC activity or translocation might contribute to the stability of advanced atherosclerotic plaques [47].

There are two main subsets of macrophages, the major cellular components of atherosclerotic plaques: the proinflammatory, M1 or classically activated macrophages, and the anti-inflammatory, M2 or alternatively activated macrophages. Buttari et al. provided the first evidence of 7-keto CH effects on human macrophage biology by skewing the M1/M2 macrophage balance toward a proinflammatory and hence, proatherogenic profile. 7-Keto CH increased the production of the proinflammatory cytokines TNF-α and IL-6, thereby leading to an incremental proinflammatory phenotype in M1 and M2 cells. Kim et al. demonstrated that treatment of macrophages with 7α-OH CH or 27-OH CH results in a significant increase in synthesis as well as secretion of TNF-α from macrophages. The cytokine TNF-α plays a critical role in the development and destabilization of atherosclerotic plaques [36,48].

Oxysterols can induce differentiation of monocytes into macrophages. Son et al. reported that treatment of human monocyticTHP1 cells with 27-OH CH and 7α-OH CH results in an increase in the number of adherent cells, and induces expression of mature dendritic cell-specific molecules, including CD40, CD80, CD83, and CD88 [49].

7-Oxysterols, especially 7β-OH CH and 7-keto CH, mediated cell death by increasing the level of cellular ROS which mediate the expression of early growth response protein 1 (Egr1) and apoptosis. Egr1 plays regulatory roles in several cardiovascular diseases and plaque progression. Egr1 also controls a wide range of genes, including the tumor-suppressor gene p53. Miah et al. suggested that ROS induced by 7-oxysterols may function as early initiators of Egr1 expression. The later induction of p53 by 7-oxysterols contributes to apoptotic cell death. Expression of p53 is associated with unstable or ruptured plaques and transient ischemic attacks in patients with carotid atherosclerosis [50]. Yuan et al. reported that 7β-OH CH and 7-keto CH induce upregulation of p53, and enhance p53-induced apoptotic cell death. These authors suggested that interaction of p53 with oxidized lipids promotes DNA damage and necrotic core formation in atherosclerosis [51].

MMPs contribute to fibrous cap thinning, plaque rupture and instability, via the degradation of extracellular matrix (ECM) components. MMP-9 plays a critical role in the development and rupture of advanced atherosclerotic lesions, and it has been found to be highly expressed in unstable human atherosclerotic plaques. Gargiulo et al. reported that an oxysterol mixture with a composition similar to that found in human carotid plaques enhances the expression of MMP-9 in human promonocytic U937 cells; this indicated that the oxysterol mixture might contribute significantly

to destabilizing the fibrotic plaque, by increasing MMP-9 expression and activity. Among the various components of the oxysterol mixture, 27-OH CH and 7α-OH CH were the main molecules responsible for the observed MMP-9 overexpression [52]. Integrin binding to ECM molecules resulted in increased expression of various matrix MMPs, e.g., MMP-1, MMP-2, MMP-3 and MMP-9. The oxysterol mixture was found to induce β1-integrin levels in cells of the macrophage lineage, which is important for monocyte recruitment to the intima and their differentiation during atherosclerotic lesion development [53].

As already noted, a large number of studies have been performed to elucidate and confirm the association between oxysterol cytotoxicity and atherosclerotic plaque formation, propagation and stability. On the other hand, only a few clinical studies have been generated to examine the potential of oxysterols as serum biomarkers for cardiovascular diseases and atherosclerotic plaque development and stability.

Several reports have indicated that plasma concentrations of 7β-OH CH are related to atherosclerotic disease progression. Increased levels of 7β-OH CH have been associated with a high risk of developing cardiovascular disease and coronary atherosclerotic plaques [54,55]. Salonen et al. examined the association between lipid oxidation and atherogenesis in humans. These authors found that a high concentration of serum 7β-OH CH is one of the strongest single predictors of progression in carotid atherosclerosis and carotid wall thickening [54]. Rimner et al. measured oxysterol levels in the sera of 42 patients with an atherogenic risk profile and symptoms of coronary artery disease (CAD), who were divided into two groups: patients without abnormal angiographic findings (control group, $n = 20$) and patients with stable CAD and stenotic coronary vessels ($n = 22$). Free plasma oxysterol levels showed a significant increase in patients with CAD compared to control patients, and of the oxysterols tested, 7β-OH CH was significantly elevated in CAD compared to control patients [56]. Yasunobu et al. investigated the relevance of oxysterols in CAD in 183 patients undergoing coronary angiography. In that study, plasma concentrations of 25-, 27- and 7β-OH CH showed an association with CAD. The concentration of the oxysterols in stenotic groups was higher than in normal groups. Furthermore, the concentrations of plasma oxysterols did not differ between subgroups with stable and unstable angina [57]. Ziedén et al. reported increased plasma 7β-OH CH concentration in a population at high risk for cardiovascular disease: among Lithuanian men between the ages of 50 and 54 years, mortality from coronary heart disease was fourfold that in Swedish men and plasma 7β-OH CH levels were found at higher concentrations [55].

In conclusion, the plasma 7beta-OH CH level can be useful as a biomarker for coronary artery disease, but plasma biomarkers distinguishing symptomatic from asymptomatic plaques have not yet been identified.

References

[1] M. Aviram, Review of human studies on oxidative damage and antioxidant protection related to cardiovascular diseases, Free Radic. Res. 33 (Suppl.) (2000) S85–97.

[2] J.A. Berliner, M. Navab, A.M. Fogelman, J.S. Frank, L.L. Demer, P.A. Edwards, A.D. Watson, A.J. Lusis, Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics, Circulation 91 (1995) 2488–2496.

[3] C.K. Glass, J.L. Witztum, Atherosclerosis. The road ahead, Cell 104 (2001) 503–516.

[4] A.J. Lusis, Atherosclerosis, Nature 407 (2000) 233–241.

[5] R. Stocker, J.F. Keaney Jr., Role of oxidative modifications in atherosclerosis, Physiol. Rev. 84 (2004) 1381–1478.

[6] B. Fuhrman, M. Shiner, N. Volkova, M. Aviram, Cell-induced copper ion-mediated low density lipoprotein oxidation increases during in vivo monocyte-to-macrophage differentiation, Free Radic. Biol. Med. 37 (2004) 259–271.

[7] A.T. Diplock, J.L. Charleux, G. Crozier-Willi, F.J. Kok, C. Rice-Evans, M. Roberfroid, W. Stahl, J. Vina-Ribes, Functional food science and defence against reactive oxidative species, Br. J. Nutr. 80 (Suppl 1) (1998) S77–112.

[8] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, fourth ed., Oxford university press, Oxford, UK, 2007.

[9] N. Khan-Merchant, M. Penumetcha, O. Meilhac, S. Parthasarathy, Oxidized fatty acids promote atherosclerosis only in the presence of dietary cholesterol in low-density lipoprotein receptor knockout mice, J. Nutr. 132 (2002) 3256–3262.

[10] J.J. Badimon, V. Fuster, J.H. Chesebro, L. Badimon, Coronary atherosclerosis. A multifactorial disease, Circulation 87 (1993) II3–16.

[11] J.L. Witztum, D. Steinberg, Role of oxidized low density lipoprotein in atherogenesis, J. Clin. Invest. 88 (1991) 1785–1792.

[12] J. Vaya, M. Aviram, S. Mahmood, T. Hayek, E. Grenadir, A. Hoffman, S. Milo, Selective distribution of oxysterols in atherosclerotic lesions and human plasma lipoproteins, Free Radic. Res. 34 (2001) 485–497.

[13] K.L. Carpenter, S.E. Taylor, C. van der Veen, B.K. Williamson, J.A. Ballantine, M.J. Mitchinson, Lipids and oxidised lipids in human atherosclerotic lesions at different stages of development, Biochim. Biophys. Acta 1256 (1995) 141–150.

[14] J.B. Marsillach, F. M. M. R. Beltran, J. Joven, J. Camp, Immunohistochemical analysis of Paraoxonase-1,2 and 3 in human atheroma plaques, 3rd International Conference on Paraoxnases (2008).

[15] B. Mackness, R. Hunt, P.N. Durrington, M.I. Mackness, Increased immunolocalization of paraoxonase, clusterin, and apolipoprotein A-I in the human artery wall with the progression of atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 17 (1997) 1233–1238.

[16] T. Saam, J. Cai, L. Ma, Y.Q. Cai, M.S. Ferguson, N.L. Polissar, T.S. Hatsukami, C. Yuan, Comparison of symptomatic and asymptomatic atherosclerotic carotid plaque features with in vivo MR imaging, Radiology 240 (2006) 464–472.

[17] J. Golledge, R.M. Greenhalgh, A.H. Davies, The symptomatic carotid plaque, Stroke 31 (2000) 774–781.

[18] F. Guardiola, R. Codony, P.B. Addis, M. Rafecas, J. Boatella, Biological effects of oxysterols: current status, Food Chem. Toxicol. 34 (1996) 193–211.

[19] A.J. Brown, W. Jessup, Oxysterols and atherosclerosis, Atherosclerosis 142 (1999) 1–28.

[20] Z.H. Feng, S. Cheng, Relationship between oxysterols and atherosclerosis, Sheng Li Ke Xue Jin Zhan 30 (1999) 23–28.

[21] J.N. Redgrave, J.K. Lovett, P.J. Gallagher, P.M. Rothwell, Histological assessment of 526 symptomatic carotid plaques in relation to the nature and timing of ischemic symptoms: the Oxford plaque study, Circulation 113 (2006) 2320–2328.

[22] M.J. Davies, P.D. Richardson, N. Woolf, D.R. Katz, J. Mann, Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth muscle cell content, Br. Heart J. 69 (1993) 377–381.

[23] A.C. van der Wal, A.E. Becker, C.M. van der Loos, P.K. Das, Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology, Circulation 89 (1994) 36–44.

[24] J.J. Boyle, Association of coronary plaque rupture and atherosclerotic inflammation, J. Pathol. 181 (1997) 93–99.

[25] J.M. Grimm, A. Schindler, T. Freilinger, C.C. Cyran, F. Bamberg, C. Yuan, M.F. Reiser, M. Dichgans, C. Freilinger, K. Nikolaou, T. Saam, Comparison of symptomatic and asymptomatic atherosclerotic carotid plaques using parallel imaging and 3 T black-blood in vivo CMR, J. Cardiovasc. Magn. Reson. 15 (2013) 44.

[26] M.M. Mughal, M.K. Khan, J.K. DeMarco, A. Majid, F. Shamoun, G.S. Abela, Symptomatic and asymptomatic carotid artery plaque, Expert Rev. Cardiovasc. Ther. 9 (2011) 1315–1330.

[27] J. Vaya, The association between biomarkers in the blood and carotid plaque composition-focusing on oxidized lipids, oxysterols and plaque status, Biochem. Pharmacol. 86 (2013) 15–18.

[28] L. Hermus, J.D. Lefrandt, R.A. Tio, J.C. Breek, C.J. Zeebregts, Carotid plaque formation and serum biomarkers, Atherosclerosis 213 (2010) 21–29.

[29] C. Meisinger, J. Baumert, N. Khuseyinova, H. Loewel, W. Koenig, Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population, Circulation 112 (2005) 651–657.

[30] H. Tavori, M. Aviram, S. Khatib, R. Musa, D. Mannheim, R. Karmeli, J. Vaya, Human carotid lesion linoleic acid hydroperoxide inhibits paraoxonase 1 (PON1) activity via reaction with PON1 free sulfhydryl cysteine 284, Free Radic. Biol. Med. 50 (2011) 148–156.

[31] E. Cohen, M. Aviram, S. Khatib, A. Rabin, D. Mannheim, R. Karmeli, J. Vaya, Increased levels of human carotid lesion linoleic acid hydroperoxide in symptomatic and asymptomatic patients is inversely correlated with serum HDL and paraoxonase 1 activity, J. Lipids 2012 (2012) 762560.

[32] K. Sato, K. Nakano, S. Katsuki, T. Matoba, K. Osada, T. Sawamura, K. Sunagawa, K. Egashira, Dietary cholesterol oxidation products accelerate plaque destabilization and rupture associated with monocyte infiltration/activation via the MCP-1-CCR2 pathway in mouse brachiocephalic arteries: therapeutic effects of ezetimibe, J. Atheroscler. Thromb. 19 (2012) 986–998.

[33] I. Bjorkhem, O. Andersson, U. Diczfalusy, B. Sevastik, R.J. Xiu, C. Duan, E. Lund, Atherosclerosis and sterol 27-hydroxylase: evidence for a role of this enzyme in elimination of cholesterol from human macrophages, Proc. Natl. Acad. Sci. USA 91 (1994) 8592–8596.

[34] E. Lund, O. Andersson, J. Zhang, A. Babiker, G. Ahlborg, U. Diczfalusy, K. Einarsson, J. Sjovall, I. Bjorkhem, Importance of a novel oxidative mechanism

for elimination of intracellular cholesterol in humans, Arterioscler. Thromb. Vasc. Biol. 16 (1996) 208–212.
[35] K.L. Carpenter, S.E. Taylor, J.A. Ballantine, B. Fussell, B. Halliwell, M.J. Mitchinson, Lipids and oxidised lipids in human atheroma and normal aorta, Biochim. Biophys. Acta 1167 (1993) 121–130.
[36] S.M. Kim, H. Jang, Y. Son, S.A. Lee, S.S. Bae, Y.C. Park, S.K. Eo, K. Kim, 27-Hydroxycholesterol induces production of tumor necrosis factor-alpha from macrophages, Biochem. Biophys. Res. Commun. 430 (2013) 454–459.
[37] M. Crisby, J. Nilsson, V. Kostulas, I. Bjorkhem, U. Diczfalusy, Localization of sterol 27-hydroxylase immuno-reactivity in human atherosclerotic plaques, Biochim. Biophys. Acta 1344 (1997) 278–285.
[38] S. Luthra, J. Dong, A.L. Gramajo, M. Chwa, D.W. Kim, A. Neekhra, B.D. Kuppermann, M.C. Kenney, 7-Ketocholesterol activates caspases-3/7, -8, and -12 in human microvascular endothelial cells in vitro, Microvasc. Res. 75 (2008) 343–350.
[39] 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 (1999) 1190–1200.
[40] E. Pedruzzi, C. Guichard, V. Ollivier, F. Driss, M. Fay, C. Prunet, J.C. Marie, C. Pouzet, M. Samadi, C. Elbim, Y. O'Dowd, M. Bens, A. Vandewalle, M.A. Gougerot-Pocidalo, G. Lizard, E. Ogier-Denis, NAD(P)H oxidase Nox-4 mediates 7-ketocholesterol-induced endoplasmic reticulum stress and apoptosis in human aortic smooth muscle cells, Mol. Cell. Biol. 24 (2004) 10703–10717.
[41] E. Nishio, Y. Watanabe, Oxysterols induced apoptosis in cultured smooth muscle cells through CPP32 protease activation and bcl-2 protein downregulation, Biochem. Biophys. Res. Commun. 226 (1996) 928–934.
[42] 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, Eur. J. Nutr. 38 (1999) 255–262.
[43] S. Lemaire-Ewing, C. Prunet, T. Montange, A. Vejux, A. Berthier, G. Bessede, L. Corcos, P. Gambert, D. Neel, G. Lizard, Comparison of the cytotoxic, pro-oxidant and pro-inflammatory characteristics of different oxysterols, Cell Biol. Toxicol. 21 (2005) 97–114.
[44] N. Shibata, C.K. Glass, Macrophages, oxysterols and atherosclerosis, Circ. J. 74 (2010) 2045–2051.
[45] L. Tesoriere, A. Attanzio, M. Allegra, C. Gentile, M.A. Livrea, Phytochemical indicaxanthin suppresses 7-ketocholesterol-induced THP-1 cell apoptosis by preventing cytosolic Ca(2+) increase and oxidative stress, Br. J. Nutr. 110 (2013) 230–240.
[46] W. Li, M. Ghosh, S. Eftekhari, X.M. Yuan, Lipid accumulation and lysosomal pathways contribute to dysfunction and apoptosis of human endothelial cells caused by 7-oxysterols, Biochem. Biophys. Res. Commun. 409 (2011) 711–716.
[47] A. Appukuttan, S.A. Kasseckert, S. Kumar, H.P. Reusch, Y. Ladilov, Oxysterol-induced apoptosis of smooth muscle cells is under the control of a soluble adenylyl cyclase, Cardiovasc. Res. 99 (2013) 734–742.
[48] B. Buttari, L. Segoni, E. Profumo, D. D'Arcangelo, S. Rossi, F. Facchiano, R. Businaro, L. Iuliano, R. Rigano, 7-Oxo-cholesterol potentiates pro-inflammatory signaling in human M1 and M2 macrophages, Biochem. Pharmacol. 86 (2013) 130–137.
[49] Y. Son, S.M. Kim, S.A. Lee, S.K. Eo, K. Kim, Oxysterols induce transition of monocytic cells to phenotypically mature dendritic cell-like cells, Biochem. Biophys. Res. Commun. 438 (2013) 161–168.
[50] S. Miah, S.N. Zadeh, X.M. Yuan, W. Li, Expression of Egr1 and p53 in human carotid plaques and apoptosis induced by 7-oxysterol or p53, Exp. Toxicol. Pathol. 65 (2013) 677–682.
[51] X.M. Yuan, E. Osman, S. Miah, S.N. Zadeh, L. Xu, C. Forssell, W. Li, P53 expression in human carotid atheroma is significantly related to plaque instability and clinical manifestations, Atherosclerosis 210 (2010) 392–399.
[52] S. Gargiulo, B. Sottero, P. Gamba, E. Chiarpotto, G. Poli, G. Leonarduzzi, Plaque oxysterols induce unbalanced up-regulation of matrix metalloproteinase-9 in macrophagic cells through redox-sensitive signaling pathways: implications regarding the vulnerability of atherosclerotic lesions, Free Radic. Biol. Med. 51 (2011) 844–855.
[53] S. Gargiulo, P. Gamba, G. Testa, B. Sottero, M. Maina, T. Guina, F. Biasi, G. Poli, G. Leonarduzzi, Molecular signaling involved in oxysterol-induced beta1-integrin over-expression in human macrophages, Int. J. Mol. Sci. 13 (2012) 14278–14293.
[54] J.T. Salonen, K. Nyyssonen, R. Salonen, E. Porkkala-Sarataho, T.P. Tuomainen, U. Diczfalusy, I. Bjorkhem, Lipoprotein oxidation and progression of carotid atherosclerosis, Circulation 95 (1997) 840–845.
[55] B. Zieden, A. Kaminskas, M. Kristenson, Z. Kucinskiene, B. Vessby, A.G. Olsson, U. Diczfalusy, Increased plasma 7 beta-hydroxycholesterol concentrations in a population with a high risk for cardiovascular disease, Arterioscler. Thromb. Vasc. Biol. 19 (1999) 967–971.
[56] A. Rimner, S. Al Makdessi, H. Sweidan, J. Wischhusen, B. Rabenstein, K. Shatat, P. Mayer, I. Spyridopoulos, Relevance and mechanism of oxysterol stereospecifity in coronary artery disease, Free Radic. Biol. Med. 38 (2005) 535–544.
[57] Y. Yasunobu, K. Hayashi, T. Shingu, T. Yamagata, G. Kajiyama, M. Kambe, Coronary atherosclerosis and oxidative stress as reflected by autoantibodies against oxidized low-density lipoprotein and oxysterols, Atherosclerosis 155 (2001) 445–453.