

Current trends in oxysterol research

William J. Griffiths^{*1}, Jonas Abdel-Khalik^{*}, Thomas Hearn^{*}, Eylan Yutuc^{*}, Alwena H. Morgan^{*} and Yuqin Wang^{*1}

^{*}College of Medicine, Grove Building, Swansea University, Singleton Park, Swansea SA2 8PP, U.K.

Abstract

In this short review we provide a synopsis of recent developments in oxysterol research highlighting topics of current interest to the community. These include the involvement of oxysterols in neuronal development and survival, their participation in the immune system, particularly with respect to bacterial and viral infection and to T_H17-cell development, and the role of oxysterols in breast cancer. We also discuss the value of oxysterol analysis in the diagnosis of disease.

Introduction

Oxysterols are oxygenated derivatives of cholesterol or its sterol precursors, e.g. 7-dehydrocholesterol (7-DHC) or desmosterol [1,2]. They are formed enzymatically in the first steps of sterol metabolism and are intermediates in the formation of the steroid hormones, bile acids and 1,25-dihydroxyvitamin D₃ [3]. Oxysterols may also be formed via non-enzymatic routes by encounters with reactive oxygen species [4,5], which provide a second pool of metabolites which also include oxidized cholesterol molecules taken from the diet [6]. A third pool may consist of oxidized cholesterol molecules generated by the gut microflora and taken up through the enterohepatic circulation. Although once thought of as inactive metabolic intermediates, the involvement of oxysterols in cholesterol homeostasis, their role as ligands to nuclear and G protein-coupled receptors and their potential as easily measured biomarkers of disease has enhanced interest in their biosynthesis, metabolism and measurement. In this review we include in the family of oxysterols the cholestenoic acids, C₂₇ carboxylated forms of cholesterol.

Oxysterols in neuronal development survival

As the mammalian central nervous system (CNS) is rich in cholesterol and oxysterols [7], it is perhaps not surprising that oxysterols play a role in the nervous system. The most

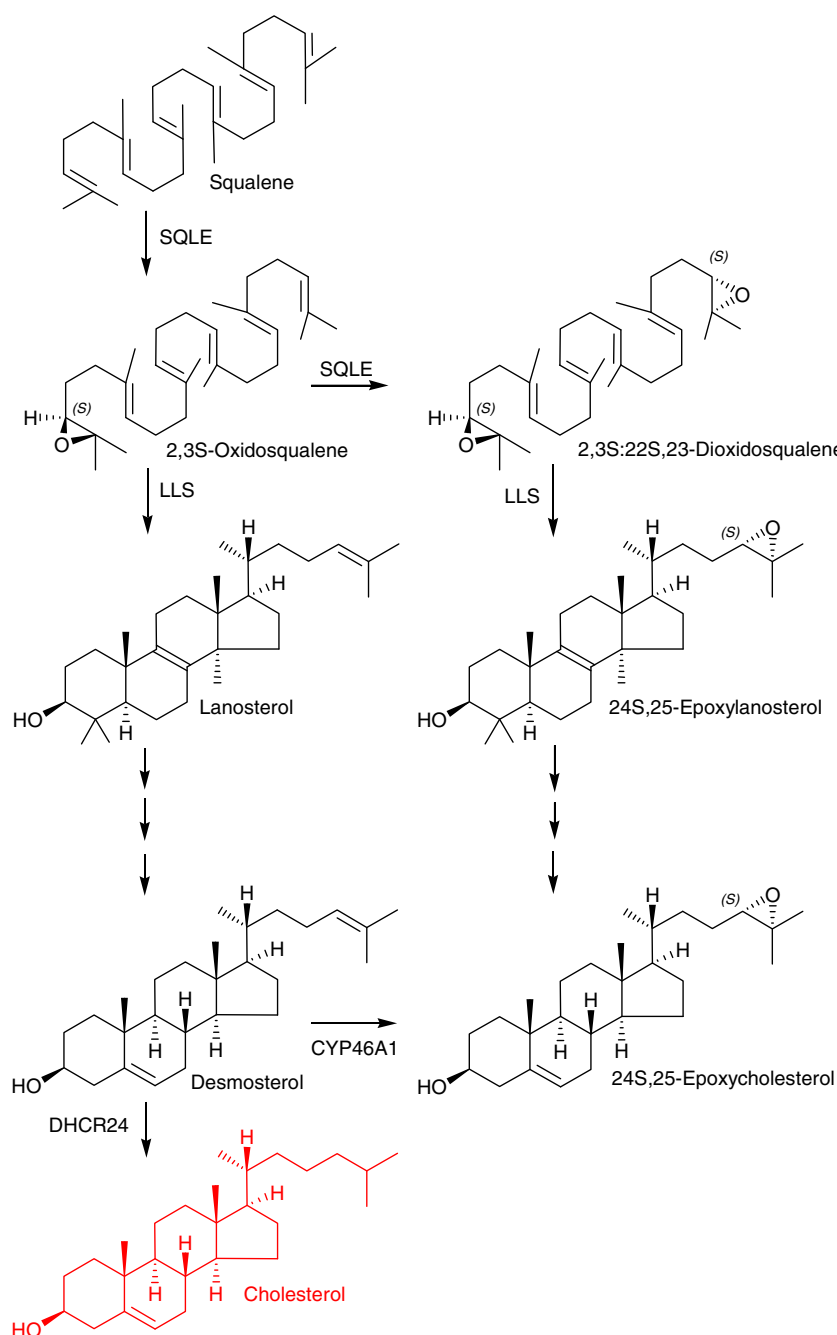
abundant oxysterol in brain is 24S-hydroxycholesterol (24S-HC), present at a level of about 20–40 ng/mg in mouse and man. This oxysterol plays a role as a cholesterol transport molecule, crossing the blood brain barrier and passing from brain to the blood stream for transport to the liver and further metabolism [8]. 24S-HC is also a ligand to the liver X receptors (LXR α and LXR β) [9], both of which are expressed in brain, and also to the endoplasmic reticulum resident protein INSIG (insulin-induced gene) which upon ligand binding anchors the transport protein SCAP (SREBP cleavage-activating protein) along with its cargo, the pro-form of the transcription factors SREBP (sterol regulatory-element binding protein), in the endoplasmic reticulum preventing its transport to the Golgi for activation [10]. The mature, or nuclear, forms of the SREBP proteins 1c and 2 are transcription factors regulating the expression of the biosynthetic enzymes of the fatty acid and cholesterol synthesis pathways respectively [11]. It is likely that side-chain oxysterols, like 24S-HC, are important for the fine tuning of cholesterol biosynthesis, whereas cholesterol itself, through direct binding to SCAP, is more important for the coarse tuning of a negative-feedback mechanism [12,13].

In foetal development in mouse, cytochrome P450 (CYP) 46A1, the enzyme responsible for the metabolism of cholesterol to 24S-HC, is weakly expressed until E18 [14], and instead 24S,25-epoxycholesterol (24S,25-EC) is a dominating oxysterol (24S,25-EC, 0.3–0.4 ng/mg; cf. 24S-HC, 0.03 ng/mg at E11.5) [15]. 24S,25-EC is an unusual oxysterol in that it is synthesized via shunt pathways in parallel to cholesterol synthesis rather from cholesterol itself (Figure 1) [12]. Either, the enzyme squalene epoxidase (SQLE), also known as squalenemonooxygenase (SM), introduces one oxygen atom to squalene to give 2,3S-oxidosqualene (squalene-2,3S-epoxide) followed by cyclisation by lanosterol synthase (LLS) to lanosterol for subsequent cholesterol biosynthesis, or rather SQLE introduces a second oxygen atom to squalene to give 2,3S:22S,23-dioxidosqualene prior to cyclisation to 24S,25-epoxylanosterol, ultimately leading to 24S,25-EC. A second pathway to 24S,25-EC synthesis is from desmosterol in a CYP46A1 catalysed reaction [16]. Interestingly, it has been shown that 24S,25-EC and desmosterol, its parallel metabolite during cholesterol

Key words: cholestenoic acid, cholesterol, hydroxycholesterol, liver X receptor (LXR), RAR-related orphan receptor gamma t (ROR γ), sterol regulatory-element binding protein (SREBP).

Abbreviations: 7-DHC, 7-dehydrocholesterol; (25R)26-HC, (25R)26-hydroxycholesterol; 24S,25-EC, 24S,25-epoxycholesterol; 24S-HC, 24S-hydroxycholesterol; 25-HC, 25-hydroxycholesterol; 26-HC, 26-hydroxycholesterol; 27-HC, 27-hydroxycholesterol; 3 β ,7 α -diHCA, 3 β ,7 α -dihydroxycholesterol-5-en-(25R)26-oic acid; 3 β -HCA, 3 β -hydroxycholesterol-5-en-(25R)26-oic acid; 5,6-EC, 5,6-epoxycholesterol; 7 α ,25-diHC, 7 α ,25-dihydroxycholesterol; 7 α ,26-diHC, 7 α ,26-dihydroxycholesterol; 7 α -HC, 7 α -hydroxycholesterol; 7 β ,26-diHC, 7 β ,26-dihydroxycholesterol; 7 β -HC, 7 β -hydroxycholesterol; CH25H, cholesterol 25-hydroxylase; ChEH, cholesterol epoxide hydrolase; CNS, central nervous system; CSF, cerebrospinal fluid; CTX, cerebrotendinous xanthomatosis; CYP, cytochrome P450; DDA, dendrogenin A; DHCR7, dehydrocholesterol reductase 7; ER, oestrogen receptor; IFN, interferon; INSIG, insulin-induced gene; LXR, liver X receptor; NPC, Niemann–Pick type C; ROR γ t, RAR-related orphan receptor gamma t; SCAP, SREBP cleavage-activating protein; SPG5, hereditary spastic paresis type 5; SQLE, squalene epoxidase; SREBP, sterol regulatory-element binding protein; TLR, Toll-like receptor.

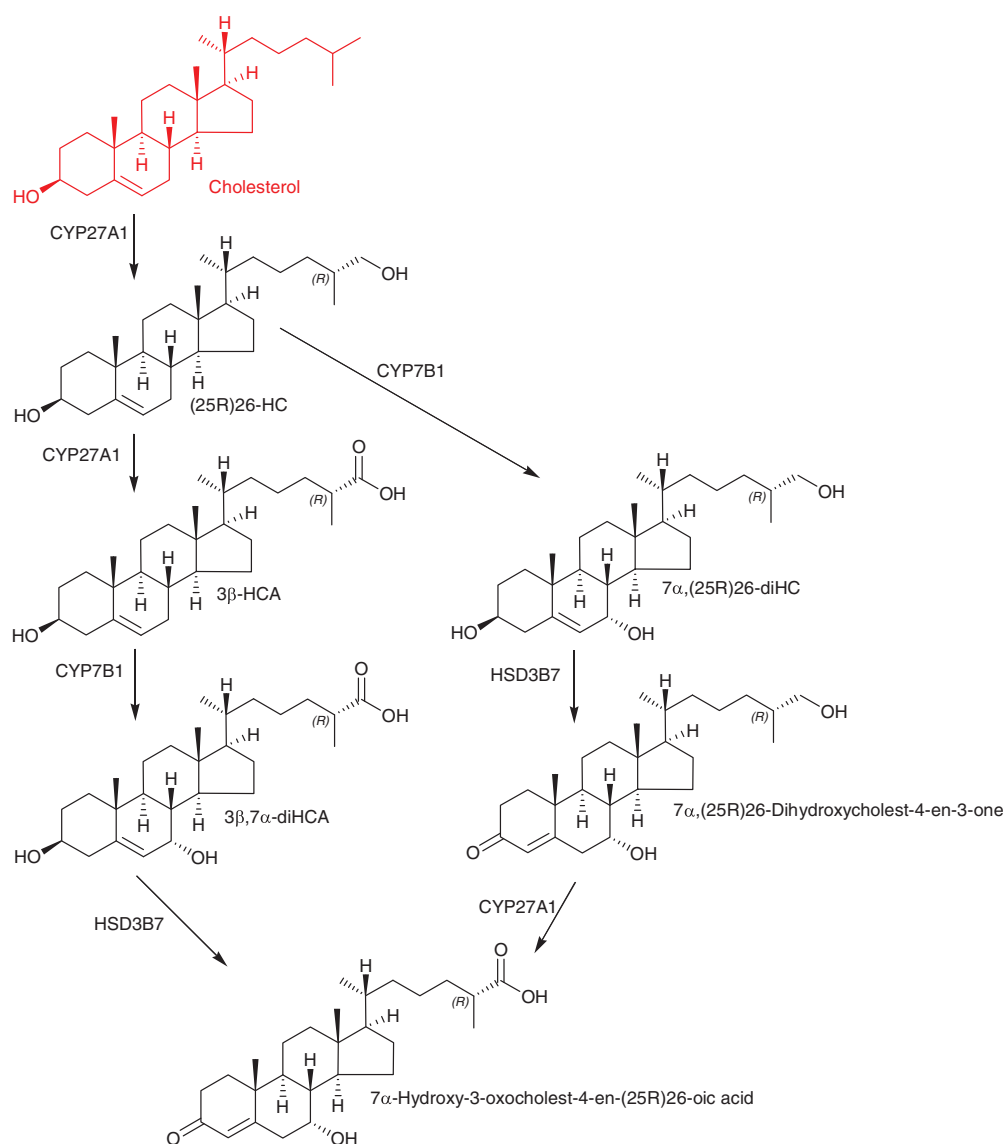
¹ Correspondence may be addressed to either of these authors (email w.j.griffiths@swansea.ac.uk or y.wang@swansea.ac.uk).

Figure 1 | Simplified pathway from squalene to cholesterol and 24S,25-epoxycholesterol

synthesis, are both reduced in concentration in brain from *Cyp46a1* knockout (*Cyp46a1*^{-/-}) mice [17]. These data can be explained by either, reduced expression of enzymes of the cholesterol biosynthesis pathway in response to removal of its export route through 24S-hydroxylation and therefore enhanced negative feedback via cholesterol, SCAP and SREBP, or alternatively, and perhaps in combination, through elimination of the desmosterol to 24S,25-EC pathway catalysed by CYP46A1. Unpublished data from the

authors and collaborators at Karolinska Institutet in Sweden indicate that 24S,25-EC is more abundant in transgenic mice overexpressing human CYP46A1, lending weight to the hypothesis portending synthesis via this enzyme. This pathway to 24S,25-EC synthesis may have importance in developing brain where despite low expression of CYP46A1 desmosterol levels are high [18].

24S,25-EC is both a ligand to INSIG, thus involved in regulation of cholesterol biosynthesis, and is also a potent

Figure 2 | The acidic pathway of cholesterol metabolism operating in the CNS

ligand to the LXRs. Its comparative high level in developing foetal mouse midbrain (0.39 ng/mg at E11.5) points to a biological activity in this region [19]. Interestingly, midbrain progenitor cells have reduced neurogenic capacity in *LxraLxrb* double knockout mice (*Lxra*^{-/-}*Lxrb*^{-/-}), whereas overexpression of *Lxrs* promotes midbrain dopaminergic neurogenesis [20]. Recent studies have identified 24S,25-EC as a midbrain LXR ligand promoting dopaminergic neurogenesis in midbrain progenitor cells and embryonic stem cell cultures [19]. These data suggest that LXR ligands may be of value in cell replacement and regenerative therapies for Parkinson's disease, a disease in which dopaminergic neurons are lost.

Adult *Lxrb*^{-/-} mice show progressive accumulation of lipids in brain and loss of spinal cord motor neurons

[21], indicating that LXRs are important for survival of neurons in the adult. Besides oxysterols, cholestenic acids are also ligands to the LXRs [22,23] and there is an expanding body of evidence indicating that cholestenic acids are synthesized in the CNS (Figure 2). Meaney et al. [24] showed that there is a net export of 7α-hydroxy-3-oxocholest-4-en-26-oic acid from human brain to the circulation, in-part compensating for a net import of (25R)26-hydroxycholesterol ((25R)26-HC) into brain from the circulation [25]. Note, we use here systematic nomenclature where hydroxylation at the terminal side chain of cholesterol is on C-26 leading to 26-hydroxycholesterol (26-HC) which may have 25R or 25S stereochemistry [26]. Unless stated otherwise 25R stereochemistry is assumed. In much of the literature (25R)26-HC is referred to 27-hydroxycholesterol

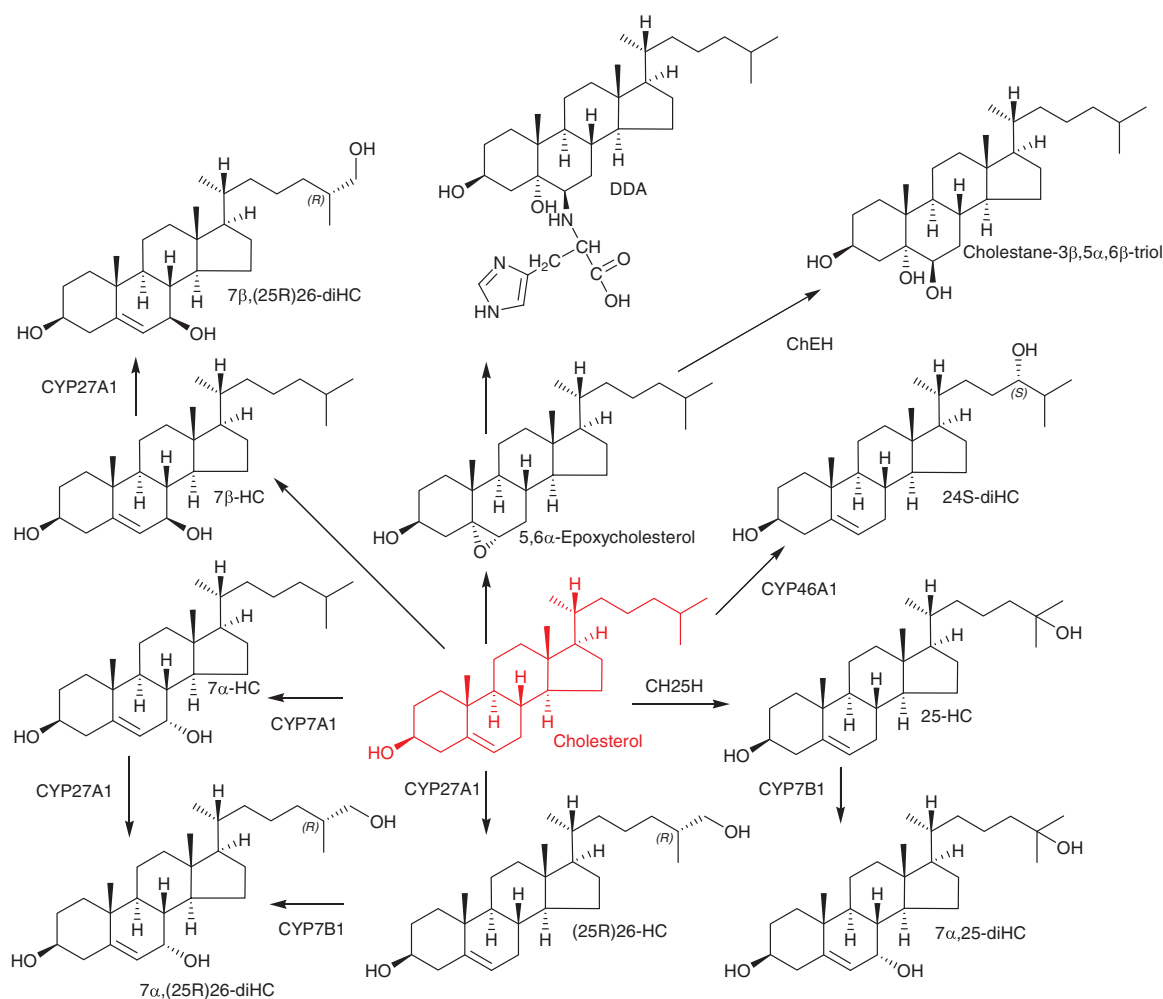
(27-HC), presumably the 25R isomer. More recently, Crick et al. [27] and Iuliano et al. [28] showed that 7α , (25R)26-dihydroxycholest-4-en-3-one, a precursor of 7α -hydroxy-3-oxocholest-4-en-(25R)26-oic acid in the pathway from (25R)26-HC is similarly exported from human brain to the circulation, and the authors group have identified low levels (0.01 ng/mg) of 3β -hydroxycholest-5-en-(25R)26-oic acid (3β -HCA) in mouse brain [29] and in collaboration with investigators at Stanford University have identified this acid and its down-stream metabolites 3β , 7α -dihydroxycholest-5-en-(25R)26-oic acid (3β , 7α -diHCA) and 7α -hydroxy-3-oxocholest-4-en-(25R)26-oic acid in porcine brain. All of these cholesterol metabolites can also be found in human cerebrospinal fluid (CSF) [29]. Using (25R)26-HC as a starting substrate in the pathway to 7α -hydroxy-3-oxocholest-4-en-(25R)26-oic acid the 7α -hydroxy group is introduced by the enzyme CYP7B1. Mutations in CYP7B1 leading to a defective oxysterol 7α -hydroxylase enzyme result in the disease hereditary spastic paresis type 5 (SPG5) [30]. Patients with this disease show upper motor neuron degeneration, linking defective cholesterol metabolism to motor neuron disorder. A second cholesterol metabolic disorder, cerebrotendinous xanthomatosis (CTX) can also present with motor neuron degeneration. In CTX the (25R)26-hydroxylase enzyme, CYP27A1, is deficient, resulting in deranged cholesterol metabolism. By profiling the plasma and CSF of CTX and SPG5 patients we found that both showed a reduced level of 3β , 7α -diHCA, whereas SPG5 patients showed high levels of 3β -HCA. Further *in vitro* and *in utero* studies in mouse identified 3β , 7α -diHCA as a neuroprotective molecule towards motor neurons whereas 3β -HCA was neurotoxic. The neuroprotective mechanism is driven through LXR, indicating that specific cholestenic acids selectively work on motor neurons to regulate the balance between survival and death [29].

Oxysterols in the immune system

25-Hydroxycholesterol (25-HC) is usually found at low levels in biological samples, and there is often doubt if it is formed enzymatically by cholesterol 25-hydroxylase (CH25H) or through *ex vivo* oxidation during sample handling and storage. However, activation of macrophages through the Toll-like receptor (TLR) by lipopolysaccharide or lipid A, mimicking bacterial infection, results in marked up-regulation of CH25H and synthesis of 25-HC both in mouse and man (Figure 3) [31,32]. Bauman et al. [31] treated naïve B-cells with nM concentrations of 25-HC and found it suppressed IL-2 mediated stimulation of B-cell proliferation, repressed activation of induced cytidine deaminase expression, and blocked class switch recombination, leading to markedly reduced IgA production. They suggested that suppression of IgA class switching in B-cells in response to TLR activation provides a mechanism for negative regulation of the adaptive immune response by the innate immune system. Blanc et al. [33] have found that 25-HC is also produced by macrophages in response

to viral infection or interferon (IFN) stimulation and acts as a paracrine inhibitor of viral infection. More recently, Reboldi et al. [34] have shown that 25-HC acts as a mediator in the negative-feedback pathway of IFN signalling on IL-1 family cytokine production and inflammasome activity. *Ch25h*^{-/-} mice were found to show increased sensitivity to septic shock, exacerbated experimental autoimmune encephalomyelitis, a mouse model for multiple sclerosis, and a stronger ability to repress bacterial growth [34]. 7α , 25-Dihydroxycholesterol (7α , 25-diHC) is a down-stream metabolite of 25-HC (Figure 3) and is also involved in the immune response. Hannedouche et al. [35] and Liu et al. [36] both identified 7α , 25-diHC as a potent agonist of the G protein-coupled receptor EBI2 (GPR183). 7α , 25-diHC was found to act as a chemoattractant for immune cells expressing EBI2 by directing cell migration. *Ch25h*^{-/-} mice failed to position activated B-cells within the spleen to the outer follicle and showed a reduced plasma cell response after immune challenge [35].

The nuclear receptor RAR-related orphan receptor γ t (ROR γ t) is required for generating IL-17-producing CD4⁺ T_h17 cells which are essential in host defence and may also play pathogenic roles in autoimmune disease. CD4⁺ T-cells comprise a heterogeneous group of effector T helper (T_h)-cells which function as the conductor, orchestrating phagocytes and B-cells to effectively clear invading pathogens. Based on their cytokine-expression profile T_h-cells can be divided into various subtypes, including the pro-inflammatory T_h1 and T_h17-cells and anti-inflammatory T_{reg}-cells. Multiple sclerosis, for example, is driven by an imbalance between T_h17, T_h1 and regulatory T_{reg}-cells. Soroosh et al. [37] have identified 7β , 26-dihydroxycholesterol (7β , 26-diHC), presumably the 25R-epimer, as a potent agonist for ROR γ t. 7β , 26-diHC and its isomer 7α , 26-diHC both enhance the differentiation of murine and human IL-17-producing T_h17-cells in a ROR γ t dependent manner [37]. Interestingly, *Cyp27a1*^{-/-} mice, deficient in the (25R)26-hydroxylase required to generate both 7β , 26-diHC and 7α , 26-diHC (Figure 3) show a significant reduction in IL-17-producing cells, including CD4⁺ cells [37]. Soroosh et al. using LC-MS based technology were able to identify 7β , 26-diHC and 7α , 26-diHC in T_h17-cells as metabolic products of exogenously added 7β -hydroxycholesterol (7β -HC) and 7α -HC respectively. Furthermore, *in vitro* differentiated T_h17-cells were found to produce 7β , 26-diHC [37]. These data are particularly interesting as a sterol 7β -hydroxylase enzyme has not been identified, although an alternative route may be reduction of a 7-oxo intermediate by the enzyme HSD11B1. In other studies, cholesterol precursors, rather than oxysterols, have been suggested to be ROR γ t ligands. Hu et al. [38] found desmosterol as a potent ROR γ t agonist and showed that desmosterol accumulates during T_h17-cell differentiation as does its sulfate ester, both serving as endogenous ROR γ t agonists, whereas Santori et al. [39] identified cholesterol precursor(s) downstream of lanosterol but up-stream of zymosterol as ROR γ t ligands.

Figure 3 | Oxysterols derived from cholesterol

Oxysterols as oestrogen receptor agonists

(25R)26-HC has been shown to be a selective oestrogen receptor (ER) modulator [40]. Recently, it has been shown by Nelson et al. [41] to be an ER ligand and to increase ER-dependent growth in mouse models of breast cancer. In addition, the expression of *CYP27A1* was found to correlate with tumour grade in breast cancer specimens, and in high grade tumours *CYP27A1* was expressed in tumour cells and also tumour associated macrophages [41]. *CYP7B1*, the enzyme which metabolizes (25R)26-HC to 7 α ,(25R)26-diHC (Figure 2) was found to be elevated at the mRNA level in several different human breast cancer data sets associated with better survival outcome in luminal A types [41]. Luminal A breast cancers generally express ER, so would be expected to be effected by the oestrogenic activity of (25R)26-HC. (25R)26-HC is also a ligand to the LXRs, and through this interaction was found to promote breast cancer metastasis [41]. It is not clear which other LXR ligands may have similar effects. Importantly, the study by Nelson et al. [41]

links the oestrogenic and metastatic activity of (25R)26-HC with hypercholesterolaemia which is a risk factor for breast cancer in postmenopausal women. A second study by Wu et al. [42] published at about the same time also found (25R)26-HC to promote ER-positive breast cancer growth. In the study of Wu et al. (25R)26-HC was found to stimulate MCF-7 cell xenograph growth in mice, whereas in ER+ breast cancer patients the level of 26-HC was found to be higher in normal tissue than in similar tissue from controls. Furthermore, the 26-HC level was higher in tumour tissue than healthy tissue. The increased 26-HC level in tumour tissue was explained by reduced *CYP7B1* expression [42]. Interestingly, neither 26-HC nor cholesterol levels in plasma were found to be significantly elevated in cancer patients compared with controls, but reduced expression of *CYP7B1* was associated with poorer patient survival [42]. These two studies by Nelson et al. [41] and Wu et al. [42] linking 26-HC to ER α and breast cancer are likely to stimulate detailed studies of the sterolome in breast and other cancers.

Dendrogenin A a steroidal alkaloid

Dendrogenin A (DDA) is the product of the aminolysis reaction between 5,6 α -epoxycholesterol and histamine (Figure 3) [43]. It has been found in mouse and human tissue at pg/mg levels and in plasma at ng/ml concentrations [43]. Importantly DDA is not detected in cancer cell lines, and its concentration in breast tumours is lower than controls, suggesting anti-tumour properties. DDA triggers tumour re-differentiation and inhibits tumour growth [43]. Interestingly, DDA is an inhibitor of cholesterol epoxide hydrolase (ChEH) the enzyme which hydrolyses 5,6-epoxycholesterols (5,6-EC) to cholestane-3 β ,5 α ,6 β -triol [43]. ChEH is a dimer of 7-dehydrocholesterol reductase (DHCR7) and 3 β -hydroxysteroid- Δ^8 - Δ^7 -isomerase (D8D7I), and acts as a high affinity binding site for the anti-tumour drug tamoxifen. Accumulation of 5,6-EC as a result of inhibition of ChEH due to tamoxifen binding is likely to contribute to tamoxifen's anti-cancer pharmacology. The discovery of DDA, a metabolite of cholesterol with anti-tumour properties, contrasts to that of (25R)26-HC, a cholesterol metabolite linked to promotion of breast cancer.

Oxysterols as markers of disease

Unsurprisingly, plasma oxysterol profiles are markers of inborn errors of cholesterol metabolism, like CTX and SPG5, and of cholesterol biosynthesis e.g. Smith–Lemli–Opitz syndrome where DHCR7 is defective [44,45]. Perhaps more surprisingly, bile acids, down-stream metabolites, are markers of the lysosomal storage disease, Niemann–Pick type C (NPC) [46]. In 2001 Alvelius et al. [46] reported an unusual pattern of bile acids in urine from a patient with NPC. They found elevated levels of 3 β -hydroxy-5-ene bile acids with a 7-oxo or 7 β -hydroxy group. More recently, Porter et al. [47] reported elevated levels of 7-oxocholesterol and cholestane-3 β ,5 α ,6 β -triol in plasma from NPC1 patients. This has been confirmed in numerous other studies and concentrations of cholestane-3 β ,5 α ,6 β -triol have also been found to be elevated in NP type A and B patients [48]. The discovery of effective biomarkers for NPC1 is particularly significant in light of 2-hydroxypropyl- β -cyclodextrin showing promise as an intrathecal medication [49].

Conclusions

Oxysterol research is currently gaining attention. The involvement of oxysterols in neuroscience, immunity and cancer highlights their importance in biology. Analysis of oxysterols is still challenging and care must be taken to avoid misinterpretation of data and confusion over isomer identification.

Funding

This work was supported by the Biotechnology and Biological Sciences Research Council [grant numbers BB/I001735/1 (to

W.J.G.) and BB/L001942/1 (to Y.W.)]; and the European Regional Development Fund/Welsh Government-funded BEACON research program.

References

- Javitt, N.B. (2008) Oxysterols: novel biologic roles for the 21st century. *Steroids* **73**, 149–157 [CrossRef PubMed](#)
- Schroepfer, Jr, G.J. (2000) Oxysterols: modulators of cholesterol metabolism and other processes. *Physiol. Rev.* **80**, 361–554 [PubMed](#)
- Russell, D.W. (2003) The enzymes, regulation, and genetics of bile acid synthesis. *Annu. Rev. Biochem.* **72**, 137–174 [CrossRef PubMed](#)
- Iuliano, L. (2011) Pathways of cholesterol oxidation via non-enzymatic mechanisms. *Chem. Phys. Lipids* **164**, 457–468 [CrossRef PubMed](#)
- Murphy, R.C. and Johnson, K.M. (2008) Cholesterol, reactive oxygen species, and the formation of biologically active mediators. *J. Biol. Chem.* **283**, 15521–15525 [CrossRef PubMed](#)
- Leonarduzzi, G., Gargiulo, S., Gamba, P., Testa, G., Sottero, B., Rossin, D., Staurenghi, E. and Poli, G. (2014) Modulation of cell signaling pathways by oxysterols in age-related human diseases. *Free Radic. Biol. Med.* **75** Suppl 1, S5 [CrossRef PubMed](#)
- Dietschy, J.M. and Turley, S.D. (2004) Thematic review series: brain lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. *J. Lipid Res.* **45**, 1375–1397 [CrossRef PubMed](#)
- Lütjohann, D., Breuer, O., Ahlborg, G., Nennesmo, I., Siden, A., Diczfalussy, U. and Björkhem, I. (1996) Cholesterol homeostasis in human brain: evidence for an age-dependent flux of 24S-hydroxycholesterol from the brain into the circulation. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 9799–9804 [CrossRef PubMed](#)
- Janowski, B.A., Grogan, M.J., Jones, S.A., Wisely, G.B., Kliewer, S.A., Corey, E.J. and Mangelsdorf, D.J. (1999) Structural requirements of ligands for the oxysterol liver X receptors LXRA and LXR β . *Proc. Natl. Acad. Sci. U.S.A.* **96**, 266–271 [CrossRef PubMed](#)
- Radhakrishnan, A., Ikeda, Y., Kwon, H.J., Brown, M.S. and Goldstein, J.L. (2007) Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: oxysterols block transport by binding to Insig. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 6511–6518 [CrossRef PubMed](#)
- Horton, J.D., Goldstein, J.L. and Brown, M.S. (2002) SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* **109**, 1125–1131 [CrossRef PubMed](#)
- Gill, S., Chow, R. and Brown, A.J. (2008) Sterol regulators of cholesterol homeostasis and beyond: the oxysterol hypothesis revisited and revised. *Prog. Lipid Res.* **47**, 391–404 [CrossRef PubMed](#)
- Wang, Y., Muneton, S., Sjövall, J., Jovanovic, J.N. and Griffiths, W.J. (2008) The effect of 24S-hydroxycholesterol on cholesterol homeostasis in neurons: quantitative changes to the cortical neuron proteome. *J. Proteome Res.* **7**, 1606–1614 [CrossRef PubMed](#)
- Tint, G.S., Yu, H., Shang, Q., Xu, G. and Patel, S.B. (2006) The use of the Dhcr7 knockout mouse to accurately determine the origin of fetal sterols. *J. Lipid Res.* **47**, 1535–1541 [CrossRef PubMed](#)
- Wang, Y., Karu, K., Meljon, A., Turton, J., Yau, J.L., Seckl, J.R., Wang, Y. and Griffiths, W.J. (2014) 24S,25-Epoxycholesterol in mouse and rat brain. *Biochem. Biophys. Res. Commun.* **449**, 229–234 [CrossRef PubMed](#)
- Goyal, S., Xiao, Y., Porter, N.A., Xu, L. and Guengerich, F.P. (2014) Oxidation of 7-dehydrocholesterol and desmosterol by human cytochrome P450 46A1. *J. Lipid Res.* **55**, 1933–1943 [CrossRef PubMed](#)
- Meljon, A., Wang, Y. and Griffiths, W.J. (2014) Oxysterols in the brain of the cholesterol 24-hydroxylase knockout mouse. *Biochem. Biophys. Res. Commun.* **446**, 768–774 [CrossRef PubMed](#)
- Jansen, M., Wang, W., Greco, D., Bellenchi, G.C., di, P.U., Brown, A.J. and Ikonen, E. (2013) What dictates the accumulation of desmosterol in the developing brain? *FASEB J.* **27**, 865–870 [CrossRef PubMed](#)
- Theofilopoulos, S., Wang, Y., Kitambi, S.S., Sacchetti, P., Sousa, K.M., Bodin, K., Kirk, J., Salto, C., Gustafsson, M., Toledo, E.M. et al. (2012) Brain endogenous liver X receptor ligands selectively promote midbrain neurogenesis. *Nat. Chem. Biol.* **9**, 126–133 [CrossRef PubMed](#)

- 20 Sacchetti, P., Sousa, K.M., Hall, A.C., Liste, I., Steffensen, K.R., Theofilopoulos, S., Parish, C.L., Hazenberg, C., Richter, L.A., Hovatta, O. et al. (2009) Liver X receptors and oxysterols promote ventral midbrain neurogenesis *in vivo* and in human embryonic stem cells. *Cell Stem Cell* **5**, 409–419 [CrossRef PubMed](#)
- 21 Andersson, S., Gustafsson, N., Warner, M. and Gustafsson, J.A. (2005) Inactivation of liver X receptor beta leads to adult-onset motor neuron degeneration in male mice. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 3857–3862 [CrossRef PubMed](#)
- 22 Ogunbare, M., Theofilopoulos, S., Lockhart, A., Hall, L.J., Arenas, E., Sjövall, J., Brenton, A.G., Wang, Y. and Griffiths, W.J. (2010) Cerebrospinal fluid steridomics: are bioactive bile acids present in brain? *J. Biol. Chem.* **285**, 4666–4679 [CrossRef PubMed](#)
- 23 Song, C. and Liao, S. (2000) Cholestenic acid is a naturally occurring ligand for liver X receptor alpha. *Endocrinology* **141**, 4180–4184 [PubMed](#)
- 24 Meaney, S., Heverin, M., Panzenboeck, U., Ekström, L., Axelsson, M., Andersson, U., Diczfalussy, U., Pikuleva, I., Wahren, J., Sattler, W. and Björkhem, I. (2007) Novel route for elimination of brain oxysterols across the blood-brain barrier: conversion into 7alpha-hydroxy-3-oxo-4-cholestenic acid. *J. Lipid Res.* **48**, 944–951 [CrossRef PubMed](#)
- 25 Heverin, M., Meaney, S., Lütjohann, D., Diczfalussy, U., Wahren, J. and Björkhem, I. (2005) Crossing the barrier: net flux of 27-hydroxycholesterol into the human brain. *J. Lipid Res.* **46**, 1047–1052 [CrossRef PubMed](#)
- 26 Fakheri, R.J. and Javitt, N.B. (2012) 27-Hydroxycholesterol, does it exist? On the nomenclature and stereochemistry of 26-hydroxylated sterols. *Steroids* **77**, 575–577 [CrossRef PubMed](#)
- 27 Crick, P.J., Beckers, L., Baes, M., Van Veldhoven, P.P., Wang, Y. and Griffiths, W.J. (2015) The oxysterol and cholestenic acid profile of mouse cerebrospinal fluid. *Steroids* **99**, 172–177 [CrossRef PubMed](#)
- 28 Iuliano, L., Crick, P.J., Zerbini, C., Tritapepe, L., Abdel-Khalik, J., Poirat, M., Wang, Y. and Griffiths, W.J. (2015) Cholesterol metabolites exported from human brain. *Steroids* **99**, 189–193 [CrossRef PubMed](#)
- 29 Theofilopoulos, S., Griffiths, W.J., Crick, P.J., Yang, S., Meljon, A., Ogunbare, M., Kitambi, S.S., Lockhart, A., Tuschl, K., Clayton, P.T. et al. (2014) Cholestenic acids regulate motor neuron survival via liver X receptors. *J. Clin. Invest.* **124**, 4829–4842 [CrossRef PubMed](#)
- 30 Arnoldi, A., Crimella, C., Tenderini, E., Martinuzzi, A., D'Angelo, M., Musumeci, O., Toscano, A., Scarlato, M., Fantin, M., Bresolin, N. and Bassi, M. (2012) Clinical phenotype variability in patients with hereditary spastic paraplegia type 5 associated with CYP7B1 mutations. *Clin. Genet.* **81**, 150–157 [CrossRef PubMed](#)
- 31 Bauman, D.R., Bitmansour, A.D., McDonald, J.G., Thompson, B.M., Liang, G. and Russell, D.W. (2009) 25-Hydroxycholesterol secreted by macrophages in response to Toll-like receptor activation suppresses immunoglobulin A production. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 16764–16769 [CrossRef PubMed](#)
- 32 Diczfalussy, U., Olofsson, K.E., Carlsson, A.M., Gong, M., Golenbock, D.T., Rooyackers, O., Flaring, U. and Björkbacka, H. (2009) Marked upregulation of cholesterol 25-hydroxylase expression by lipopolysaccharide. *J. Lipid Res.* **50**, 2258–2264 [CrossRef PubMed](#)
- 33 Blanc, M., Hsieh, W.Y., Robertson, K.A., Kropp, K.A., Forster, T., Shui, G., Lacaze, P., Watterson, S., Griffiths, S.J., Spann, N.J. et al. (2013) The transcription factor STAT-1 couples macrophage synthesis of 25-hydroxycholesterol to the interferon antiviral response. *Immunity* **38**, 106–118 [CrossRef PubMed](#)
- 34 Reboldi, A., Dang, E.V., McDonald, J.G., Liang, G., Russell, D.W. and Cyster, J.G. (2014) Inflammation. 25-Hydroxycholesterol suppresses interleukin-1-driven inflammation downstream of type I interferon. *Science* **345**, 679–684 [CrossRef PubMed](#)
- 35 Hannedouche, S., Zhang, J., Yi, T., Shen, W., Nguyen, D., Pereira, J.P., Guerini, D., Baumgarten, B.U., Roggo, S., Wen, B. et al. (2011) Oxysterols direct immune cell migration via EBI2. *Nature* **475**, 524–527 [CrossRef PubMed](#)
- 36 Liu, C., Yang, X.V., Wu, J., Kuei, C., Mani, N.S., Zhang, L., Yu, J., Sutton, S.W., Qin, N., Banie, H. et al. (2011) Oxysterols direct B-cell migration through EBI2. *Nature* **475**, 519–523 [CrossRef PubMed](#)
- 37 Soroosh, P., Wu, J., Xue, X., Song, J., Sutton, S.W., Sablad, M., Yu, J., Nelen, M.I., Liu, X., Castro, G. et al. (2014) Oxysterols are agonist ligands of RORgamma and drive Th17 cell differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 12163–12168 [CrossRef PubMed](#)
- 38 Hu, X., Wang, Y., Hao, L.Y., Liu, X., Lesch, C.A., Sanchez, B.M., Wendling, J.M., Morgan, R.W., Aicher, T.D., Carter, L.L. et al. (2015) Sterol metabolism controls T(H)17 differentiation by generating endogenous RORgamma agonists. *Nat. Chem. Biol.* **11**, 141–147 [CrossRef PubMed](#)
- 39 Santori, F.R., Huang, P., van de Pavert, S.A., Douglass, Jr, E.F., Leaver, D.J., Haubrich, B.A., Keber, R., Lorbek, G., Konijn, T., Rosales, B.N. et al. (2015) Identification of natural RORgamma ligands that regulate the development of lymphoid cells. *Cell Metab.* **21**, 286–297 [CrossRef PubMed](#)
- 40 Umetani, M., Domoto, H., Gormley, A.K., Yuhanna, I.S., Cummins, C.L., Javitt, N.B., Korach, K.S., Shaul, P.W. and Mangelsdorf, D.J. (2007) 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. *Nat. Med.* **13**, 1185–1192 [CrossRef PubMed](#)
- 41 Nelson, E.R., Wardell, S.E., Jasper, J.S., Park, S., Suchindran, S., Howe, M.K., Carver, N.J., Pillai, R.V., Sullivan, P.M., Sondhi, V. et al. (2013) 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. *Science* **342**, 1094–1098 [CrossRef PubMed](#)
- 42 Wu, Q., Ishikawa, T., Sirianni, R., Tang, H., McDonald, J.G., Yuhanna, I.S., Thompson, B., Girard, L., Mineo, C., Brecken, R.A. et al. (2013) 27-Hydroxycholesterol promotes cell-autonomous, ER-positive breast cancer growth. *Cell Rep.* **5**, 637–645 [CrossRef PubMed](#)
- 43 de Medina, P., Paillasse, M.R., Segala, G., Voisin, M., Mhamdi, L., Dalenc, F., Lacroix-Triki, M., Filleron, T., Pont, F., Saati, T.A. et al. (2013) Dendrogenin A arises from cholesterol and histamine metabolism and shows cell differentiation and anti-tumour properties. *Nat. Commun.* **4**, 1840 [CrossRef PubMed](#)
- 44 Clayton, P.T. (2011) Disorders of bile acid synthesis. *J. Inher. Metab. Dis.* **34**, 593–604 [CrossRef PubMed](#)
- 45 Shackleton, C.H. (2012) Role of a disordered steroid metabolome in the elucidation of sterol and steroid biosynthesis. *Lipids* **47**, 1–12 [CrossRef PubMed](#)
- 46 Alvelius, G., Hjalmarson, O., Griffiths, W.J., Björkhem, I. and Sjövall, J. (2001) Identification of unusual 7-oxygenated bile acid sulfates in a patient with Niemann-Pick disease, type C. *J. Lipid Res.* **42**, 1571–1577 [PubMed](#)
- 47 Porter, F.D., Scherrer, D.E., Lanier, M.H., Langmade, S.J., Molugu, V., Gale, S.E., Olzeski, D., Sidhu, R., Dietzen, D.J., Fu, R. et al. (2010) Cholesterol oxidation products are sensitive and specific blood-based biomarkers for Niemann-Pick C1 disease. *Sci. Transl. Med.* **2**, 56ra81 [CrossRef PubMed](#)
- 48 Klinke, G., Rohrbach, M., Giugliani, R., Burda, P., Baumgartner, M.R., Tran, C., Gautschi, M., Mathis, D. and Hersberger, M. (2015) LC-MS/MS based assay and reference intervals in children and adolescents for oxysterols elevated in Niemann-Pick diseases. *Clin. Biochem.* **48**, 596–602 [CrossRef PubMed](#)
- 49 Maarup, T.J., Chen, A.H., Porter, F.D., Farhat, N.Y., Ory, D.S., Sidhu, R., Jiang, X. and Dickson, P.I. (2015) Intrathecal 2-hydroxypropyl-beta-cyclodextrin in a single patient with Niemann-Pick C1. *Mol. Genet. Metab.* **116**, 75–79 [CrossRef PubMed](#)

Received 25 January 2016

doi:10.1042/BST20150255