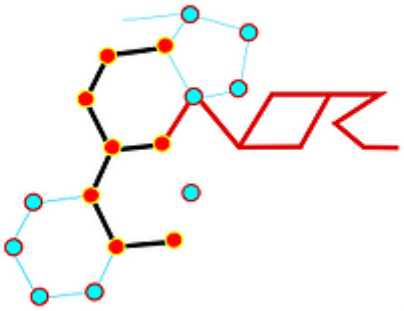

European Network for Oxysterol Research (ENOR)

Program

10th ENOR Symposium – Web Meeting

2021

September 16th (whole day)- September 17th (morning)

Organizing commitee

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Invited speakers

Peter Tontonoz

(University of California, Los Angeles, USA)

New Pathways in Cellular and Systemic Lipid Transport

Jiang Xuntian

(Washington University School of Medicine in St. Louis, USA) ;

Newborn Screening for Niemann-Pick C Disease

Program

10th ENOR Symposium – Web Meeting
16-17 September 2021

All times CET

Oral communications (10 minutes presentation + 5 minutes questions)
Flash talks (5 min per talk + 10 minutes at the end of presentations)

Day 1
16th September

08:30-08:40 Opening messages from Home Chair (Gérard Lizard and Luigi Iuliano) and information regarding digital communication (Hanne Røberg-Larsen and James Thorne)

Session 1: Metabolism of oxysterols and phytosterols

08:40-09:40 Oral communications Session 1 – Chair: Dr. Silvère Baron

OC1 **S. Baumgartner** - Plasma oxyphytosterols most likely originate from hepatic oxidation and subsequent spill-over in the circulation

OC2 **K. Ronacher** - Oxysterols in the lung: implications for respiratory infection outcomes and opportunities for more therapeutics

OC3 **S. Maioli** - Sex-dependent effects of CYP46A1 overexpression on cognitive function during aging

OC4 **J.H. Taskinen** - Global effects of pharmaceutical inhibition of OSBP in HUVECs

09:40-10:15 Flash Talk Session 1 – Chair: Imen Ghzaiel

FT1 **F. Canzoneri** - Cholesterol oxidation products as markers of nutritional quality of milk and milk products

FT2 **L. Griffiths** - Analysing cholesterol in mouse and human brain tissue using mass spectrometry imaging.

FT3 **V.M.S. Kjær** - Discovery of GPR183 Agonists Based on an Antagonist Scaffold

FT4 **F. Stellaard** - Serum markers of cholesterol absorption and synthesis in a young mixed dyslipidemic population with moderately enhanced serum cholesterol concentrations

FT5 **M. Ali Asgari** - Sterol and Oxysterol Markers of Huntington's Disease: The 24S-Hydroxycholesterol Pathway

10:15-10:40 Coffee break / comfort break

10:40-11:25 Oral Communication Session 2 – Chair: Prof. Jogchum Plat

OC5 **N. Zhan** - Paradoxical regulation of cholesterol metabolism by fucosterol and saringosterol

OC7 **H. Røberg-Larsen** - Oxysterols secreted from a Non-Alcoholic Fatty Liver Disease (NAFLD) induced liver organoids

OC8 **G. Testa** - 24-Hydroxycholesterol reduces tau levels through proteasome activation via SIRT1/PGC1α/Nrf2 pathway: a promising strategy to counteract Alzheimer's disease.

11:25-12:10 Flash Talk Session 2 – Chair: Eylan Yutuc

FT6 P. de Médina - Side chain hydroxylation of cholestane-3β,5α,6β-triol and Oncosterone lead to the production of new endogenous oxysterols potentially involved in breast cancer development.

FT7 P. de Médina - Inhibition of oncosterone biosynthesis as a new pharmacological anticancer strategy in breast cancer.

FT8 A. Websdale - Pharmacologic and genetic inhibition of cholesterol esterification reduces tumour burden: a pan-cancer systematic review and meta-analysis of preclinical models

FT9 M.G. Nasoni - Secosterol-B triggers oxidative-inflammatory response associated with alterations of eNOS/Cav-1 expression in HMEC-1 cells

FT10 S. Giannelli - Attractive targets to fight Alzheimer's disease: from oxysterol profile alteration to SIRT-1 and Nrf2 decline

FT20 M. Roumain - Investigation of the Cyp27a1-mediated hydroxylation of 4β-hydroxycholesterol

12:15-13:00 Lunch

Session 2: Sterol chemistry and methodology advancements in oxysterol research

13:00-14:00 Plenary 1 - Jiang Xuntian

14:00-15:00
Oral Communication Session 3 – Chair: Prof. Yuqin Wang

OC9 E. Yutuc and M. Pacciarini - Sterolomic profiling of human CSF and plasma to reveal altered cholesterol metabolism in Parkinson's Diseases

OC10 C. Soteriou - Dietary phytosterols impair oncogene signalling via modulation of membrane lipid organisation

OC11 K. Borah - Assessment of oxysterol outcomes in immune cells targeted with reactive oxygen species (ROS) amplifiers

OC12 H. Schaller - High phytosterol variants towards improved feedstocks and biofortification of crops

15:00-15:35 Flash Talk Session 3 – Chair: Alex Websdale

FT11 P. Holy - Comprehensive assessment of germline and somatic variants in oxysterol-related genes in breast cancer patients

FT12 E. Staurenghi - Oxysterols present in Alzheimer's disease brain induce synaptotoxicity by activating astrocytes: a major role for lipocalin-2

FT13 A. Yammine - Prevention by oleic and docosahexaenoic acids of 7-ketocholesterol-induced mitochondrial and peroxisomal alteration on murine neuronal N2a cells

FT14 T. Nury - Prevention of 7-ketocholesterol-induced oxiapoptophagy by sulfo-N-succinimidyl oleate (SSO) on murine oligodendrocytes 158N

FT15 D. Abed-Vieillard - 7-Ketocholesterol effects on survival and growth in Drosophila melanogaster

15:35-16:00 Coffee break / comfort break

16:00-16:45
Oral Communication Session 4 – Chair: Dr. Irundika Dias

OC13 M Poirot - Characterization of a cholesterol metabolic switch controlling breast carcinogenesis
OC14 K. Hawkins - Distribution and Abundance of Oxysterols in the Human Multiple Sclerosis Brain
OC15 V. Leoni - Plasma and tissue distribution of 27-hydroxycholesterol after oral administration to mice.

16 :45-17:00 Coffee break / comfort break

Session 3.1: Oxysterols in Health and Disease

17:00-18:00 Plenary 2 - Peter Tontonoz

Day 1 Close

Day 2
17th September

Session 3.2: Oxysterols in Health and Disease (cont.)

08:45 Welcome to second day

09:00-9:45 Oral Communication Session 5 – Chair: Prof. Maria Teresa Rodriguez Estrada

OC16 I.H.K. Dias - Parallel changes occur in systemic oxysterol levels and retinal vascular function in ageing
OC17 P. Lianto - Alternative splicing that disrupts ligand binding domains in liver x receptors predicts survival in triple negative breast cancer
OC18 A. Spalenkova - The influence of 7-ketocholesterol on tamoxifen efficacy in breast cancer

9:45-10:15
Flash Talk Session 4 – Chair: Stian Kogler

FT16 C. Mounier - LXR signalling in the striatum and neuroprotection in Huntington's Disease
FT17 M. Ksila - In vitro evaluation of the effect of lemon essential oils on 7-ketocholesterol-induced cytotoxicity
FT18 N. Mankeviciute-Delporte - Characterization of LXRb specific modulators
FT19 S. Ghosh - Bioremediation of 7-Ketocholesterol and subsequent biotransformation by cholesterol oxidase nano-conjugates

10:15-10:30 Coffee break / Comfort break

10:30-12:00
Oral Communication Session 6 – Chair: Prof. Steven Wilson

OC19 I. Ghzaiel - 7β-hydroxycholesterol-induced oxidative stress, mitochondrial and peroxisomal dysfunctions: attenuation with Milk thistle seed oil
OC20 K. Diallo - New targeting in oxysterol metabolism for triple negative breast cancer therapy
OC21 Y. Urano - 25-Hydroxycholesterol induces ferroptosis via downregulation of mevalonate pathway in Schwann cells
OC22 L. Arendholz - The oxysterol receptor EBI2 is involved in the pathogenesis of murine contact hypersensitivity
OC23 F. Ruiz - Endothelial cells-derived Oxysterols promotes neuroinflammation trough suppression of Myeloid-Derived Suppressor Cells
OC24 S. Dallel - Liver X receptors and ovarian hyperstimulation syndrome

12:00-13:00 Lunch

13:00-13:30 Oral communications and flash talks awards

13:30-14:00 ENOR's general assembly and concluding remarks

NB: *people doing either a flash talk or doing an oral presentation will have the possibility to submit a paper (research paper, review) in Steroids (ENOR Special Issue: deadline end of march 2022)*

OC1

Plasma oxyphytosterols most likely originate from hepatic oxidation and subsequent spill-over in the circulation

S. Baumgartner[1], D. Lütjohann[2], C. Husche[2], A. Kerksiek[2], A.K. Groen[3], R.P. Mensink[1] and J. Plat[1]

[1]Department of Nutrition and Movement Sciences. NUTRIM School of Nutrition and Translational Research in Metabolism. Maastricht University, Maastricht, 6200 MD, The Netherlands. [2]Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, D-53127, Germany. [3]Amsterdam Diabetes Center and Department of Vascular Medicine, Amsterdam University Medical Center, Amsterdam, 1105 AZ, The Netherlands.

We evaluated oxyphytosterol (OPS) concentrations in plasma and various tissues of two genetically modified mouse models with either increased cholesterol (apoE KO mice) or increased cholesterol and plant sterol (PS) concentrations (apoExABCG8 dKO mice). Sixteen female apoE KO and 16 dKO mice followed the same standard, low OPS-chow diet. Animals were euthanized at 36 weeks to measure PS and OPS concentrations in plasma, brain, liver and aortic tissue. Cholesterol and oxysterol (OS) concentrations were analyzed as reference for sterol oxidation in general. Plasma campesterol (24.1±4.3 vs. 11.8±3.0 mg/dL) and sitosterol (67.4±12.7 vs. 4.9±1.1 mg/dL) concentrations were severely elevated in the dKO compared to the apoE KO mice ($p<0.001$). Also, in aortic and brain tissue, PS levels were significantly elevated in dKO. However, plasma, aortic and brain OPS concentrations were comparable or even lower in the dKO mice. In contrast, in liver tissue, both PS and OPS concentrations were severely elevated in the dKO compared to apoE KO mice (sum OPS: 7.4±1.6 vs. 4.1±0.8 ng/mg, $p<0.001$). OS concentrations followed cholesterol concentrations in plasma and all tissues suggesting ubiquitous oxidation. Despite severely elevated PS concentrations, OPS concentrations were only elevated in liver tissue, suggesting that OPS are primarily formed in the liver and plasma concentrations originate from hepatic spill-over into the circulation.

OC2

Oxysterols in the lung: implications for respiratory infection outcomes and opportunities for more therapeutics.

Minh Dao Ngo[1], Cheng Xiang Foo[1], Stacey Bartlett[1], Helle Bielefeld-Ohmann[2,3], Roma Sinha[1], Liv von Voss Christensen[4], Kirsty R. Short[2,3], Thomas Mandrup-Poulsen[4], Mette Marie Rosenkilde[4], Katharina Ronacher[1,2]

1. Mater Research Institute, The University of Queensland, Translational Research Institute, Brisbane, QLD, Australia. 2. Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, QLD, Australia. 3. School of Biomedical Sciences, The University of Queensland, QLD, Australia. 4. Department of Biomedical Sciences, University of Copenhagen, DK.

Our team previously reported that expression of the oxidised cholesterol-sensing receptor GPR183 is significantly downregulated in blood leukocytes from tuberculosis (TB) patients with diabetes compared to TB patients without co-morbidities and that this is associated with tuberculosis disease severity on chest-x-ray [1]. We therefore aimed to study the role of GPR183 during *M. tuberculosis* (Mtb) and viral respiratory infections in the lung.

Consistent with observations in humans, mice with dysglycemia and infected with Mtb had more severe lung damage [2]. We found that the 7□□25OHC-producing enzymes CH25H and CYP7B1 were highly upregulated upon Mtb infection in the lungs of normal chow diet (NCD)-fed mice, and this was associated with increased expression of GPR183 indicative of effective recruitment of GPR183-expressing immune cells to the site of infection. Using immunofluorescence staining on lung sections, we showed that CYP7B1 was predominantly expressed by Siglec F^+ positive alveolar macrophages in the centre of TB granulomatous structures. Expression of CH25H and CYP7B1 was significantly blunted in lungs from HFD-fed dyglycemic animals which coincided with delayed recruitment of macrophages to the lung during early infection and more severe lung pathology. While GPR183KO mice were more susceptible to Mtb during early infection (before initiation of an adaptive immune response), they were more resistant to pneumonia virus of mice (PVM) infections with a 50% reduction in PVM titers, and lower concentrations of inflammatory cytokines; the latter was also observed in the lungs of influenza A virus (IAV) infected GPR183 KO mice vs. WT. The GPR183 antagonist NIBR189 [3] reduced transmigration of immune cells to IVA infected lungs and inflammatory cytokine production at the site of infection.

Together our data suggest that oxidised cholesterols produced by alveolar macrophages upon Mtb infection play an important role in positioning of GPR183+ immune cells in TB granulomas. We provide evidence that HFD-induced dysglycemia disturbs the oxysterol/GPR183 axis and impairs macrophage recruitment which is associated with more severe TB. Finally, we demonstrate that lack of GPR183 has differential pathogen-specific effects.

[1] GPR183 regulates interferons, autophagy and bacterial growth during *Mycobacterium tuberculosis* infection and is associated with TB disease severity. Bartlett S, Gemiarto AT, Ngo MD, Sajiir H, Hailu S, Sinha R, Foo CX, Kleynhans L, Tshivhula H, Webber T, Bielefeldt-Ohmann H, West NP, Hiemstra AM, MacDonald CE, Christensen LVV, Schlesinger LS, Walzl G, Rosenkilde MM, Mandrup-Poulsen T, Ronacher K. Front Immunol 2020 doi: 10.3389/fimmu.2020.601534

[2] Pre-diabetes increases tuberculosis disease severity, while high body fat without impaired glucose tolerance is protective. Sinha R, Ngo MD, Bartlett S, Bielefeldt-Ohmann H, Keshvari S, Hasnain SZ, Donovan ML, Kling J, Blumenthal A, Chen C, Short KR, Ronacher K. Front Cell Infect Microbiol 2021 doi: 10.3389/fcimb.2021.691823

[3] Identification and characterization of small molecule modulators of the EBI2 Receptor. Gessier F, Preuss I, Rosenkilde MM, Laurent S, Endres R, Chen YA, Marsilje T, Seuwen K, Nguyen DG, Sailer A. J. Med. Chem 2014 doi: 10.1021/jm4019355

OC3

Sex-dependent effects of CYP46A1 overexpression on cognitive function during aging

Latorre-Leal M, Rodriguez-Rodriguez P, FranchiniL, Daniilidou M, Eroli F, Winblad B, Blennow K, Zetterberg H, Kivipelto M, Pacciarini M, Wang Y, Griffiths J, Björkhem I, Sandebring Matton A, Merino-Serrais P, Cedazo-Minguez A, Maioli S

Division of Neurogeriatrics, NVS Department, Center for Alzheimer Research, Stockholm, Sweden

Cholesterol turnover and CYP46A1 regulation are reported to be crucial for memory functions. An increasing body of evidence shows that CYP46A1 activation is able to reduce Alzheimer's Disease (AD) pathological processes. In this study we report for the first time that CYP46A1 overexpression and increase of 24S-hydroxycholesterol (24OH) induces sex-specific changes in synaptic functions in aged mice, being beneficial in females while detrimental in males. The positive effects on cognition in aged CYP46A1 overexpressing female mice were accompanied by morphological changes in dendritic spines and enhancement of estrogen receptor signaling in hippocampus. In aged males, CYP46A1 overexpression leads to anxiety-like behavior and worsening of spatial memory, followed by decreased dendritic spine density and higher 5α-dihydrotestosterone (DHT) levels in hippocampus. Further, analysis of cerebrospinal fluid (CSF) from AD, mild cognitive impairment and healthy patients revealed that 24OH was negatively associated to markers of neurodegeneration in women but not in men. Based on our results, CYP46A1 activation may represent a pharmacological target that could specifically enhance brain estrogen receptor signaling in women at risk of developing AD. Finally, this study highlights the importance of taking into account the sex-dimension in both preclinical and clinical studies of neurodegenerative diseases like AD.

OC4

Global effects of pharmaceutical inhibition of OSBP in HUVECs

Juuso H. Taskinen, Vesa M. Olkkonen

Minerva Foundation Institute for Medical Research, Biomedicum 2U, Helsinki, Finland

Background: OSBP (oxysterol-binding protein) is a cholesterol/PI4P exchanger at contact sites of the endoplasmic reticulum with *trans*-Golgi, late endosomes, and recycling endosomes. The function of this ubiquitously expressed protein has mainly been studied in immortalized or cancer-derived cell lines. Several central endothelial cell (EC) functions depend on an adequate distribution of cholesterol in the cellular membranes; however, the role of OSBP in this clinically crucial cell type has thus far been ignored.

Methods: OSBP was in inhibited in HUVECs for 24 h with 25 nM Schweinfurthin G (SWG) or Orsaponin (OSW1), followed by analysis of cellular free cholesterol, immunofluorescence microscopy and western blot analysis of OSBP. We also employed a battery of hypothesis free omics methods, such as mass spectrometric lipidomics, as well as next-generation RNA sequencing of the SWG-treated and control HUVECs.

Results: RNA sequencing of HUVEC treated for 24 h with 25 nM SWG revealed altered expression of ER stress and angiogenesis related pathways, especially the Alzheimer, Huntington and Parkinson's disease related pathways, as well as cilia-related and the VEGFA-VEGFR2 signaling pathway. SWG inhibition also significantly downregulated cholesterol homeostatic genes such as *LDLR*, *DHCR24*, *HMGCR* and *SREBF2*, consistent with an ER cholesterol exit defect. Global lipidomic analysis of the SWG-treated HUVECs revealed a significant decrease in PS species and mild but not significant reductions in the cellular concentrations of several PC and PE species, as well as a significant increment of cholesterol esters (ChoE) as compared to vehicle (DMSO) controls, while unesterified cholesterol was reduced. The OSW1-treated cells showed a similar pattern, but the magnitude of upregulation in ChoE and downregulation of PS was greater than for SWG, but reduction in unesterified cholesterol was milder. Immunofluorescence imaging revealed that, as previously reported for HUVECs, OSBP was already associated with the TGN (Trans-Golgi Network) in the absence of the inhibitor treatments. In SWG- or OSW1-treated cells OSBP disappeared from the TGN, which was apparently fragmented, in a time-dependent manner, coinciding with reduction of the OSBP protein on western blots. Moreover, we observed that 24-h treatment with OSW1 or SWG hampered the adherence of HUVEC on fibronectin/gelatin-coated substratum, coinciding with a respective 25 or 20% reduction of metabolic activity as measured with MTT assay. Of note, both inhibitors slowed down HUVEC angiogenic tube formation *in vitro* as compared to vehicle control or to naïve HUVECs.

Conclusion: Inhibition of OSBP with SWG modifies the HUVEC transcriptome including genes related to ER stress, cilia, angiogenesis and cholesterol homeostasis. Subsequent ER stress might be due to altered lipid composition of ER membranes and fragmentation of the TGN. Cilia-related genes may be affected due to perturbed cholesterol homeostasis, ciliary function depending on cholesterol. Disturbed cholesterol homeostasis and ER-stress likely compromise the cells' capacity of angiogenic tube formation. Thus, OSBP is a crucial regulator of EC lipid homeostasis, ER maintenance and lipid-mediated signals that control EC adhesion, metabolic activity and angiogenesis.

OC5

Paradoxical regulation of cholesterol metabolism by fucosterol and saringosterol

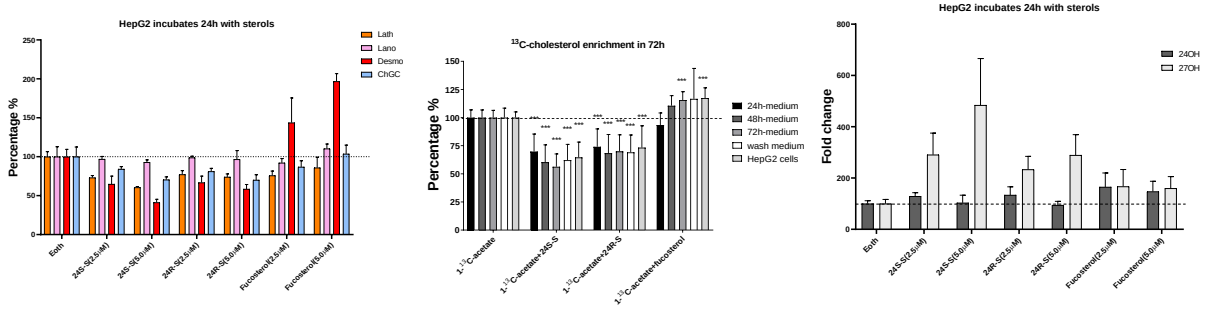
Na Zhan[1,2,4], Silvia.Friedrichs[2], Gardi J. Minderman-Voortman[1], Nikita Martens[1,3], Tim Vanmierlo[3], Hong-Bing Liu[4], Dieter Lütjohann[2], Monique T. Mulder[1]

[1]Department of Internal Medicine, Laboratory Vascular Medicine, Erasmus MC University Rotterdam, Ee-816, Wytemaweg 80, 3015CN Rotterdam, The Netherlands; [2]Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, Bonn, Germany; [3]Neuro-Immune Connect & Repair Lab, Biomedical Research Institute, Hasselt University, Hasselt, Belgium; [4]Key Laboratory of Marine Drugs, Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao, China.

Accumulating evidence indicates a key role for a disturbed cholesterol transport in the brain during the development and progression of Alzheimer's disease (AD). We have reported that memory of AD mice improves when their brain cholesterol turnover is being activated by T0901317, a synthetic activator of liver x receptors α and β (LXRα/β). LXRs are promising well-studied therapeutic targets for increasing cholesterol turnover and decreasing neuroinflammation in AD. However, synthetic LXR agonists induce undesirable side effects like hypertriglyceridemia and hepatic steatosis, hampering the use of synthetic LXR agonists in the clinic. The phytosterol fucosterol and its oxidation product saringosterol, are natural compounds present in Phaeophyta and other algae. Saringosterol and fucosterol have been reported to activate LXRs, although the latter to a lesser extent. Our recent studies showed that saringosterol can traverse the blood-brain barrier (BBB) and prevent memory decline in Alzheimer mice, possibly by affecting cholesterol turnover. Neuroprotective effects of fucosterol which crosses the BBB to a limited extent have also been reported. However, the in-depth biological mechanisms supporting its neuroprotective effect is poorly understood. Both fucosterol and saringosterol may affect cholesterol turnover at multiple points.

In this study, we focused on the cellular internalization of fucosterol and saringosterol, and illustrate molecular pathways through which they affect cholesterol turnover. We found that fucosterol and 24S- or 24R-Saringosterol were taken up by HepG2, SH-SY5Y and CCF-STTG1 cells. The percentage of fucosterol accumulating in cells is 15% after 24h incubation, which is half that of 24S-or 24R-Saringosterol. Desmosterol, as an endogenous LXR agonist is an immediate precursor of cholesterol in the Bloch pathway. 24S-or 24R-Saringosterol down-regulated, while fucosterol upregulated desmosterol level in HepG2 cells as measured by GC-MS (**Figure 1**). The more obvious decrease or increase effects were observed in the higher concentration groups. When we incorporated 1-^{13}C-acetate into newly synthesized cholesterol and calculated the newly enrichment of cholesterol, we found that the enrichment of ^{13}C-cholesterol of the 24S- or 24R-Saringosterol or fucosterol group was conversely affected compared with the control group (**Figure 2**). However, 24S- or 24R-Saringosterol greatly promotes the synthesis of 27-hydroxycholesterol (**Figure 3**). Presently, we are taking the hypothesis that fucosterol inhibits DHCR24 or promotes DHCR7, resulting in the rapid accumulation of the substrate desmosterol. Both 24S- or 24R-Saringosterol increased 27-hydroxycholesterol, thereby reducing desmosterol and cholesterol levels. Possibly, 27-hydroxycholesterol may also reduce desmosterol via acting as a negative feed back regulation of cholesterol biosynthesis. Alternatively, saringosterol may inhibit DHCR7, thus synthesis of cholesterol was interrupted at the level of its immediate precursor.

Our data show opposite effect of saringosterol and fucosterol on cholesterol metabolism, decreasing and increasing levels of desmosterol, respectively. Fucosterol may indirectly activate LXR through upregulating desmosterol.



OC7

Oxysterols secreted from a Non-Alcoholic Fatty Liver Disease (NAFLD) induced liver organoids

Henriette Nordli[1], Aleksandra Aizenshtadt[2], Stefan Krauss[2],Steven R Wilson[1, 2] and Hanne Røberg-Larsen[1]

[1] *Department of Chemistry, University of Oslo, Norway*
[2] *Hybrid Technology Hub – Centre of Excellence, Institute of Basic Medical Science, University of Oslo, Norway*

Non-Alcoholic Fatty Liver Disease (NAFLD) is a spectrum of heterogenous liver disorders with rapidly growing worldwide prevalence. The stages of the disease are differentiated by the severity of the conditions, ranging from fatty liver and fibrosis (both reversible) to irreversible cirrhosis. Today's most used and accurate diagnostic tool for disease progression is an invasive liver biopsy. Hence there is a need to find biomarkers of disease progression that can be monitored in a non-invasive way compared to liver biopsy.

Organoids are simplified three-dimensional tissue models of human organs. The organoids can be generated from induced pluripotent stem cells (iPSC) or adult tissue-resident cells and better reflect the physiological conditions and complexity in humans, compared to animal models and immortalized cell lines.

We have established NAFLD *in vitro* modes using human primary hepatocyte (pHLO) and iPSC-derived liver organoids (iHLO). pHLO and iHLO have been cultured in the presence of pathophysiological concentrations of free fatty acids, insulin, and glucose to induce steatosis. Using our established derivatization with Girard T reagent and UHPLC-MS/MS method, modified for analysing cell culture medium samples, we were able to quantify the LXR active 24S-OHC, 25-OHC and 26-OHC secreted from healthy and NAFLD induced liver organoids. The steatotic organoids secret an increased amount of these oxysterols compared to healthy organoids. The steatotic organoids also secrets a larger amount of di-hydroxycholesterol (the identification process is ongoing). In a (blank) cell culture medium sample not exposed to organoids, all these oxysterols were below detection limits.

Organoids have the potential of becoming an important tool in disease monitoring, drug development, and personalized medicine. The larger secretion of oxysterols from NAFLD induced organoids, together with proteomics data, will be used to evaluate if oxysterols are suitable a diagnostic tool for the NAFLD disease progression and to better help us understanding liver organoids as NAFLD disease models.

OC8

24-Hydroxycholesterol reduces tau levels through proteasome activation via SIRT1/PGC1α/Nrf2 pathway: a promising strategy to counteract Alzheimer's disease.

Testa G., Giannelli S., Gamba P., Staurenghi E., Sottero B., Poli G., Leonarduzzi G.

Department of Clinical and Biological Sciences, University of Torino, San Luigi Hospital, Orbassano (Torino), Italy.

It has become clear that cholesterol dysmetabolism in the brain is involved in Alzheimer's disease (AD) development: it is now believed that oxysterols, cholesterol oxidation products, are the link connecting altered brain cholesterol metabolism to AD. Regarding the pathological hallmarks of AD, much is known about the link between cholesterol dysmetabolism and deposition of amyloid-beta (Aβ), while its relationship with the accumulation of neurofibrillary tangles (NFTs) made of hyperphosphorylated tau is currently almost unknown.

Previously, we showed that the oxysterol 24-hydroxycholesterol (24-OHC) up-regulates the levels of the neuroprotective enzyme sirtuin 1 (SIRT1) in neuroblastoma SK-N-BE cells, consequently preventing the intracellular accumulation of insoluble tau aggregates. Interestingly, we observed that the levels of SIRT1 markedly decrease in the brain with AD progression, in parallel with the loss of 24-OHC and accumulation of NFTs. It has been hypothesized that 24-OHC favors tau degradation by inducing SIRT1-dependent deacetylation of tau.

We are now investigating whether 24-OHC might reduce tau levels via proteasome activation. We have observed that 24-OHC, via SIRT-1/PGC1α activation, induces a significant up-regulation of both Nrf2 expression and synthesis and leads to a decrease of Nrf2/Keap1 complex inducing a nuclear translocation of Nrf2. Importantly, as a consequence of Nrf2 activation by 24-OHC, we observed a significant increase of tau deacetylation and ubiquitination, key events leading to tau proteasomal degradation, with the consequent decrease of both phosphorylated and total tau levels.

These data suggest the importance of preventing the loss of 24-OHC in the brain during the course of AD. The administration of 24-OHC, able to promote tau proteasomal degradation, may provide a promising therapeutic strategy aimed at reducing NFTs accumulation.

OC9

Sterolomic profiling of human CSF and plasma to reveal altered cholesterol metabolism in Parkinson's Diseases

Eylan Yutuc[1], Manuela Pacciarini[1], Anders Öhman[2], Lars Forsgren[2], Miles Trupp[2], Yuqin Wang[1], William J. Griffiths[1]

[1]*Institute of Life Science 1, Swansea University Medical School, SA2 8PP, Swansea, United Kingdom*
[2]*Umea University, Umea, Sweden*

Parkinson's disease (PD) is a neurodegenerative disorder characterised by a progressive and uncontrollable deterioration of dopaminergic cells from the nigrostriatal pathway, resulting in typical motor symptoms like tremor, rigidity and movement imbalance and non-motor manifestations, including neuropsychiatric symptoms. The complex and multifactorial nature of this condition makes the aetiology still uncertain, with no specific and effective treatments available.

However, recent findings link disordered brain cholesterol metabolism to PD development, pointing out a plausible central role for sterol molecules in the main PD pathological pathways, represented by oxidative stress, endosomal-lysosomal dysfunction and neuroinflammation.

To shed a light on the contribution of sterols to PD, human plasma and CSF samples from PD patients (n=100) and non-PD controls (n=102) have been analysed through a targeted lipidomic strategy focusing on the sterols content.

The method consists of a multi-step procedure starting from sterol extraction, chromatographic separation, enzymatic assisted derivatisation to LC-MS qualitative/quantitative analysis.

A total of 19 CSF and 22 plasma sterols/oxysterols have been partially or fully identified, ranging a concentration from 0.01 to 11,000 ng/mL in CSF and from 0.07 to 700,000 ng/mL in plasma.

Of the 19 CSF sterols/oxysterols, 2 represent cholesterol precursors, 1 C_{24} bile acid, 6 hydroxysterols and 9 cholestenoic acids, while for plasma 2 cholesterol precursors, 13 hydroxysterols and 6 cholestenoic acids have been identified. Mann-Whitney and Kolmogorov-Smirnov tests were used to compare patients and controls CSF and plasma sterols/oxysterol levels.

Identifying relevant differences in the cholesterol metabolite content between PD patients and non-PD individuals could serve as a starting point for novel R&D strategies as well for the identification of clinically significant biomarkers for disease risk, onset, and progression.

OC10

Dietary phytosterols impair oncogene signalling via modulation of membrane lipid organisation

C.Soteriou[1,2,3], A.C.Kalli[2], S.D.Connell[3], A.I.I.Tyler[1], J.L.Thorne[1]

[1]*School of Food Science and Nutrition, University of Leeds, Leeds LS29JT, UK*
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The plasma membrane acts as a barrier and conveyor of information from the external environment to the cell. In cancer cells the lipid composition of the plasma membrane is dramatically altered in a way that permits enhanced initiation of oncogenic signalling cascades[1]. One such oncogene, AKT, depends on phosphatidylinositol (PIP) lipids for activation. PIPs are located in cholesterol-rich domains[2] of the plasma membrane, thus linking AKT to cholesterol rich regions. Phytosterols (PSS) are plant derived compounds commonly found in nuts and seeds. PSS disrupt formation of cholesterol-rich domains due to their structural and biophysical similarity to cholesterol[3]. We recently found PSS impair AKT activity *in vivo*[4] in an array of cancer types ($p<0.001$), yet the underpinning mechanisms remain obscure. The hypothesis that phytosterols impair AKT by disruption of the cholesterol-rich membrane domains essential for PIP-dependent recruitment, tethering, and stabilisation of AKT is explored here.

The effect of phytosterols on cholesterol-rich and saturated lipid-rich domain formation, and how AKT recruitment and activation was altered, was measured using inter-disciplinary methods, including: molecular dynamic simulations of computational membranes; atomic force microscopy (AFM) and small-angle X-ray scattering (SAXS) analysis of synthetic membranes; and protein- and lipid-labelling of biological membranes in cancer cells.

Computational modelling indicated formation of sterol-rich domains essential for AKT recruitment was prevented if phytosterols replaced 10% of membrane sterol. AFM and SAXS validated these observations. Sterol-rich domain formation was also significantly reduced, by 38% ($p<0.001$), and membrane thickness reduced by 1.9Å. *In vitro*, unsaturated side-chain phytosterols such as stigmasterol reduced AKT phosphorylation ($p<0.05$) indicating recruitment and or tethering had been disrupted.

This inter-disciplinary approach performed at overlapping temporal and spatial scales, from *in silico* to cell, suggests cancer severity could be ameliorated by phytosterols. These nutraceuticals are already clinically recommended for lowering cardio-vascular disease risk in patients with elevated LDL-cholesterol and can be distributed to the general population and in clinics at a low cost and with high safety.

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OC11

Assessment of oxysterol outcomes in immune cells targeted with reactive oxygen species (ROS) amplifiers

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Reactive Oxygen species (ROS) are key signalling molecules in inflammation and are involved in the pathogenesis of inflammatory and autoimmune diseases. The effects of ROS may be either harmful and beneficial depending upon their cellular concentrations. Previous work showed that loss of function polymorphisms in neutrophil cytosolic factor 1(Ncf1), a gene encoding for NADPH oxidase that produces oxygen radicals in phagocytic cells, increases disease severity in an animal model of rheumatoid arthritis. Restoring production of ROS was shown to reduce disease severity. Cholesterol oxidation by ROS to form oxysterols may mediate the anti-inflammatory effects of ROS. Here we aim to elaborate the effects of ROS amplifiers in oxysterol production. We used three ROS amplifier prodrugs to investigate their effects on ROS and inflammatory signalling in *in vitro* THP-1 macrophage models. We measured cell viability, mitochondrial activity and quantified oxysterols in macrophages after 24h exposure to 20µM Mis43, HX040 and VR79, noting that VR79 increases mitochondrial ROS production specifically[1,2,3]. Mitochondrial activity was impaired by Mis43 and HX040 but not VR79. 7-ketocholesterol was higher in the cells exposed to the prodrugs, with VR79 eliciting the greatest~5 fold increase. Similarly, the anti-inflammatory oxysterol, 25-hydroxycholesterol, was significantly elevated in all treated cells with VR79 having the greatest effect. In contrast the total cholesterol content was reduced in drug-treated macrophages. Our results demonstrate that all three produgs elicit changes to oxysterol levels in inflammatory macrophages.

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OC12

High phytosterol variants towards improved feedstocks and biofortification of crops

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Phytosterols are valuable biomolecules used as nutrients and as feedstock materials for the pharmaceutical and cosmetic industries especially for their health-beneficial properties. The innovative and fundamental challenge biologists and breeders are faced with is to develop valuable crops dedicated to the large scale production of phytosterols, which are currently extracted from agricultural waste materials or from oil crops as by-products.

The goal of the current work is to identify master regulators of phytosterol biosynthesis and accumulation. In fact, phytosterols have essential cellular functions in plant growth and development, acting as key membrane components and reinforcers, as signals in development, and as precursors of a class of oxysterol growth regulators known as brassinosteroids. The concentration of phytosterols is therefore tightly regulated in order to comply with plant cellular homeostasis.

Barley (*Hordeum vulgare*), tobacco (*Nicotiana tabacum*), and thale cress (the model *Arabidopsis thaliana*) genetic resources generated in the laboratory led to the biochemical and molecular characterization of plant variants displaying (*i*) novel phytosterol profiles acting on plant growth, and (*ii*) phenotypes of possible agro-economic interest.

OC13

Characterization of a cholesterol metabolic switch controlling breast carcinogenesis

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Cholesterol can be converted into the tumor suppressor dendrogenin A (DDA)[1] or the tumor promoter 6-oxo-cholestan-3β,5α-diol (OCDO, oncosterone)[2] via cholesterol-5,6-epoxides[3], establishing a molecular link between cholesterol and breast cancer (BC). The accumulation of OCDO in BC was found to be due to the upregulation and to the appearance of its biosynthetic enzymes, the cholesterol-5,6-epoxide hydrolase and the 11β-hydroxysteroid dehydrogenase of type 2 respectively[2]. Preliminary studies showed that DDA levels decreased[1] in BC for an unknown reason. The aim of the present study is thus to identify the DDA synthase (DDAS).

Pharmaco-genomic screening combined with molecular modeling studies led to the identification of glutathione S transferase A1 (GSTA1) as a probable candidate. Transcriptome database analyses showed that GSTA1 was expressed in human breast and disappeared in BC. Interestingly, high expression of GSTA1 (Affy ID:215766_at) in all BC subtypes increased significantly the relapse free survival in patients (hazard ratio: 0.71; p<0.0001; 37 datasets, n=4930) suggesting a protective effect of GSTA1. Analyses of GSTA1 expression at the protein level in patient biopsies by immunohistochemistry shows a decrease of GSTA1 protein in BC tissue compared to paired normal adjacent tissues (n=52, p<0.001). Furthermore, GSTA1 was found to be selectively expressed in epithelial cells from lactating ducts in the normal breast. The decrease in GSTA1 expression in BC paralleled the decrease in DDA level. The majority of breast cancers (up to 95 percent) begin in the breast duct epithelial cells. While epithelial cells are at the root of the cancer, these cells account for just 2 to 10 percent of total breast cells. Together, these data support an important role of DDA metabolism in breast carcinogenesis.

Biochemical analyses performed on pure recombinant hGSTA1 showed, as predicted, that it catalyzes DDA biosynthesis. The K_m found for 5,6α-EC and histamine is in the range of their endogenous concentrations in healthy tissues producing DDA. Pharmacological studies showed that B-ring oxygenation products of cholesterol and 7-dehydrocholesterol such as 5,6β-EC, cholestane-3β,5α,6β-triol, DHCEO and oncosterone are potent inhibitors of DDAS with IC_{50} in the µM range, while side chain oxysterols do not inhibit DDAS up to 20µM. Given that DDA is a cell differentiation agent and a tumor suppressor, the accumulation of B-ring oxysterols could contribute to an oncometabolic deregulation through the inhibition of DDA biosynthesis at the DDAS level.

Studies on the 5,6-EC metabolic branch on the transgenic mouse model of spontaneous mammary cancer (mmtv-PYMT) revealed a similar imbalance between oncosterone and DDA to that found in human BC. Feeding mice with DDA delayed mammary tumor development and blocked lung metastasis, confirming the tumor suppressor and chemopreventive activities of DDA on cancer.

Collectively these data established that DDA is produced by hGSTA1 in the breast, that the decrease in DDA level in BC is associated with a decreased expression in GSTA1 and an increase in DDAS inhibitors such as oncosterone. Together these data showed that the cholesterol-5,6-epoxide branch plays an unprecedented role in BC development.

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OC14

Distribution and Abundance of Oxysterols in the Human Multiple Sclerosis Brain

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Background

Multiple sclerosis is a disease of the central nervous system characterised by demyelination, neuroinflammation and neurodegeneration, for which there are few prognostic biomarkers and limited therapeutics for those with long-standing disease. Oxysterols can regulate inflammation, myelination and neuronal survival and plasticity. The concentrations of various oxysterols have been found to differ in a range of neurodegenerative diseases, including multiple sclerosis, but less is known about the abundance and location of oxysterols in the human brain or their contribution to multiple sclerosis disease.

Method

Post-mortem, snap frozen brain tissue from controls (n=5, females=2, mean age=61.2 years) and multiple sclerosis (n=9 progressive multiple sclerosis, females=6, mean age=49.7 years; study approval 13/WA/0292) were used. Areas of pathological interest were separated via macro-dissection and cryosectioned to collect enriched fractions. Oxysterols were extracted and prepared for enzyme-assisted derivatisation for sterol analysis–liquid chromatography–mass spectrometry (EADSA-LC-MS). The presence of specific oxysterols was confirmed using MS^3, their concentration determined and direct comparison made.

Results

We have found evidence suggestive of cholesterol biosynthesis pathway dysregulation in the multiple sclerosis brain. Concentrations of cholesterol precursors (including 8,9-dehydrocholesterol and desmosterol), cholesterol itself and downstream metabolites (including 24S-hydroxycholesterol (24S-HC), 25-HC and (25R)26-HC) differed between multiple sclerosis and control brain, and areas of active damage. For example, 24S-HC differed significantly between controls and areas of multiple sclerosis white matter pathology: 15.05ng/mg control white matter, 7.83ng/mg multiple sclerosis white matter, 1.91ng/mg multiple sclerosis white matter lesion centres, 3.27ng/mg multiple sclerosis white matter lesion edge ($p<0.05$; Kruskal Wallis and Dunn's post-test).

Conclusion

Oxysterols are druggable targets; identification of differences in the cholesterol biosynthesis and metabolism pathways will improve our understanding of multiple sclerosis pathogenesis and may lead to predictive biomarkers and new treatments.

OC15

Plasma and tissue distribution of 27-hydroxycholesterol after oral administration to mice.

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Background and Rationale. The side chain oxysterol 27-hydroxycholesterol (27OHC) has been demonstrated *in vitro* to exert a broad spectrum inhibitory effect on the replication of both enveloped and non enveloped viruses. With the aim to confirm and further investigate in experimental animals the antiviral property of 27OHC, a pilot pharmacokinetic study was carried in CD-1 male mice receiving a single oral dose of 27OHC (25 mg/kg).

Methods. The oxysterol was dissolved in a glycerol solution containing (2-hydroxypropyl)-β-cyclodextrin (2HPβCD) and administered by gavage. At the selected time points, 0, 1, 4, 8, 24h (n=4 mice per time point), animals were exhanguinated under anesthesia, then plasma, small intestine, liver, lungs and brain were collected. 27OHC was quantified by gas chromatography-isotope dilution mass spectrometry (GC-MS).

Results. Following the oral 25 mg/kg dosing, plasma levels of 27OHC appeared to increase rapidly and reach a Cmax of 928 ng/mL (2.3 µM), followed by elimination with a total AUC of 6,578 ng*h/mL (16.3 µMh), corresponding to an average steady-state concentration of 274 ng/mL (0.68 µM). The obtained kinetcs data clearly indicate that 27OHC is a high hepatic extraction drug, possibly with an extrahepatic component contributing to the total clearance. The concentration of 27OHC recovered in the lungs transiently doubled the basal one after 1h dosing, then slowly returned to basal values after 8h time. The single oral administration of 27OHC just slightly modified the oxysterol's basal concentration in the brain, showing a pretty modest and transient peak at 4 h time point.

Conclusions. As such, the 27OHC 25 mg/kg dose level in the mouse provides systemic steady-state concentrations very similar to those exerting half maximal effect (EC50) in vitro against the replication of Herpes Simplex, Rhinovirus, Rotavirus or Papillomavirus. Improvement of the 27OHC-2HPβCD combination and/or increase of the oral dose of 27OHC, plus the increment of the kinetics time points, will be afforded, before achieving pharmacokinetic data from other animal species.

OC16

Parallel changes occur in systemic oxysterol levels and retinal vascular function in ageing

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Vascular aging impairs vascular function and causes end organ damage, predominantly in the heart, brain, and kidney. It has been, however, admitted that individuals do not age at similar pace. This observation has led to the concept of biological aging, which is a measure of bodily functional decline. Oxysterols, oxygenated derivatives of cholesterol, are known to induce endothelial dysfunction and promote the pathogenesis of atherosclerosis by accelerating the vascular ageing process. The microvasculature has higher endothelial cell turnover and is the first to be affected in the course of cardiovascular disease (CVD). Indeed, in individuals without any other CVD risk, there are age-related differences in flicker-induced retinal vessel diameter changes throughout the entire retinal microvascular function. This observation is extremely important as it has been already advocated that biological vascular age should be used for the selection of the individuals in need for early CVD prevention as demonstrated by the presence of early dysfunction measurable at this level. To date, a link between oxysterols and microvascular function in apparently healthy individuals, free of vasoactive medication and of various age groups have never been assessed. Methods: Blood was collected from healthy controls (n=42) and categorised into; group 1: 19–30 years (10 male: 6 female), group 2: 31–50 years (8 male:8 female), and group 3: 51–70 years (4 male: 6 female). Retinal vessel reactivity was assessed using the dynamic retinal vessel analyzer (DVA, IMEDOS GmbH, Jena, Germany) in accordance with an established protocol. Fasting plasma total cholesterol (CHOL) and low-density lipoprotein cholesterol (HDL-C) using the Reflotron Desktop Analyser. Plasma was separated, and lipids were extracted. Quantification of monohydroxy oxysterols (25-OHC, 27-OHC, 4β-OH, 7β-OHC, and 7-keto-OHC) and dihydroxysterols (7α-25- dihydroxycholesterol and 7α-27-dihydroxycholesterol) was performed by LC-MS/MS (ABSciex 5500) following reverse phase chromatography. Results: In all participants, the levels of 7-keto-OHC, 25-OHC and 7β-OHC correlated significantly and positively with the maximum retinal artery diameter (tMD) (r=0.4318, p=0.025; r=0.0301, p=0.742; r=0.4029, p=0.037 respectively). In addition, 25-OHC and 7β-OHC negatively correlated to the maximum vessel dilation diameter (r=-0.3879, p=0.046; r=-0.5324, p=0.004 respectively). A negative correlation was observed for 27-OHC and 7β-OHC with arterial constriction (r=-0.2291, p=0.0250; r=-0.4576, p=0.016, respectively). Conclusion: These data suggest that oxysterol profile is altered with ageing towards oxidative phenotype and it correlates with age-related changes in retinal vascular function. Such link could be a step towards the so-called “composite biomarker predictors”, which are supposed to offer better individual estimates for ageing and risk for CVD.

OC17

Alternative splicing that disrupts ligand binding domains in liver x receptors predicts survival in triple negative breast cancer

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Introduction: The nuclear receptors LXRα/NR1H3 and LXRβ/NR1H2 are sensors and regulators of cholesterol metabolism and modify breast cancer (BCa) pathophysiology[1, 2]. Upon binding of side-chain hydroxycholesterols to the LXR ligand-binding (LB) domain protein conformation is altered, and co-repressors are replaced by co-activators. Transcriptional activation of target genes ensues. We previously found co-factors were critical for determining differential LXR function between oestrogen receptor positive (ER+) and negative (ER-) breast cancers (BCa)[3]. An array of LXR splice variants are annotated in genome (NCBI, ENSEMBL) and proteome (UNIPROT) databases. Several of these variants show truncations or alternations in the LB and activation function (AF) domains, both essential for co-factor recruitment. Several LXR splice variants have been detected that have alterations in these domains[4-7], but recent updates to genomic databases suggest that several more may exist. The aim of this study was to systematically evaluate the pattern and clinical significance of LXR splicing in BCa.

Method: Expression of canonical and predicted LXR mRNA splice variants was determined in: *i)* a public repository (the Cancer Genome Atlas Splicing Variant database (TSVdb)); *ii)* a panel of BCa cell lines representing ER-positive (MCF7, BT474), claudin-low triple negative BCa (TNBC; MDA.MB.231, MDA.MB.157) and claudin-high TNBC (MDA.MB.468, MDA.MB.453) subtypes subtypes; and *iii)* tumours from 38 patients with TNBC (15/HY/0025). RNA expression was determined using a panel of 26 SYBR-green primer pairs targeting unique and shared exon-exon junctions. Expression of LXR splice variants at protein level was surveyed in the same cell lines and patient samples, using immunoblotting and S-trap coupled mass-spectrophotometry (MS). Isoform identify was confirmed using RNA-protein correlation analysis (spearman rank), targeted siRNA knockdown (origene trisilencer), and MS with unique peptide mapping (Expasy PeptideMass tool).

Results

Mining genomic and proteomic databases indicated forty-eight potential LXRα transcripts encoding 26 proteins and 11 LXRβ transcript variants encoding nine proteins. Nomenclature used in previous studies was not consistent with database nomenclature; the NCBI and UNIPROT naming systems for all LXR splice variants is used here. LXRα1.1, α1.2, α3.1, and LXRβ1.1 transcript were detected in the TSVdb. Across all BCa cell lines and tumour samples seven LXRα/LXRβ splice variants were detected (LXRα1-5, LXRβ1/β4); of which three (LXRα4, LXRα5, LXRβ4) have not been recorded previously. Full length LXRα1 was the predominant LXRα splice variant in TNBC cell lines and in tumour samples, and its expression was significantly (Log-rank Mantel Cox test: p=0.0005) associated with shorter disease-free survival (DFS) in TNBC patients. ER-positive samples were different, the major isoform was LXRα5, a previously unreported variant. LXRα5 has deletions in the AF and LB domains and, although absent from TNBC cell lines, was expressed in tumour samples and associated with significantly longer DFS (p=0.04). LXRβ1 (p=0.0023) and the novel LXRβ4 (p=0.037) were associated with longer DFS.

Conclusion: We conclude that there are at least five LXRα and two LXRβ splice variants expressed in breast tumour cell lines and primary tissues. Canonical LXRα function is likely to be an oncogenic driver

in triple negative tumour pathophysiology. In tumours this may be countered by high expression of LXRα variants that harbour disruptions to the LBD, or high expression of LXRβ.

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OC18

The influence of 7-ketocholesterol on tamoxifen efficacy in breast cancer

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Oxygenated derivatives of cholesterol (oxysterols) are known as important players in different cellular processes, as well as pathological phenotypes, including cancerous diseases. In last decades, many studies demonstrated their role in carcinogenesis, proliferation, or modulation of cell death. Apart from that, different oxysterols were shown to modulate the efficacy of anti-cancer drugs, such as doxorubicin, vincristine, cisplatin, or 5-fluorouracil. Our previous study has shown that circulating levels of 7-ketocholesterol reflect the presence of tumour cells in breast cancer patients. In this study, we aimed to analyse the role of 7-ketocholesterol in breast carcinoma cell response to tamoxifen.

The study was performed on three breast carcinoma cell lines – two oestrogen receptor (ER) positive – MCF7 and T47D, and one ER negative – BT-20. Firstly, we analysed cellular response to tamoxifen, 7-ketocholesterol, or their combination by analysing cell viability (CellTiter-Blue® Cell Viability Assay) and caspase 3/7 activity (Caspase-Glo® 3/7 Assay System) in each cell line. Secondly, we aimed to determine differences in cell cycle induced in the presence of tamoxifen, 7-ketocholesterol, or mix of both substances using flow cytometry (BD FACSVerse). In the last part of the study, we evaluated differences in gene expression of key factors from tamoxifen metabolism in cells co-incubated with both substances or cultured with each substance separately (TaqMan™ Gene Expression Assays and ViiA 7 Real-Time PCR System).

When co-incubated with tamoxifen and 7-ketocholesterol, MCF7 cells showed slightly, but repeatedly significantly lower sensitivity to the presence of tamoxifen. In T47D cell line, there was no difference in cellular response to tamoxifen between cells cultured with both substances or tamoxifen alone. BT-20 cells showed higher sensitivity to tamoxifen, when co-incubated with both substances. Analysis of caspase activity showed similar results – there was higher caspase activity in BT-20 cells when cultured with 7-ketocholesterol only or with combination of 7-ketocholesterol and tamoxifen. There was no significant difference in caspase 3/7 activity either in MCF7, or T47D cells. Cell cycle analysis showed significant decrease of cells in S phase in MCF7 cells when incubated with tamoxifen or combination of both substances after 72 hours, however, there was no such effect in T47D and BT-20 cells. Gene expression analysis showed deregulation of some tamoxifen-metabolism enzymes with *CYP1A1* and *CYP1B1* being interesting candidates that are differently deregulated in MCF7 and BT-20 cells.

Taken together, this study brings new perspective on the role of 7-ketocholesterol, which is different in each cell line making 7-ketocholesterol an interesting candidate for future studies. This study was supported by project from the Czech Ministry of Education, Youth and Sports INTER-COST LTC19020 (COST Action CA17104 STRATAGEM).

OC19

New targeting in oxysterol metabolism for triple negative breast cancer therapy

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Breast cancer (BC) is the leading cause of cancer death in women worldwide. Great strides have been made in BC treatment using targeted therapies such as hormonotherapy for BC expressing estrogen and progesterone receptors or agents targeting overexpressed Her2. Tumors that express neither ER, PR nor HER2 (named triple negative or TN) have currently limited effective targeted therapy. Therefore, there is an urgent need to develop alternative therapies for TNBC.
We have shown that the metabolism of 5,6-epoxycholesterol (5,6-EC) is deregulated in BC, and it controls BC development (Voisin et al, PNAS 2017). Indeed, in normal breast tissues, the 5,6-EC α and β are transformed by the cholesterol epoxide hydrolase (ChEH) into cholestane-3β,5α,6β-triol (CT). In BC, CT is further transformed into 6-oxo-cholestan-3β,5α-diol (OCDO or oncosterone) by the 11β-hydroxysteroid dehydrogenase-type 2 enzyme (11βHSD2). 11βHSD2 is known to regulate glucocorticoid metabolism by converting the active glucocorticoid receptor agonist cortisol into inactive cortisone. We established that oncosterone promotes ER-positive and TN breast cancer cell proliferation by binding to the glucocorticoid receptor (GR). Patient BC samples showed significant increased oncosterone levels and greater ChEH and 11βHSD2 protein expression compared with adjacent normal breast tissues and 11βHSD2 and ChEH overexpression correlated with a higher risk of patient death, highlighting that oncosterone biosynthetic pathway is of major importance to BC pathology. Moreover, GR overexpression is correlated with poor progression-free and overall survival in patients with TNBC. Interestingly, the study of 11βHSD2 showed that a phytosterol analogue of oncosterone (called OPDO) is produced by 11βHSD2 in TNBC cells incubated with phytosterol analogues of CT or 5, 6-EC. Thus, this metabolic pathway could also generate a phytosterol analogue of oncosterone from food. The promoter or suppressor properties of OPDO remain to be studied.
The aim of the present study was to decipher the signaling pathway involved in the proliferative activity of oncosterone and the effect of OPDO and to identify “anti-oncosterone” therapies in TNBC.

Two TNBC cell lines were tested, MDA-MB231 cells that express the tumor suppressor retinoblastome (Rb) and MDA-MB468 cells that are deleted for Rb expression.We showed that oncosterone and OPDO activated the phosphorylation of JNK and of its substrate, c-jun. The knock-out of JNK1 expression, in both cell lines, inhibited oncosterone and OPDO-induced JNK phosphorylation. Pharmacological inhibition of JNK phosphorylation with the JNK inhibitor, SP600125 or AS602801, abolished oncosterone-induced JNK phosphorylation as well as cell cycle progression and proliferation. Importantly, *in vivo*, AS602801 and SP600125, inhibited oncosterone-induced MDA-MB231 and MDA-MB468 tumor growth respectively. Together these data indicate that JNK activation is essential in mediating oncosterone proliferative activity in both tumor cell lines, independently of Rb expression.
In conclusion, targeting JNK activation may be a new opportunity to treat TNBC deregulated in the oncosterone biosynthetic pathway.

OC20

25-Hydroxycholesterol induces ferroptosis via downregulation of mevalonate pathway in Schwann cells

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by a progressive degeneration of motor nerve cells in the brain and spinal cord that lead to muscle atrophy. A link between dysregulated lipid metabolism and ALS has been proposed, and several lines of evidence suggest that alterations of cholesterol 25-hydroxylase and 25-hydroxycholesterol (25-OHC) are linked to the development of ALS. It has been reported that levels of 25-OHC are elevated nearly ten-fold in the spinal cords of ALS model mice, and are also significantly increased in cerebrospinal fluid of ALS patients. Furthermore, 25-OHC induces apoptosis in a motor neuron cell line. Although it is indicated that dysfunction of not only motor neurons but also non-neuronal glia cells play critical roles in the pathogenetic process of ALS, the effect of 25-OHC on cell viability in glial cells is uncertain.
In our present study, to investigate the molecular mechanism of 25-OHC-induced cell death in glial cells, we used immortalized adult mouse Schwann cells (IMS32). As a result of our study, we found that 25-OHC induced not only apoptosis but also ferroptosis which is characterized by iron-dependent peroxidation of polyunsaturated phospholipids on cell membranes. We confirmed that 25-OHC induced lipid peroxide accumulation. 25-OHC-induced cell death was significantly suppressed by treatment with vitamin E (□-tocopherol and □-tocotorienol), ferroptosis inhibitor ferrostatin-1 and iron chelator deferoxamine. The mRNA levels of several genes related to mevalonate pathway were decreased in 25-OHC-treated cells. We further found that 25-OHC treatment significantly decreased the protein expression levels of glutathione peroxidase 4 (GPx4) which is an antioxidative enzyme and a major regulator of ferroptosis. 25-OHC-induced GPx4 decrease and cell death were significantly suppressed by cotreatment with mevalonate. Collectively, these results suggest that 25-OHC-induced downregulation of mevalonate pathway caused decrease of GPx4 protein levels, resulting in induction of lipid peroxidation-mediated ferroptosis.

OC21

The oxysterol receptor EBI2 is involved in the pathogenesis of murine contact hypersensitivity

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Atopic contact dermatitis (ACD) is an allergic reaction in the skin to contact with an allergen in humans. Contact hypersensitivity (CHS) reaction is the murine model for ACD, during which antigen-specific T cells mediate a local inflammation at the site of allergen contact. T cell migration is guided by a variety of chemotactic signals including those which activate the G-protein coupled receptor EBI2 (GPR183). The main ligand of EBI2, 7α,25-dihydroxycholesterol (7α,25-OHC), is biosynthesized from cholesterol by serial hydroxylations via CH25H and CYB7B1. Since both of these enzymes are upregulated in the inflamed skin of CHS patients suffering from allergic contact dermatitis, we analyzed 2,4,6-trinitro-1-chlorobenzene (TNCB)-induced CHS in the mouse model system. Indeed, we found that both ligand-generating enzymes are upregulated in inflamed ears with CHS. In agreement, we found that EBI2 deficient mice developed an ameliorated inflammation in three different models of TNCB-induced CHS. Adoptive T cell transfer experiments showed that EBI2 on T cells promotes TNCB-specific CHS of the ear. Our data therefore indicate that EBI2 is involved in the pathogenesis of the CHS reaction and ACD.

OC22

Endothelial cells-derived Oxysterols promotes neuroinflammation trough suppression of Myeloid-Derived Suppressor Cells

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Background
The development and progression of multiple sclerosis (MS) results in part from a dysbalance between pathogenic effector T cells and negative regulation imposed by regulatory cells. Oxysterols, can modulate the immune response during MS and its animal model, the experimental autoimmune encephalomyelitis (EAE). Immunoregulatory oxysterols include 25-hydroxycholesterol produced by Cholesterol-25-hydroxylase (Ch25h). Ch25h deficient mice display an attenuated EAE. However, the cellular source of Ch25h during neuroinflammation is not elucidated and how oxysterols promote inflammation is largely unknown.
We here propose to assess the source of Ch25h expression and oxysterol production during EAE and further examined their function and mechanisms of action during autoimmunity.
Material and methods
We generated a floxed reporter-ch25h knock-in mouse and evaluated the impact of Ch25h endothelial-specific deletion during EAE development. Reporter expression in CNS endothelial cells at baseline and during EAE and CNS infiltrating leukocytes were assessed by flow cytometry. We further used in-vitro mouse brain endothelial cells at baseline and under by IL-1β stimulation and combined transcriptomic and lipidomic approaches to evaluate the impact of Ch25h deletion in CNS endothelial cells during an inflammatory challenge.
Results
Ch25h reporter expression was increased in CNS endothelial cells during EAE. Selective Ch25h deletion in blood but not in lymphatic endothelial cells was sufficient to attenuate EAE. Moreover IL-1β stimulation increased Ch25h expression and production of 25-OHC in brain endothelial cells in-vitro. RNA sequencing of brain endothelial cells stimulated by IL-1β and lipidomic analysis combined with in-vitro experiments revealed that Ch25h deletion induced a lipid remodeling favoring Granulocytic Myeloid-Derived Suppressor Cells (G-MDSC) expansion. G-MDSC have been shown to attenuate EAE. Evaluation of G-MDSC by flow cytometry in the CNS during EAE revealed an increased infiltration of G-MDSC and a decreased proliferation of CD4 T cells in Ch25h endothelial-deleted mice.
Collectively, our results reveal a central role of endothelial-derived oxysterols in attenuation of neuroinflammation through expansion of suppressive myeloid populations in CNS.

OC23

Liver X receptors and ovarian hyperstimulation syndrome

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Liver X Receptors (LXRs), oxysterol-activated nuclear receptors, are regulators of cholesterol homeostasis, steroidogenesis and inflammation. These 3 processes are essential for ovarian physiology. Previously published results from our team demonstrated the development of an ovarian hyperstimulation syndrome (OHSS) in Lxr-deficient mice.
We show that this phenotype is rescued by re-expressing Lxrß in granulosa cells upon hormonal stimulation by Follicle Stimulating Hormone (FSH). In parallel, we aim to identify the molecular mechanisms by which LXRß is involved in the response to hormonal stimulation with in granulosa cells by FSH using two transgenic mouse models (*Lxrαß*$^{-/-}$ or *Lxrαß*$^{-/-}$-AMH-*Lxrß*).
By RNA-seq approaches, we showed that after FSH stimulation, LXRß exerts a control of the NFκB pathway and the inflammatory response in granulosa cells, especially at the end of folliculogenesis. We also observed that the cholesterol biosynthesis pathway and numerous genes involved in the synthesis of LXR ligands are controlled by FSH.
Altogether, the production by CYP51A1 of FF-MAS, an endogenous ligand of LXRs, could modulate the trancriptional activity of LXRß in granulosa cells.
We thus point out that LXRs are major regulators of the hormonal response in the ovary.

OC24

7β-hydroxycholesterol-induced oxidative stress, mitochondrial and peroxisomal dysfunctions: attenuation with Milk thistle seed oil

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Background and aim : Mitochondria and peroxisomes are among the main intracellular sources for reactive oxygen species. Despite obvious differences, these organelles play an important role in several cellular pathways, especially in lipid and RedOx homeostasis. Failure in the function of both organelles is linked to oxidative stress and accelerated aging. In this work, i) we aimed to study the ability of 7β-hydroxycholesterol (7β-OHC), which is frequently increased in patients with age-related diseases (including sarcopenia), to trigger oxidative stress, mitochondrial and peroxisomal dysfunction in murine C2C12 myoblast cells, and ii) we evaluated the capacity of Milk thistle seed oil (MTSO) to counteract the toxic effects of 7β-OHC.

Methods : An in vitro study was carried out on murine C2C12 myoblast cells. The impacts of 7β-OHC (50 µM for 24 h), in the presence or absence of MTSO (100 µg/mL), on mitochondria were studied using several criteria: measurement of succinate dehydrogenase activity with the MTT test, evaluation of transmembrane mitochondrial potential (ΔΨm) and mitochondrial superoxide anions ($O2^{\bullet-}$) production using DiOC6(3) and MitoSOX dyes, respectively; quantification of mitochondrial mass with Mitotracker Red. The effects on peroxisomes were characterized with complementary techniques: fluorescence microscopy and flow cytometry to quantify the peroxisomal mass with Abcd3 antibodies; evaluation of the peroxisomal topography, shape, and size by transmission electron microscopy; measurement by GC/MS of very long-chain fatty acids (C24:0; C26:0) to evaluate peroxisomal β-oxidation.

Results : Our results indicate that MTSO prevents mitochondrial and peroxisomal dysfunction induced by 7β-OHC and favors the restoration and normalization of mitochondrial and peroxisomal mass and/or activity. Thus, in the presence of MTSO, succinate dehydrogenase enzyme activity, ΔΨm, mitochondrial $O2^{\bullet-}$ productions, as well as mitochondrial and peroxisomal mass are normalized in 7β-OHC-treated cells.

Conclusion : These results underline the interest of MTSO to attenuate 7β-OHC-induced cytotoxic effects, associated with mitochondrial and peroxisomal dysfunction in myoblasts. This data support that MTSO could be of interest in the prevention of sarcopenia which is a frequent age-related disease.

Keywords: aging, C2C12 cells, 7β-hydroxycholesterol, mitochondria, myoblast, oxidative stress, Milk thistle seed oil, peroxisome, sarcopenia.

FT1

Cholesterol oxidation products as markers of nutritional quality of milk and milk products

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Oxysterols are products of enzymatic and/or chemical cholesterol oxidation. While some of the former possess broad antiviral activities, the latter mostly originate from the deterioration of the nutritional value of foodstuff after exposure to heat, light, radiation and oxygen. This raises questions about their potential health risks, considering that their action is related to several pathologies. We evaluated the presence of selected oxysterols in bovine colostrum, mature milk and milk-containing products and monitored their evolution throughout an industrial-scale production chain and after industrially employed storage procedures. Our work highlights for the first time the presence of the enzymatic oxysterol 27-hydroxycholesterol (27OHC) in concentrations of antiviral interest in bovine colostrum that decreased during the first postpartum days, pointing to a passive transfer of innate immunity. Of note, this oxysterol is also observed in milk and milk products and is not negatively affected by industrial processing or storage. We further highlight a relevant increase of the nonenzymatic oxysterols 7β-hydroxycholesterol (7βOHC) and 7-ketocholesterol (7KC) in both whole (WMPs) and skimmed milk powders (SMPs) during prolonged storage, confirming their role as reliable biomarkers of cholesterol oxidation over time. After 12 months, 7βOHC reached in both SMPs and WMPs amounts that have been found to be potentially toxic in vitro. Interestingly, industrial processes appeared to affect the generation of 7βOHC and 7KC differently, depending on the presence of fat in the product: while their ratios increased significantly after skimming and processing of skimmed milk and milk products, this was not observed after processing whole milk and milk cream. Lastly, composite products also show a differential presence of both enzymatic and chemical oxysterols, reflecting their shelf-life and the freshness of the milk ingredients used in dose. The measurement of enzymatic and non-enzymatic oxysterols could represent a useful tool to monitor and increase the commercial and nutritional value of both milk ingredients and finished products, highlighting their quality and freshness in relation to the processing and storage procedures applied.

FT2

Analysing cholesterol in mouse and human brain tissue using mass spectrometry imaging.

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Cholesterol is an essential molecule in the central nervous system (CNS) with 25% of the body's cholesterol found in the CNS. It is required for the organisation of cell membranes and formation of lipid rafts. With cholesterol unable to cross the blood brain barrier it is synthesised in situ. Cholesterol dysregulation has been linked to several neurodegenerative diseases including Alzheimer's, Huntington's and multiple sclerosis, where analysis of biofluid and brain tissue samples have shown altered levels of sterols compared to control. The most conventional method for sterol analysis from tissue samples is homogenisation followed by solid phase extraction or liquid-liquid extraction, where the extract is usually analysed using liquid chromatography or gas chromatography coupled with mass spectrometry (LC-MS or GC-MS). However, this process means a loss of structural information from the tissue. Recent techniques using mass spectrometry imaging (MSI) allow for the analysis of lipids across intact tissue sections for precise analysis in pathological and anatomical regions of interest. MALDI-MSI, typically in vacuum, is fast becoming one of the most popular methods for lipid analysis, but despite cholesterol being a critical molecule in the CNS and reports of dysregulation linking to neurodegeneration, MSI of cholesterol has not fully been explored primarily due to the difficulty of ionizing cholesterol. We have optimised a platform to visualise cholesterol using atmospheric pressure MALDI with an on-tissue enzymeassisted derivatisation method and applied it to several tissue types, including human multiple sclerosis brain and several mouse models. Images of MS/MS/MS (MS3) fragments on tissue confirmed identification of cholesterol, creating normalised images at the MSn -level. We have preliminary data for absolute quantification of cholesterol in distinct anatomical regions of control and Huntington's Q150+ mouse model brain tissue, and control and multiple sclerosis human brain tissue.

FT3

Discovery of GPR183 Agonists Based on an Antagonist Scaffold

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The G protein-coupled receptor GPR183/EBI2, which is activated by oxysterols, is a therapeutic target for inflammatory and metabolic diseases where both antagonists and agonists are of potential interest. Using the piperazine diamide core of the known GPR183 antagonist (*E*)-3-(4-bromophenyl)-1-(4-(4-methoxybenzoyl) piperazin-1-yl)prop-2-en-1-one (NIBR189) as starting point, we identified and sourced 79 structurally related compounds that were commercially available. *In vitro* screening of this compound collection using a Ca2+ mobilization assay resulted in the identification of 10 compounds with agonist properties. To enable establishment of initial structure-activity relationship trends, these were supplemented with five inhouse compounds, two of which were also shown to be GPR183 agonists. Taken together, our findings suggest that the agonist activity of this compound series is dictated by the substitution pattern of one of the two distal phenyl rings, which functions as a molecular efficacy-switch.

FT4

Serum markers of cholesterol absorption and synthesis in a young mixed dyslipidemic population with moderately enhanced serum cholesterol concentrations

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Elevated serum cholesterol (CH) concentrations (SCHCs) are associated with an increased risk for cardiovascular disease. CH absorption (CHA) and synthesis (CHS) are the major input fluxes into the endogenous cholesterol pool and are potential causes of elevated SCHCs. The first line CH lowering therapies are based on inhibition of CHS (statins) and CHA (ezetimibe). The aim of this study was to examine whether CHA and CHS are enhanced in mixed young dyslipidemic patients with elevated SCHC (>200 mg/dl).

Back-up serum samples from patients examined from the former Institute of Clinical Pharmacology were analyzed for total SCHC, cholestanol (cholol), lathosterol (lath), campesterol (camp), and sitosterol (sit) by gas chromatography-flame ionization detection. The ratios of the non-cholesterol sterols to cholesterol were interpreted as markers for CHA (R_cholol, R_camp, R_sit) and for CHS (R_lath).

Serum samples of 624 subjects (age from 0.1 to 26 years, 282 females, 342 males) were available. SCHC varied from 66 to 397 mg/dl of which 51% >200 mg/dl. In this group mean SCHC was 60% higher ($P<0.0001$), R_cholol 9% lower ($P < .0001$) and R_lath 11% ($P = .026$), R_camp 14% ($P = 0.012$), and R_sit 14% ($P = .015$) higher than in the group with SCHC < 200 mg/dl. In the highest quartile of SCHC, mean SCHC was 100% elevated compared to the lowest quartile whereas R_lath, R_Camp and R_sit were 11%, 14% and 14% higher, respectively. SCHC was positively correlated with R_camp and R_sit ($P <.0001$ and $P =.001$, respectively), but negatively associated with R_cholol ($P < 0.0001$). R_lath only tended to be positively associated with SCHC ($P= .098$). Significant positive correlations with age were found for SCHC ($P < .0001$) and R_lath ($P < .0001$) and a negative correlation with R_cholol ($P < .0001$).

R_lath is determined by age and only weakly associated with SCHC. R_camp and R_sit are associated with SCHC unrelated to age. R_cholol appears affected by an age related decreased formation and to not reflect CHA. Markers for CHA and CHS are only moderately enhanced in the group of patients with elevated SCHCs.

FT5

Sterol and Oxysterol Markers of Huntington's Disease: The 24S-Hydroxycholesterol Pathway

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Huntington's disease (HD) is an inherited neurodegenerative disorder caused by a CAG triplet repeat expansion in the gene encoding the huntingtin protein (HTT). HD is clinically characterised by a variable phenotypic expression of motor, cognitive and psychiatric symptoms, with typical age-at-onset in the thirties or forties and a slow disease progression.

Oxysterols are bioactive molecules. The side-chain oxysterols are established ligands to the liver X receptors (LXRα, NR1H3; LXRβ, NR1H2), inhibitors of the processing of SREBP-2 (sterol regulatory element-binding protein-2) to its active form as the master transcription factor for expression of genes in the mevalonate pathway of cholesterol biosynthesis and potent allosteric modulators of the *N*-methyl-d-aspartate (NMDA) receptors.

Previous research has demonstrated that HD significantly affects brain cells, and changes in cholesterol levels and oxysterol concentrations are reported in brain. Some studies indicate that mutated HTT decreases brain cholesterol synthesis by inducing inhibition of a series of essential genes that are responsible for cholesterol biosynthesis (e.g. HMG–CoA reductase and 7-dehydroxycholesterol reductase). However, other studies have demonstrated cholesterol accumulation and lipid droplets in HD neurons. 24(S)-Hydroxycholesterol (24S-HC) formation in HD brain would be expected to be decrease because of reduced 24-hydroxylase levels on account of a reduced number of active neurons, which should lead to a lower efflux of 24S-HC from the brain to the circulation. Lower brain and plasma 24S-HC levels have been observed in several rodent models of HD. Similarly, plasma 24S-HC levels were reported to be reduced in HD patients compared to healthy subjects.

In this study, we are assessing the level of cholesterol, a broad spectrum of oxysterols and cholesterol precursors in human CSF and plasma samples from HD patients and the controls using LC-MS with the aim of determining the main sterol and oxysterol markers of HD.

FT6

Side chain hydroxylation of cholestane-3β,5α,6β-triol and Oncosterone lead to the production of new endogenous oxysterols potentially involved in breast cancer development.

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Background and Purpose. A growing body of evidence supports that oxysterols metabolism impact the development of breast cancer (BC). Indeed, we recently reported that cholestane-3β,5α,6β-triol (CT) is oxidized by the 11β-hydroxysteroid dehydrogenase of type 2 (HSD2) into 6-oxo-cholestan-3β,5α-diol (OCDO, Oncosterone)[1], an oncometabolite that displays tumour promoter properties on BC[2]. Our results highlight that HSD2 is confirmed as a pharmacological target for the BC treatment[3]. Other groups showed that 27-hydroxycholesterol (27-HC) and to a lesser extend 25-hydroxycholesterol (25-HC) also act as tumor promoter in BC[2]. 25-HC and 27-HC are produced respectively by the cholesterol-25-hydroxylase (CH25H) and the sterol-27-hydroxylase (Cyp27A1) that are both expressed in breast cancers. We postulate that side chain hydroxylation on CT and OCDO at position 25 and 27 lead to the production of endogenous oxysterols potentially involved in BC development.

Key Results. We have performed the chemical synthesis of cholestane-3β,5α,6β, 25-tetraol (25OH-CT), cholestane-3β,5α,6β, 27-tetraol (27OH-CT), 6-oxo-cholestan-3β,5α, 25-triol (25OH-OCDO) and 6-oxo-cholestan-3β,5α, 27-triol (27OH-OCDO). Using these compounds as chromatographic standards, we have compared the metabolism CT by two BC cells lines: MDAMB-231 and MCF-7 that respectively expressed and do not expressed CH25H and Cyp27A1. We found by using TLC autoradiography of BC cell extracts that the radiolabelled [^{14}C]-CT is metabolized into compounds that co-migrated with 25/27OH-CT and 25/27OH-OCDO in MDAMB-231 while no metabolism was observed in MCF-7 cells. We next showed that 25OH-CT and 27OH-CT targeted HSD2 and inhibited the production of OCDO on both cell lysate and whole cell assays. Interestingly, 27OH-CT was found more potent than CT to inhibit OCDO synthase activity on whole cell assay suggesting it is a better substrate.

Conclusions and Implications. Our results strongly support the existence of new endogenous oxysterols metabolites arising from side chain hydroxylation of CT and OCDO. The impact of these new oxysterols on nuclear receptor signalling and BC development will deserve further investigations.

[1] Voisin et al, *PNAS*, 2017; [2] Silvente-Poirot et al, *Cancer Research*, 2018 ; de Medina et al, *Br J Pharmacol*, 2020 ; de Medina et al, MS in preparation

FT7

Inhibition of oncosterone biosynthesis as a new pharmacological anticancer strategy in breast cancer.

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Background and Purpose. We recently discovered in breast cancer (BC) the presence of oncosterone (6-oxo-cholestan-3β,5α-diol, OCDO), an oncometabolite derived from cholesterol that displays tumour promoter properties[1]. The oncosterone biosynthesis enzyme was identified as the 11β-hydroxysteroid dehydrogenase of type 2 (HSD2)[1,2,3], the enzyme known to inactivate cortisol. We investigated herein if the production of selective oncosterone biosynthesis inhibitors on HSD2 was possible and if they can control BC development.

Experimental Approach. The analysis of HSD2 expression in cancer and patient survival in public databases was done. The HSD2 activity for cortisol and cholestane-3β,5α,6β-triol (CT) oxidation *in vitro* and on whole cell assays using HEK293T cells expressing HSD2 were studied. We have compared the pharmacological profile of HSD2 for cortisone and oncosterone production highlighting differences and structure-function studies were performed. Selective oncosterone biosynthesis inhibitors were tested *in vitro* and *in vivo* for anticancer activities.

Key Results. The over-expression of the oncosterone biosynthesis pathway is associated with a poor survival in BC and several other cancers. Biochemical, structure-function and pharmacological studies reveal that cortisol and CT binding sites are different in HSD2. Pharmacological studies revealed that B-ring oxysterols are selective inhibitors of oncosterone biosynthesis with no inhibition of cortisol oxydation. Indeed, 6-ketocholestanol inhibits tumour growth and this effect was reversed by oncosterone addition.

Conclusions and Implications. We report that the inhibition of oncosterone biosynthesis is possible without affecting cortisol metabolism. These data paved the way to the development of selective inhibitors of oncosterone biosynthesis and give evidences that HSD2 represent a new promising target for BC treatment.

[1]Voisin et al, *PNAS*, 2017; [2]Poirot et al, *Biochimie*, 2018 ; [3]de Medina et al, *Br J Pharmacol*, 2021

FT8

Pharmacologic and genetic inhibition of cholesterol esterification reduces tumour burden: a pan-cancer systematic review and meta-analysis of preclinical models

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Cholesterol esterification proteins Sterol-O acyltransferases (SOAT) 1 and 2 are emerging prognostic markers in many cancers. These enzymes utilise fatty acids conjugated to coenzyme A to esterify cholesterol. Cholesterol esterification is tightly regulated and enables formation of lipid droplets that act as storage organelles for lipid soluble vitamins and minerals, and as cholesterol reservoirs. In cancer, this provides rapid access to cholesterol to maintain continual synthesis of the plasma membrane. In this systematic review and meta-analysis, we summarise the current depth of understanding of the role of this metabolic pathway in pan-cancer development. A systematic search of PubMed, Scopus, Web of Science and Cochrane Library for preclinical studies identified eight studies where cholesteryl ester concentrations were compared between tumour and adjacent-normal tissue (PROSPERO: CRD42020202409). Eight studies evaluating cholesterol ester concentration between tumour and matched non-tumour tissue and 24 studies evaluating genetic or pharmacological inhibition of esterification were included for data extraction. Tumour tissue had a significantly greater concentration of cholesterol esters than non-tumour tissue (SMD=1.29; 95% CI: 0.68 to 1.90; I^2=31%; p<0.0001). Pharmacological or genetic inhibition of SOAT1/2 was associated with significantly smaller tumours across many cancer groups (SMD=-2.1; 95% CI: -2.56 to -1.64; p<0.00001). Furthermore, by utilising experiments assessing time to animal sacrifice for ethical consideration, we calculated hazard ratios that describes the risk of euthanasia of preclinical models (HR = 0.15; 95% CI: 0.08 to 0.28; I^2 = 63%; p<0.00001). Additionally, reduced tumour volume was accompanied with increased apoptosis (SMD=5.64; 95% CI: 1.57 to 9.71; I^2 = 83%; p=0.0004), CD8+ immune infiltration (SMD=1.12; 95% CI: 0.46 to 1.77; I^2 = 0%; p=0.0009) and reduced Ki67 expression (MD=-14.43; 95% CI: -22.32 to -6.55; I^2 = 98%; p=0.0003). We noted a significant risk of publication bias; trim and fill method indicated our observed effect size may be an overestimate by as much as 33%. Shifting the balance of cholesterol-cholesteryl esters changes multiple tumour growth metrics. Mechanistically, signalling by the AKT and ERK oncogenes was inhibited and invasion of the tumours by cytotoxic T-cells was potentiated following inhibitor treatment. We found that five pharmacological inhibitors of cholesterol esterification used in pre-clinical anti-cancer studies have also been reported as safe and tolerable in human clinical trials for other diseases. These agents remain largely unevaluated in the clinical cancer setting and thus are potential drug-repurposing candidates.

FT9

Secosterol-B triggers oxidative-inflammatory response associated with alterations of eNOS/Cav-1 expression in HMEC-1 cells

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Introduction: The endothelium acts as gatekeeper of blood vessel integrity regulating the trafficking between endothelium and extracellular environment. Endothelial dysfunction (ED) is a key feature of early atherosclerotic lesions in both human and animal models and is characterized by a change in the balance of vasomotor factors released by the endothelium, such as nitric oxide (NO) and endothelin-1 (ET-1). Oxysterols are a family of 27-carbon cholesterol oxidation products found in LDL and atherosclerotic plaques where they trigger several biological responses involved in the initiation and progression of atherosclerosis. Several evidence suggest that oxysterols contribute to ED due to their ability to alter membrane fluidity and thus signalling leading to inflammation, oxidative stress and apoptosis. The aim of the present study was to investigate the effects of Secosterol-B (SEC-B) -an autoxidation product of cholesterol found in atherosclerotic plaques- on NO production focusing on eNOS/Cav-1 modulation, oxidative stress and cytokine release which are known to be involved in endothelial dysfunction and eNOS dysregulation.

Materials and methods: HMEC-1 (human microvascular endothelial cells) were treated with SEC-B 20 µM for 4 and 24 hours. Cell viability and inhibition of proliferation were evaluated by WST-8 and wound healing assay, respectively. Intracellular reactive oxygen species (ROS) and NO levels were analysed using DCF-DA and DAF-DA probes, respectively. NOX4 and eNOS expression were evaluated by RT-PCR. The expression and release of cytokines and proinflammatory factors, such as IL-6, TWEAK and ET-1 were assessed by RT-PCR and ELISA immunoassay, respectively. Cav-1 modulation was investigated by confocal microscopy. Monocyte recruitment was investigated by the cell adhesion assay. The expression of intercellular cell adhesion molecule-1 (ICAM-1) was evaluated by flow cytometry and RT-PCR.

Results: Our results highlight that SEC-B is able to activate HMEC-1 by inducing ROS and NO production within the first 4 hours of treatment. Besides, SEC-B stimulates the expression and secretion of IL-6, and TWEAK, while it significantly downregulates ET-1 expression and extracellular release. Exposure to SEC-B up to 24 hours brings persistent activation of NOX4 coupled with deregulation of eNOS expression and downmodulation of CAV-1 protein levels. This proinflammatory state leads to impairment of endothelial function characterized by high levels of ICAM-1 expression and increased monocyte recruitment on activated HMEC-1 cells and reduced capability to repair the damaged tissue.

Conclusion: These findings add new knowledge on the pro-inflammatory role of SEC-B in ED and its implication in progression of atherosclerotic disease.

FT10

Attractive targets to fight Alzheimer's disease: from oxysterol profile alteration to SIRT-1 and Nrf2 decline

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Introduction: In spite of the efforts made over the last decades, the etiology of Alzheimer's disease (AD) remains to be fully elucidated. However, it is becoming increasingly clear that the disturbance in brain cholesterol metabolism is a critical key to the onset and progression of AD. Cholesterol oxidation products, named oxysterols, play a pivotal role in AD pathogenesis by promoting oxidative stress, intraneuronal neurofibrillary tangles of hyperphorylated tau protein and extracellular senile plaques of amyloid beta. In this context, the activation of the nuclear factor erythroid 2-related factor 2 (Nrf2), the main switch of antioxidant defense system and proteostasis, may be a reasonable strategy to attenuate oxidative stress and counteract misfolded protein accumulation. In addition, a growing body of studies supports the neuroprotective role of sirtuin-1 (SIRT-1), a broad-spectrum deacetylase that is able to exert different beneficial effects.

Methods: A systematic analysis of oxysterols in *post-mortem* human AD brains, classified by the Braak staging system of neurofibrillary pathology, was performed by isotope dilution mass spectrometry. SIRT-1 and Nrf2 levels were evaluated by molecular biology techniques.

Results: It emerged that, among oxysterols of enzymatic (i.e. 24-hydroxycholesterol and 27-hydroxycholesterol) and non-enzymatic origin (i.e. 7-ketocholesterol, 7β-hydroxycholesterol), only the levels of 24-hydroxycholesterol markedly reduced with the progression of the disease while those of others increased significantly. Moreover, a marked reduction of SIRT-1 transcriptional levels and synthesis has been demonstrated during AD progression; in contrast, Nrf2 expression increased while its synthesis declined, suggesting a possible failure in the post-transcriptional regulation mechanisms.

Conclusions: Our study elucidates the pathogenic association between level changes of oxysterols in AD brains and the progression of the disease. Moreover, the simultaneous loss of SIRT-1 and Nrf2 might amplify and sustain neuronal death, exacerbating amyloidopathy and tauopathy. In order to find a better approach to prevent or reduce AD progression, further studies are needed to investigate whether the marked decrease of 24-hydroxycholesterol may modulate the SIRT-1- and Nrf2-depending pathways, accelerating the disease development.

FT11

Comprehensive assessment of germline and somatic variants in oxysterol-related genes in breast cancer patients

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Introduction: Oxysterols are involved in a number of metabolic and signalling pathways and have been implicated in multiple pathologies, including breast cancer, where the link is especially prominent. There are several groups of genes whose members are either modulated by or their protein products interact with oxysterols, e.g., enzymes of the biosynthesis and metabolism of cholesterol and other sterols, sterol transporters, and nuclear and cell membrane receptors. Based on existing *in vitro* and/or *in vivo* evidence in the literature, we assembled a gene panel (n=113) containing those genes and those that are functionally closely related. While their expression has been studied in connection with breast cancer, detailed data on genetic variability of the full panel in breast cancer patients is missing.

Methods and results: Target-enriched libraries from 101 non-tumour (97x blood and 4x tissue) and 99 paired tumour DNA samples from breast cancer patients were sequenced on the Illumina platform with high coverage (187.1 ± 38.8x for non-tumour and 665.9 ± 238.3 for tumour samples). Bioinformatic analysis was performed by a custom pipeline based primarily on the Genome Analysis Toolkit (GATK) in order to obtain high confidence single nucleotide substitutions (SNV) and indels.

Germline variants were further filtered by removing those with minor allele frequency < 5% and those that did not conform to Hardy-Weinberg equilibrium, leaving 67 288 variants (1 415 unique variants across 101 samples, including non-coding). Analyses with clinical data of patients revealed SNVs impacting patient survival (either overall, disease-free or both) negatively (n=23, in *ABCA1*, *ABCA8*, *ABCC1*, *GPR183*, *LDLR*, *MBTPS1*, *NR1I2*, *OSBPL2*, *OSBPL3*, *OSBPL5*, *OSBPL6*, *RORA*, *RORC*) or positively (n=7, in *ABCA8*, *ABCG2*, *HSD3B7*, *PPARGC1A*, *SREBF1*).

There were 1 177 somatic variants found (1 090 unique across 99 samples), including 2 hypermutated samples with approx. 300 mutations each. Associations with clinical data were evaluated based on various groups of related genes being mutated rather than on individual variants. Mutations in *CYP46A1* and related genes (by STRING algorithm), as well as *ARNT*, *NR1I2*, the *OSBP* gene group and several other groups negatively impacted survival, with and without hypermutated samples included.

Conclusions: Candidate germline and somatic variants were found that could influence prognosis of breast cancer patients. Validation genotyping study in a larger cohort, as well as gene expression functional studies, are to follow.

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FT12

Oxysterols present in Alzheimer's disease brain induce synaptotoxicity by activating astrocytes: a major role for lipocalin-2

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Among Alzheimer's disease (AD) brain hallmarks, the presence of reactive astrocytes was demonstrated to correlate with neuronal loss and cognitive deficits. Evidence indeed supports the role of reactive astrocytes as mediators of changes in neurons, including synapses. However, the complexity and the outcomes of astrocyte reactivity are far from being completely elucidated. Another key role in AD pathogenesis is played by alterations in brain cholesterol metabolism. Oxysterols are crucial for brain cholesterol homeostasis and we previously demonstrated that changes in the brain levels of various oxysterols correlate with AD progression. In order to deepen the role of oxysterols in AD, we investigated whether they could contribute to astrocyte reactivity, and consequently impact on neuronal health. Methods: Two oxysterol mixtures were used, whose composition is based on our previous oxysterol quantification in mild (Early AD mix) and severe (Late AD mix) AD brain samples. Mouse primary astrocytes were treated with the oxysterol mixtures (10µM) at different time points. Mouse primary neurons were co-cultured with astrocytes previously treated with oxysterols. The transient knockdown of lipocalin-2 (Lcn2) gene was performed in order to investigate Lcn2 involvement in the synaptotoxic effect exerted by oxysterol-treated astrocytes. Results: Results showed that oxysterols present in mild or severe AD brains induce a clear morphological change in mouse primary astrocytes, accompanied by the upregulation of some reactive astrocyte markers, including Lcn2. Moreover, astrocyte conditioned media analysis revealed a significant increase in the release of Lcn2, cytokines, and chemokines in response to oxysterols. A significant reduction of postsynaptic density protein 95 (PSD95) and a concurrent increase in cleaved caspase-3 protein levels have been demonstrated in neurons co-cultured with oxysterol-treated astrocytes, pointing out that mediators released by astrocytes have an impact on neurons. Among these mediators, Lcn2 has been demonstrated to play a major role on synapses, affecting neurite morphology and decreasing dendritic spine density. Conclusions: These data demonstrated that oxysterols present in the AD brain promote astrocyte reactivity, determining the release of several mediators that affect neuronal health and synapses. Lcn2 has been shown to exert a key role in mediating the synaptotoxic effect of oxysterol-treated astrocytes.

FT13

Prevention by oleic and docosahexaenoic acids of 7-ketocholesterol-induced mitochondrial and peroxisomal alteration on murine neuronal N2a cells

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Background and aim: 7-ketocholesterol (7KC), a major cholesterol oxidation product plays key roles in the development of age-related diseases including Alzheimer's disease (AD), eye diseases (cataract, age related macular degeneration (ARMD)), and cardiovascular diseases since elevated levels of 7KC are found in tissue lesions, cerebrospinal fluid and/or plasma of patients with these diseases. Hence, it is important to find molecules capable of counteracting the detrimental effects and the cellular dysfunctions caused by 7KC. Mediterranean diet, which is rich in polyphenols and several fatty acids, is associated with improved age-related health due to its antioxidant and anti-inflammatory properties.

The aim of this work was to evaluate the cytoprotective effects of two fatty acids present in significant amounts in the Mediterranean diet: oleic acid (OA; C18:1 n-9) found in vegetable oils such as olive, argan, canola and sunflower, and docosahexaenoic acid (DHA; C22:6 n-3) present mainly in blue fishes and seafood, against 7KC-induced mitochondrial and peroxisomal dysfunctions on murine neuronal N2a cells.

Methods: After 48 h of treatment, flow cytometric analyses were carried out with propidium iodide to quantify dead cells and with $DiOC_6(3)$ to assess transmembrane mitochondrial potential ($\Psi\Delta m$). The effects of 7KC (50 µM) alone, or associated with OA or DHA (25 µM) on ABCD3 levels, a marker of peroxisomal mass, were also quantified by flow cytometry. Transmission electron microscopy (TEM) was used to simultaneously visualize peroxisomes and mitochondria in N2a cells in the absence or in the presence of 7KC, without or with OA and DHA.

Results: Our results show that 7KC at 50 µM, induces a disruption of $\Psi\Delta m$ with an increase in plasma membrane alterations and cell death. In addition, a significant increase of the percentage of cells with reduced ABCD3 level was shown under treatment with 7KC (50 µM). Mitochondrial and peroxisomal morphological modifications were illustrated by changes of mitochondrial structure and shape associated with damaged peroxisomes in the cytoplasm of 7KC (50 µM)-treated cells. Our results show that OA and DHA at 25 µM, were able to prevent 7KC-induced cytotoxicity by attenuating both morphological and functional mitochondrial and peroxisomal alterations on N2a cells.

Conclusion: Our data underlines the beneficial effect of OA and DHA present in the Mediterranean diet to counteract 7KC-induced mitochondrial and peroxisomal dysfunctions which are involved in the development of several age-related diseases, including neurodegenerative diseases.

Keywords: age-related diseases; docosahexaenoic acid; 7-ketocholesterol; Mediterranean diet; mitochondria; N2a cells; oleic acid; peroxisome.

FT14

Prevention of 7-ketocholesterol-induced oxiapoptophagy by sulfo-N-succinimidyl oleate (SSO) on murine oligodendrocytes 158N

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Background: 7-ketocholesterol (7KC) is a cytotoxic oxysterol increased in many chronic inflammatory diseases and age-related diseases (cardiovascular diseases, bowel diseases, ocular diseases (age related macular degeneration, cataract) as well as neurodegenerative diseases (Alzheimer's disease, multiple sclerosis, X-ALD, Niemann Pick). Inhibiting the toxicity of 7KC is a major challenge in treating 7KC-associated diseases.

Experimental approach: The 158N oligodendrocyte model was used to evaluate the cytoprotective effects of sulfo-N-succinimidyl oleate (SSO) a chemical molecule derived from oleic acid versus oleic acid and α-tocopherol, both known as cytoprotective compounds against oxiapoptophagy (Oxidative stress + apoptosis + autophagy) induced by 7KC on various cell types.

The ability of these molecules to inhibit 7KC-induced toxicity (plasma membrane alteration, loss of ΔΨm, peroxisomal dysfunctions, reactive oxygen species overproduction, induction of apoptosis and autophagy) was quantified.

Key results: Results showed that SSO (50 µM), oleic acid (100 µM) and α-tocopherol (400 µM) are cytoprotective against oxidative stress, plasma membrane permeability, mitochondrial damages, peroxysomal dysfunctions, autophagy and apoptosis activation induced by 7KC (50 µM) on 158N cells. Noteworthy, at the opposite of oleic acid, the cytoprotective activities of SSO and α-tocopherol are not associated with an accumulation of lipid droplets.

Moreover, SSO is cytoprotective at lower concentration (50 µM) than oleic acid (100 µM) and α-tocopherol (400 µM).

Conclusions: These different characteristics of SSO make it possible to envisage its use for therapeutic purposes in diseases where 7KC level is greatly increased.

Keywords: 7-ketocholesterol, α-tocopherol, oleic acid, oxiapoptophagy, sulfo-N-succinimidyl oleate (SSO)

FT15

7-Ketocholesterol effects on survival and growth in *Drosophila melanogaster*

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7-ketocholesterol (7KC) is one of the most abundant cholesterol oxides formed by cholesterol auto-oxidation and its cytotoxicity has been demonstrated *in vitro* in different types of mammalian cells. However, few studies have been conducted *in vivo* (Vejux *et al.*, 2020). Zong *et al.* (2014) have shown in *Caenorhabditis elegans* that 7KC shortened life span in a dose-dependent manner and reduces reproductive capacity.

Here, we investigated the effects of 7KC on the development, survival and starvation resistance in the fruit fly, *Drosophila melanogaster*. Individuals were reared on media enriched with 7KC versus standard media (STD) or cholesterol-enriched media (CHO).

Our results reveal that larvae develop perfectly and no significant difference was observed in the number of pupae and adults that developed from larvae reared on 7KC-enriched media regardless of the concentration used. However, pupae from larvae reared on 7KC-enriched medium at a 1,000 µM concentration are significantly heavier, wider and longer than pupae reared on CHO-enriched or STD-enriched media. Adults that emerge do not exhibit morphological abnormalities. There is no difference in male survival in 7KC (50 µM)- or CHO-enriched but male life span is significantly shortened in 1,000 µM enriched-CHO and 7KC media versus standard media (STD). Opposite results are found in females. Female life span being significantly shortened in the 7KC (50 µM)- or CHO-enriched media but not in the 7KC (1,000 µM)- or CHO-enriched media. Furthermore, 7KC (1000 µM)-enriched medium seems to benefit to female during the 50 first days as they survived significantly more than on standard medium. In addition, starvation resistance experiments were conducted on adult males and females hatched from larvae raised on the different enriched media. There is no difference in starvation resistance in males regardless of larval rearing environment. However, starvation resistance is significantly lower in females hatched from larvae reared in 50 µM CHO and 7KC media but not in 1000 µM CHO and 7KC media.

Our results clearly show that 7KC has no deleterious effects on the development and survival of *Drosophila melanogaster*, even at high concentrations. Furthermore 7KC high concentration may benefit to female as it improve survival during the first 50 days. These data suggest that *Drosophila melanogaster* could constitute a usefull model to study the effects of 7KC on the metabolism and development.

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FT16

LXR signalling in the striatum and neuroprotection in Huntington's Disease

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Huntington's disease (HD) is a rare neurodegenerative disease characterized by neuropsychiatric, motor and cognitive symptoms, with no disease modifying treatment. HD is caused by expanded polyglutamine in Huntingtin (mHTT), inducing many cellular dysfunctions, including cholesterol metabolism deregulation. The main pathway of cholesterol elimination is its catabolization by the neuronal 24-hydroxylase enzyme (CYP46A1) into 24S-hydroxycholesterol (24S-OHC), a ligand of the nuclear receptors Liver X Receptor (LXR). CYP46A1 level is decreased in HD, and its restoration induces a neuroprotection and a restoration of cholesterol metabolism including an increase of 24S-OHC levels. CYP46A1 restoration in HD mice is also associated with an upregulation of LXR target genes involved in cholesterol metabolism. Therefore, we hypothesized the involvement of LXR in CYP46A1 mediated neuroprotection. A therapeutic interest was raised for the LXR in several neurodegenerative disease. There are two LXR isoforms, LXRα mainly expressed in liver and LXRβ enriched in the brain for cholesterol metabolism regulation. Commercialized LXR agonists suffer from side effects on lipogenesis due to the activation of LXRα in the liver. The aim of the project is to take advantage of new LXRβ agonists, derivated from phytosterols, to investigate the role of LXR activation in HD. Primary cultures of striatal neurons and astrocytes were treated with LXR agonists to validate their bioactivity and study their neuroprotective role in a HD cellular model. In neurons and astrocytes culture, LXRα, LXRβ and commercial agonists induce an increase of mRNA level of LXR target genes, involved in cholesterol metabolism and known to be downregulated in HD. The LXR agonists induce a neuroprotection in HD striatal neurons in culture, with a decrease of mHTT aggregates and an increase of cell survival. When treated with inhibitor of proteasome or autophagy machinery, the neuroprotective role induced by LXR agonists is reversed. To study LXR effect on HD mice, administration root of LXR commercial agonist and protocol have been compared on Wild Type mice. These results support the biological efficacy of these new LXR compounds and their neuroprotective role in HD striatal neurons. The next step will be to explore their effect in HD mice model.

FT17

In vitro evaluation of the effect of lemon essential oils on 7-ketocholesterol-induced cytotoxicity

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Background and aim: Several age related diseases (cardiovascular diseases, Parkinson's and Alzheimer's diseases, eye diseases (cataract, age related macular degeneration)) have in common an increase level of 7-ketocholesterol (7KC) in biological fluids and/or in the tissue lesions. It is well admitted that 7KC favors oxidative stress, organelle dysfunction (lysosome, mitochondria, peroxisome, endoplasmic reticulum) which can contribute to trigger cell death (oxiapoptophagy).

To prevent and treat such diseases, mixture of molecules as well as natural and synthetic molecules can be used. The use of essential oils is well known in traditionnal medecine and in aromatherapy. However, nothing is known on the impact of essential oils on 7KC-induced cytotoxicity.

By using different nervous cell lines (N2a, SH-SY5Y et SK-N-BE) treated by different compounds inducing an oxydative stress such as Amyloid β, H_2O_2 and 7KC, our objective was to determine whether the cytotoxic effects of these compounds was attenuated or not by lemon essential oils. Alpha-tocopherol was used as positive control to prevent 7KC-induced cell death.

Methods: Lemon essential oils were extracted and further identified by gas chromatography-mass spectrometry. The *in vitro* cytoprotective activity of lemon essential oils was determined using the MTT and Sulforhodamine 101 (SR101) assays to evaluate the impact on cell growth and cell viability. Flow cytometric analyses were also realized to measure plasma membrane permeability and cell death by staining with Propidium Iodide (PI). As tu évalué le stress oxidant???? Si oui, completer

Results: Lemon essential oils composition varied with the phenological stage. The main chemical classes were D-Limonene (65.78%) with the most representative compound being β-pinene (16.54%). No cytoprotective effects of lemon essential oils were observed against 7KC-induced cell death as well as against Amyloid β and H_2O_2.

Conclusion: Whereas lemon essential oils have anti-bacterial and anti-inflammatory properties, no cytoprotective effects were observed against Amyloid β, H_2O_2 and 7KC

Keywords: lemon essential oils, cytoprotection, 7-ketocholesterol.

FT18

Characterization of LXRβ specific modulators

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Nuclear receptors for oxysterols LXRα (NR1H3) and LXRβ (NR1H2) are two transcription factors that could activated by numerous natural ligands, usually derived from oxidized derivatives of cholesterol. Both LXRs are associated to the control of numerous physiological functions. They are thus putative pharmacological targets for the treatment of homeostasis deregulations such as dyslipidemia, type 2 diabetes, skin disorders, and cancers (prostate, breast, bones, colon-rectum, brain, skin…). To date, only few synthetic compounds have been used in human therapy. This is mainly due to the high sequence identity between LXRα and LXRβ and the serious side effects.

For the screening of specific modulators, we have used two complementary approaches: the study of the LXRβ receptor by molecular modelling in order to identify the interactions necessary to obtain an agonist effect, and the test of these molecules in transfection assay using a specific chimeric receptor.

The molecular modelling data has pointed out that the hydrophobic ligand-binding pocket is larger than what has been suspected so far, which explains why slight modifications within the molecular interactions could change a strong agonist into an agonist. Besides, we have been able to propose new molecules that could modulate LXRβ transcriptional activity from docking assays. The transfection assays have confirmed the pertinence of this screening.

This screening tool gives hence new opportunities for the study of LXRβ modulators and to decipher the molecular characteristics of molecules that could differentiate the transcriptional activity of LXRα vs. LXRβ.

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FT19

Bioremediation of 7-Ketocholesterol and subsequent biotransformation by cholesterol oxidase nano-conjugates

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The oxidation of cholesterol results in the formation of oxysterols such as 7-ketocholesterol (7KC), which are implicated in a number of age-related disorders such as atherosclerosis, Alzheimer's disease and macular degeneration. 7KC can be majorly absorbed from animal-origin food products or produced endogenously in the body. Thus, at the very outset of the thesis, Indian milk products were assessed for their 7KC content. According to preliminary studies, 7KC levels were detected to be higher in milk powder samples than in raw milk. However, it was detected in negligible amounts in cheese and ghee samples. Thus, the nature of cooking/processing and temperature involved plays an important role in 7KC formation.
Current modalities against 7KC mediated cytotoxicity use antioxidants and other natural or synthetic molecules to reduce 7KC-induced cytotoxity. The alternative application of enzymes from microbial sources to degrade oxysterols in vitro and in vivo is an innovative 'medical bioremediation' approach. During initial screening, Pseudomonas aeruginosa PseA and Rhodococcus erythropolis MTCC 3951 were found to be potential degrader strains using 7KC as a sole carbon source. Under optimized conditions, they were able to degrade 88% (within 25 days) and 91% (within 15 days) respectively of an initial concentration of 1g/L (1000ppm) 7KC. Preliminary in vitro studies with extra-cellular extracts showed degradation of the compound, thus reinforcing the occurrence of suitable enzymatic systems involved in the process. The strains produced cholesterol oxidase, lipase, dehydrogenase and reductase in the presence of 7KC, with cholesterol oxidase being reported as the major enzyme involved in the degradation pathway. Some of the intermediates were also identified to predict the degradation pathway. The extracellular extracts of both the strains decreased the 7KC content when added to milk products, which may form a strategy for dealing with 7KC cytotoxicity at source.
In order to increase the applicability, cholesterol oxidase (ChOx) enzyme from P. aeruginosa PseA (ChOxP) and R. erythropolis MTCC 3951 (ChOxR) strains and a commercial variant from Streptomyces sp. (ChOxS) were immobilized on functionalized magnetic Iron (II, III) oxide (MNP) and Silica nanoparticles (SNP). The MNP-nanobiocatalysts in case of ChOxP, ChOxR and ChOxS, retained 71, 91 and 86% of cholesterol oxidase activity respectively. In case of SNPs, the immobilization efficiency was calculated as 68, 86, 83% for ChOxP, ChOxR and ChOxS respectively. The catalytic efficiency of the immobilized enzyme was found to be almost 2.0 times higher than free enzyme, along with increase in stability over a wide range of temperature (10-70 °C) and pH (4.0-9.0). However, the pH (7.5) and temperature (30 °C) optima were found to remain unchanged. The nanobioconjugates were reusable upto 10th cycle of operation. The immobilization of the enzyme on nanoparticles was confirmed by FTIR, SEM and TEM. Pharmaceutically important molecules, 4-cholesten-3-one and 4-cholesten-3,7-dione, were produced through biotransformation of cholesterol and 7KC respectively using the nanobioconjugates.

FT20

Investigation of the Cyp27a1-mediated hydroxylation of 4β-hydroxycholesterol

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Oxysterols are oxidized derivatives of cholesterol that are formed by enzymatic processes or through the action of reactive oxygen species. 4β-hydroxycholesterol, an LXR agonist, is the most abundant circulating oxysterol in mice. It is synthesized by the human CYP3A4 and CYP3A5 or their murine orthologues Cyp3a11 and Cyp3a13. 4β-hydroxycholesterol levels are decreased in inflammatory pathologies such as Crohn's disease and obesity. Surprisingly, apart from its long half-life of several days, little is known about its catabolic pathway.

27-hydroxycholesterol, another oxysterol formed by CYP27A1, is also implicated in inflammatory processes through its action on LXR, ERα and RORγ. As 4β-hydroxycholesterol, its levels were decreased in patients suffering from inflammatory bowel diseases (IBD), which was directly correlated with the decreased expression of CYP27A1, CYP3A4 and CYP3A5.

In this context, we decided to investigate the catabolism of 4β-hydroxycholesterol, and more specifically its 27-hydroxylation by Cyp27a1.

First, we set up an in vitro Cyp27a1 activity assay to measure the 27-hydroxylation of 4β-hydroxycholesterol. Mitochondria-enriched fractions were isolated from mouse liver and the protocol was validated using cholesterol as a substrate. Then, the protocol was applied to the 27-hydroxylation of 4β-hydroxycholesterol. Using an HPLC-MS Orbitrap with high mass resolution, we observed four peaks corresponding to hypothetical 4β-hydroxycholesterol metabolites. Then, using cyclosporin A as an inhibitor of Cyp27a1, we observed a decreased signal of one peak.

Finally, we observed an increased signal of this peak following intraperitoneal administration of 4β-hydroxycholesterol in mice. These results support the identification of 4β,27-dihydroxycholesterol in vivo.

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