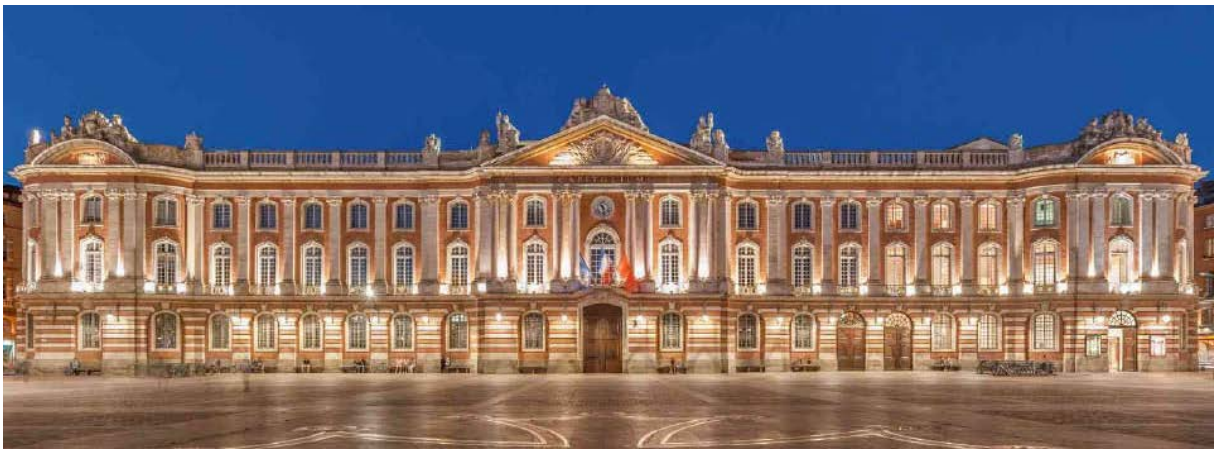
12th meeting of the European Network for Oxysterol Research (ENOR)

Importance of oxysterols in nutrition, Cancer and other degenerative diseases



21st – 22nd September 2023

Toulouse, France

September 21th (whole day) - September 22nd (Morning)

IUCT-Oncopole
Amphithéatre Claudius Regaud

https://www.oxysterols.net/
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Local organizing committee

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University of Strasbourg, France

William J Griffiths, PhD
University of Swansea, UK

Marc Poirot, PhD
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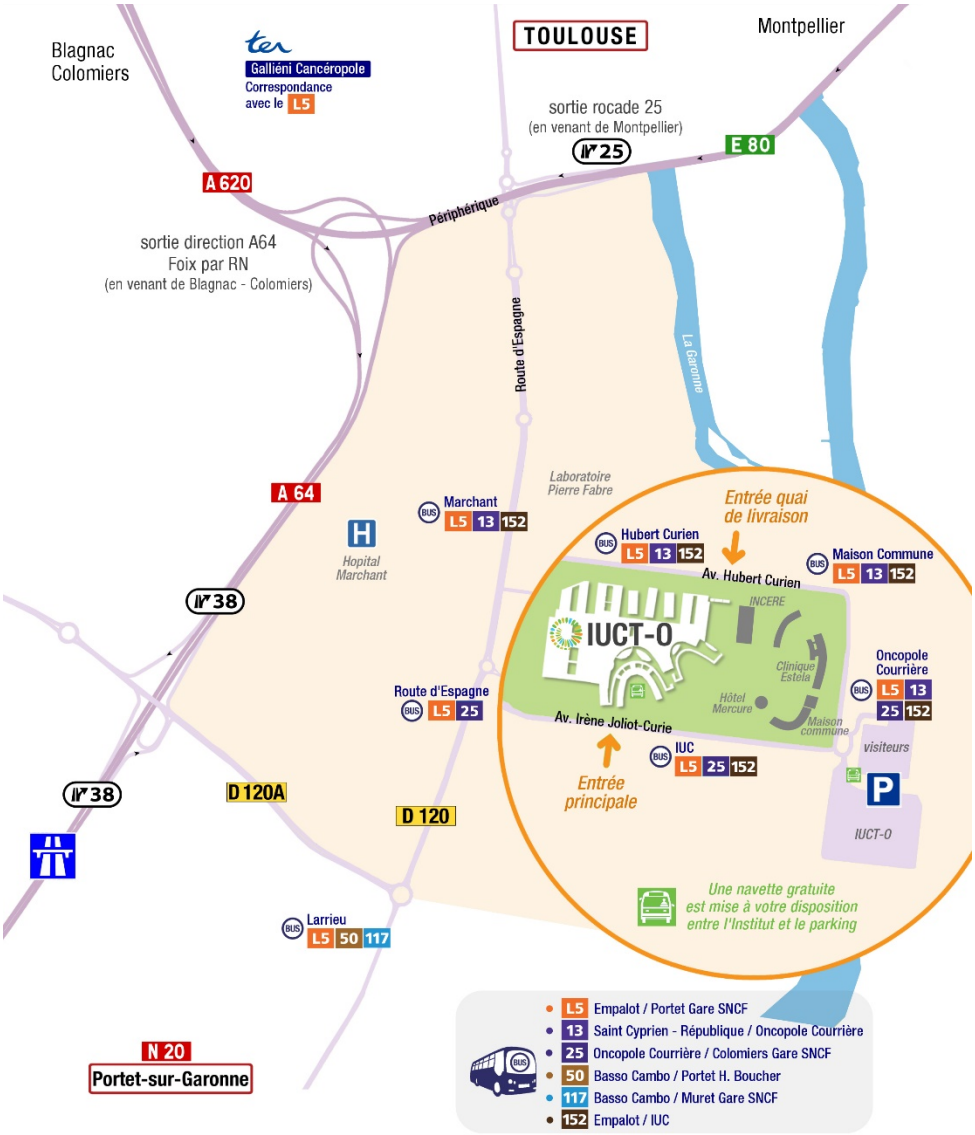

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Program
12th ENOR Symposium - *21-22 September 2023*

Thursday, September 21rst

08:30-08:40 Opening messages
Luigi Iuliano, Gérard Lizard, Marc Poirot, & Sandrine Silvente-Poirot

Session 1: Metabolism of oxysterols and phytosterols (chair: P de Medina & G Lizard)

8:40-9:40 Plenary 1 - Hubert Schaller
Phytosterol metabolism: genetic analysis of the mevalonate / isoprenoid/phytosterol pathways.

09:40-10:00 Oral communication (15' + 5' questions)

Philippe de Médina / Silia Ayadi - Metabolic switching from tumor promoter activities of oncosterone and 27-hydroxycholesterol to 27-hydroxy-oncosterone with antiproliferative properties

10:00-10:30 Flash presentations (5' + 5' questions)

Yvonne Benatzy - ALOX15B controls macrophage cholesterol homeostasis via lipid peroxidation, ERK1/2 and SREBP2.

Philippe de Médina - Chemical synthesis and biochemical properties of cholestane-5α,6β-diol-3-sulfonate, a non-hydrolysable analogue of cholestane-5α,6β-diol-3β-sulfate.

Kersti Karu - Importance of oxysterols, phytosterols in nutrition and possible biomarkers of Sepsis-induced brain dysfunction (SIBD).

10:30-11:00 Coffee break / comfort break

11:00 -11:20 Oral communication (15' + 5' questions)

Silia Ayadi - Biochemical and structural evidences that the human glutathione S-transferase A1 catalyzes the biosynthesis of dendrogenins.

11:20-11:40 Flash presentation (5' + 5' questions)

Maria Teresa Rodriguez-Estrada - Unraveling the bioaccessibility of plant sterol oxidation products from plant sterol-enriched rye bread under young adult and senior digestion conditions.

Hannah Nudds - Role of the cholesterol-oxysterol-bile acid pathway in maintenance of neuronal health.

12:00-14:00 Lunch

Session 2: Sterol metabolism and physiolopathology
(Chair: Y Wang & S Silvente-Poirot)

14:00-15:00 **Plenary 2 - William J Griffiths**, Eylan Yutuc, Manuela Pacciarini, Lauren Griffiths, Mohsen Ali Asgari, Yuqin Wang
Cholesterol Biosynthesis and Metabolism: Implications in Immunity and Neurodegeneration

15:00-15:40 **Oral communications (15' + 5' questions)**

Irina Pikuleva - Primary processes integrating the multiplicity of CYP46A1 activity brain effects.

Cashikar Anil - Microglial 25-hydroxycholesterol mediates neuroinflammation and neurodegeneration in a mouse model of tauopathy.

15:40-15:50 Flash presentation (5' + 5' questions)

Isabelle Delton / Clara Hennot - Antiparasitic activity of 25-OH, 27-OH and 7-KC on macrophages infection by Leishmania infantum parasite.

15:50-16:20 **Coffee break / comfort break**

16:20-17:00 **Oral communications (15 min +5 min questions)**

Dieter Lütjohann - Role of CYP39A1 deficiency and 24S-hydroxycholesterol in the pathogenesis of pseudoexfoliation syndrome/glaucoma.

Sandrine Betuing - Liver X Receptor (LXR) activation is associated with behavioural and neuropathological improvements in Huntington's disease.

17:00-17:10 **Flash presentations (5' + 5' questions)**

Paola Gamba - Altered brain cholesterol homeostasis in a Down syndrome murine model: could it correlate with early Alzheimer's disease onset?

17:10-18:00 **ENOR's general assembly.**

20:00 **Gala dinner**

Day 2
Friday, September 22nd

Session 3 : Sterols, cancer and other diseases
(D Lütjohan & L Iuliano)

08:30 Welcome to day 2

08:30-9:30 **Plenary 3 - Marc Poirot** & Sandrine Silvente-Poirot
Characterization of a new branch in the cholesterol pathway centered on cholesterol-5,6-epoxides: the story of dendrogenins and oncosterone discoveries

9:30-10:10 **Oral communications (15 min + 5 min questions)**

Silke Matysik - Cerebrotendinous Xanthomatosis: 13-year follow-up case report of LDL-apheresis therapy.

Julio Bunay - The oxysterol dendrogenin A increases the activation of migratory dendritic cells by acting through the LXRβ.

10:10-10:30 **Flash presentations (5 min + 5 min questions)**

Meriam Messedi / Gérard Lizard - Plasma circulating oxysterol levels in patients with metabolic syndrome.

Monique Mulder - Identification of side chain oxidized sterols as novel liver X receptor agonists with therapeutic potential in the treatment of cardiovascular and neurodegenerative diseases.

10:30-11:00 **Coffee break / Comfort break**

11:00-11:40 **Oral communications (15 min + 5 min questions)**

Jean-Marc Lobaccaro / **Norberta Delporte, Aurélie Lagarde** - Development of new synthetic molecules modulating Liver X receptors.

Ljerka Delac - Molecular phenotyping of an App knock-in mouse model of Alzheimer's disease with CYP46A1 upregulation.

11:40-12:00 **Flash presentations (5 min +5 min questions)**

Fatima Nigro - Cholenamides and Homocholenamides as Herpetic Antivirals: From Identification by a Library Screening toward Rational Optimization.

Marc Poirot / Farid Khallouki - Synthesis and cellular evaluation of new bi-specific estrogen receptor modulators and ACAT/SOAT inhibitors as anticancer agents.

12:00-13:30 **End of the meeting, concluding remarks and lunch**

Dear ENOR's members,

In 2023, the ENOR meeting will again be held in person. The theme for this year's meeting is the "Importance of Oxysterols in Nutrition, Cancer, and Other Degenerative Diseases." We eagerly anticipate reconnecting with colleagues, exchanging ideas, and engaging in face-to-face discussions. The 12th ENOR meeting will be hosted locally by Marc and Sandrine Poirot, along with their dedicated team and the logistical support from the University of Toulouse.

The event is scheduled to take place on **21-22 September 2023** in the beautiful city of Toulouse, specifically at the Toulouse Oncopole. This gathering will serve as a valuable platform for researchers across various disciplines in the life sciences to share their findings and insights on oxysterols and phytosterols. Moreover, we aim to explore how these molecules can be applied in diverse domains, ranging from agri-food to medicine.

As in previous years, we encourage young researchers to present their work through oral communications and posters. A selection of these presentations will be chosen for flash talk sessions. Additionally, we will be recognizing outstanding contributions by awarding two prizes for the best works presented. Furthermore, following our tradition, there will be an opportunity to publish the works presented at the meeting in a Special Issue of a highly esteemed scientific journal. This initiative aims to promote the quality of research conducted within the ENOR network and showcase the dynamic nature of our group.

We eagerly anticipate the participation of many of you at this 12th ENOR symposium. We guarantee a congenial and fraternal atmosphere where you can enjoy excellent scientific exchanges. Let us come together and contribute to advancing our understanding of oxysterols and phytosterols while fostering fruitful collaborations.

Marc Poirot
and local organizing committee

NB: *people selected for poster / flash communication or oral presentation can submit a paper (research paper, review, short communication) in J Steroid Biochem Mol Biol (ENOR Special Issue, deadline for submission: end of march 2024)*

Cholesterol Biosynthesis and Metabolism : Implications in Immunity and Neurodegeneration

William J Griffiths, Eylan Yutuc, Manuela Pacciarini, Lauren Griffiths, Mohsen Ali Asgari, Yuqin Wang

Swansea University Medical School, Swansea, Wales, UK.

Cholesterol is a key molecule in both the immune and nervous systems. This is due to its essential role in membranes and as a precursor of signalling molecules. The primary route of cholesterol metabolism is towards the formation of bile acids although steroid hormones are generated in steroidogenic tissue. While the neutral pathway of bile acid biosynthesis starting with 7αhydroxylation of cholesterol is hepatic other pathways are initiated extra-hepatically. Importantly, these latter pathways produce signalling molecules, e.g. as ligands to nuclear and G protein-coupled receptors and as modulators of SREBP processing. In terms of the immune system 25-hydroxycholesterol (25-HC) appears to be of central importance. Unlike other monohydroxycholesterols which are formed from cholesterol by cytochrome P450 (CYP) enzymes, cholesterol 25-hydroxylase (CH25H) is not a CYP. 25-HC is formed by cells of the immune system and has been ascribed both anti-bacterial and anti-viral activities. 7α,25-Dihydroxycholesterol (7α,25- diHC) formed by the action of CYP7B1 on 25-HC is a ligand to GPR183 and also has a role in the immune system acting as a chemoattractant to cells expressing this receptor. In this paper we will describe cholesterol metabolism in a group of patients where the CH25H gene is deleted along with the adjacent LIPA gene and discuss CH25H-indipendent mechanisms of 7α,25-diHC formation. Cholesterol metabolism is also linked with neurodegeneration, here 24S-hydroxycholesterol (24S-HC) is the focal molecule, but metabolism via other pathways may also be important. The potential of using 24S-HC and other oxysterols as markers of neurodegeneration will be discussed.

Characterization of a new branch in the cholesterol pathway centered on cholesterol-5,6-epoxides: the story of dendrogenins and oncosterone discoveries.

Marc Poirot & Sandrine Silvente-Poirot

University of Toulouse 3 / INSERM, France

The 20th century was marked by the characterization of the human genome, transcriptome and proteome. The elucidation of the human metabolome is still in its infancy but remains an extraordinary challenge for the future. This is mainly because it requires specific analytical methods dedicated to different structural classes and subclasses of metabolites that display very different physico-chemical properties. The sterolome constitutes a subdivision of the lipidome, which is in expansion. Apart from steroids (defining the steroidome), the sterolome includes cholesterol and its steroidal precursors, plant and other living organisms sterols, bile acids, and oxysterols in their free or conjugated forms. Oxysterols are mono- or poly-oxygenation products of sterols known to exist in free form or as conjugates with fatty acids, sulfate, or glucides. The characterization of known sterols is still ongoing and revealed very specific structuredependent biological properties suggesting that each oxysterol may have a specific biological function in a given physiological context. The identification of new bioactive sterol metabolites remains a very challenging but important objective for the future. We have developed a targeted and rational approach to identify new bioactive oxysterols that are 5,6-epoxycholestanols metabolites. To do this, we have: 1) identified the cholesterol-5,6-epoxide hydrolase as a putative check point metabolic enzyme, 2) conceived putative 5,6-epoxycholestanols derivatives, 3) chemically synthesized them, 4) developed specific analytical methods to confirm their existence as endogenous metabolites in mammals, 5) characterized their biological properties, 6) identified their cognate receptors, 7) identified their biosynthesis enzymes, 8) and studied the deregulation of their metabolism in cancers. We will expand on the 5,6-EC metabolic branch that was uncovered, and led to the discoveries of the oncometabolite and tumor promoter oncosterone and of the tumor suppressor metabolite dendrogenin A, both involved in the control of carcinogenesis.

Phytosterol metabolism: genetic analysis of the mevalonate/isoprenoid/phytosterol pathways.

Hubert Schaller

Institut de biologie moléculaire des plantes (IBMP), CNRS, Université de Strasbourg, 12 rue du Général Zimmer, Strasbourg, France

Phytosterol metabolism produces a vast and diverse array of compounds across organisms and species. This includes sterols and their fatty acyl or glycosidic conjugates, stanols, and the various types of oxidized sterols (oxyphytosterols) found in plants like brassinosteroids, ecdysteroids, secosteroids (vitamin D), withanolides, steroidal alkaloids, and cardenolides. Whereas phytosterols and brassinosteroids exhibit essential biological functions in plant development and responses to environmental biotic and abiotic stresses, other classes of steroids like e.g. steroidal alkaloids are produced through specialization and display particular ecological functions.

The coordination of growth and metabolism and responses to the environment implies regulatory mechanisms acting at the level of phytosterol biosynthesis. In contrast to the well documented mechanism of cholesterol homeostasis in mammals, knowledge about phytosterol homeostasis is more limited. A common aspect between plant and mammal sterol metabolism is a crucial role of upstream enzymes implied in the mevalonate/isoprenoid pathway. To identify major components at play in isoprenoid and phytosterol homeostasis, we have designed genetic screens searching for second site suppressor mutations of loss-of-function mutants hampered in the production of C5 isoprenic building blocks. Genetic and bulk segregant NGS analyses led to the identification of a gene of which null alleles are causing a growth restoration of a mutant hmg1 deficient in HMGR (3-hydroxy-3-methylglutaryl-coenzyme A-reductase) therefore coined ROGH (restoration of growth of hmg1; Villette, Darnet, et al) and also known as HISE (high sterol ester; Shimada et al, 2019). Loss of function of HISE/ROGH in a wild-type background increases HMGR by a yet unknown mechanism and results in enhanced sterol biosynthesis. This is reminiscent of previous knowledge regarding the up-regulation of the biosynthetic flux in the mevalonate pathway, resulting in a strong and steady accumulation of sterols in the form of sterol esters that are deposited in cytoplasmic lipid droplets.

Finally, the search for regulatory component(s) acting on the production of phytosterol may open new perspectives for breeding or metabolic engineering strategies for the production of bioactive phytosterol derivatives.

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Alphabetical order first author

Microglial 25-hydroxycholesterol mediates neuroinflammation and neurodegeneration in a mouse model of tauopathy.

Cashikar Anil∗†1, Danira Toral-Rios1, Justin Long1, Jinsheng Yu1, Jason Ulrich1, David Holtzman1, and Steven Paul1

[1] *Washington University School of Medicine in St. Louis – USA*

In response to inflammatory stimuli myeloid cells overexpress Ch25h, which encodes cholesterol 25-hydroxylase - an enzyme that converts cholesterol to 25-hydroxycholesterol (25HC). 25HC has been shown to modulate cholesterol metabolism and have immunomodulatory effects. We and others have previously observed that Ch25h is overexpressed in disease-associated microglia (DAM) in AD brain and AD mouse models of amyloid beta and tau aggregation. Our lab demonstrated a pro-inflammatory role for 25HC in augmenting interleukin 1-beta production in mouse microglia stimulated with lipopolysaccharide. We also showed that in mouse astrocytes, 25HC regulates cholesterol metabolism and modulates levels of extracellular ApoE. To investigate the role of 25-HC in vivo, PS19 tau transgenic mice expressing human P301S mutant tau and PS19 mice lacking the Ch25h gene were generated and aged to 9.5 months before assessing neuroinflammation and neurodegeneration. Similarly aged wildtype and ch25h knockout mice were used as controls. Our results show that in the absence of Ch25h and 25HC, there is a striking reduction in age-dependent neuroinflammation and neurodegeneration in the hippocampus and entorhinal/piriform cortex. Transcriptomic analyses revealed that Ch25h deficiency in PS19 mice reduced NFkB and Stat3 signaling to suppress proinflammatory cytokine signaling and restored sterol synthesis as well as trans-synaptic signaling. Our results suggest Ch25h may be a novel therapeutic target for primary tauopathies, AD, and other neuroinflammatory diseases.

Keywords : Ch25h, 25-hydroxycholesterol, neuroinflammation, neurodegeneration, tauopathy, Alzheimer's disease

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Metabolic switching from tumor promoter activities of oncosterone and 27-hydroxycholesterol to 27-hydroxy-oncosterone with antiproliferative properties

Silia Ayadi, Sylvia Friedrich, Régis Soulès, Laly Pucheu, Dieter Lütjohann, Sandrine Silvente-Poirot, Marc Poirot*[1] and Philippe De Médina†‡

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Oncosterone (6-oxo-cholestan-3β,5α-diol ; OCDO) is an oncometabolite and a tumor promoter on estrogen receptor alpha positive breast cancer (ERα+ BC) and triple negative breast cancers (TNBC). OCDO is an oxysterol formed in three steps from cholesterol by oxygenation of its delta-5,6 double bond : 1) oxygen addition at the double bond to give a- or b- isomers of 5,6-epoxycholestanols (5,6-EC), 2) hydrolyses of the epoxide ring of 5,6-ECs to give cholestane-3β,5α,6β-triol (CT), and 3) oxidation of the C6 hydroxyl of CT to give OCDO. On the other hand, cholesterol can be hydroxylated by CYP27A1 at the ultimate methyl carbon of its side chain to give 27-hydroxycholesterol (27HC), which is a tumor promoter for ERα+ BC. Whether OCDO and its precursors can be hydroxylated at C27 by CYP27A1 is currently unknown so do is the impact on such modification on ERα+ and TNBC cell proliferation. We investigate herein if 27-hydroxylated-5,6-ECs, -CT and -OCDO exist as metabolites and can be produced by CYP27A1 expressing cells. We report for the first time that these compounds exist as human metabolites. We give pharmacological and genetic evidences that CYP27A1 is responsible for their production. Importantly, we found that 27H-OCDO inhibits BC cells proliferation showing that these metabolic conversions commute the proliferative properties of OCDO and 27HC into antiproliferative ones. These data highlight an unprecedent role of CYP27A1 in the control of breast carcinogenesis that mitigates the tumor promoter activities of oncosterone and 27HC.

Keywords : CYP27A1 ; oxysterols ; cholesterol, 5, 6, epoxides ; cholestane ; triol, ; oncosterone ; metabolism ; breast cancer.

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Biochemical and structural evidences that the human glutathione S-transferase A1 catalyzes the biosynthesis of dendrogenins

Silia Ayadi*, Georges Ndikoum-Matip, Philippe De Médina, Régis Soulès, Lionel Mourey, Virginie Nahoum, Laurent Maveyraud†, Sandrine Silvente-Poirot‡, and Marc Poirot*§[1]

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Dendrogenins constitute a new class of bioactive cholesterol/oxysterol metabolites extending the sterolome content with new bioactive steroids. Dendrogenins are formed through the enzymatic conjugation of the 5,6 α-epoxycholesterol (5,6α-EC) with small molecular weight biological nucleophiles at C6 of the steroid backbone through a *trans*-diaxial opening of the epoxide ring. Among dendrogenins, dendrogenin A (DDA) is of peculiar interest because it is a tumor suppressor metabolite, which level strongly decreased during breast carcinogenesis evidencing a deregulation of its metabolism in breast cancers (BC). In order to get more insight into DDA metabolism we have investigated the identification of the DDA synthase. While the enzymatic conjugation of an epoxide with an amine has never been reported in databases, a similar reaction with the nucleophilic mercaptant group (SH) of glutathione was reported to be catalyzed by the rat glutathione S-transferase A1 (rGSTA1) to give a compound we named dendrogenin C (DDC). In addition: 1) the analyse of transcriptomic databases shows a strong decrease in human GSTA1 (hGSTA1) expression in BC, 2) the immuno detection of hGSTA1 in BC biopsies confirmed this decrease at the protein level, 3) hGSTA1 is selectively expressed in epithelial cells from lactating ducts and terminal units, those cells that are at the origin of 70% of BC, 4) hGSTA1 expression is a good pronosis for patient survival for all BC subtypes (HR= 0.75, n= 4929, 37 datasets, https://kmplot.com). We thus investigated if hGSTA1 can catalyze the biosynthesis of dendrogenins.

We have produced recombinant hGSTA1 in *E Coli* and purified it by affinity chromatography. We have defined specific but different biochemical conditions that enable the production of DDA and DDC respectively, evidencing a context-dependent production of DDA and DDC. These data shows that hGSTA1 is a dendrogenin synthase, and this was confirmed through genetic and pharmacological approaches.

To get more insight into the enzymatic activity of hGSTA in dendrogenin biosynthesis, we synthesized hydrosoluble forms of 5,6α-EC, DDA and DDC and used them for cristallisation purposes. We obtained several high resolution crystalls of the complexes giving structural insight for the dendrogenin synthase activity.

Together these data showed that the promiscuous type II xenobiotic metabolism enzyme hGSTA1 is responsible for an unprecedented reported production of endogenous conjugated oxysterols metabolism leading to the production of at least two dendrogenins.

Keywords : dendrogenin, oxysterol, epoxycholestanol, conjugation, enzymology, pharmacology, structure

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The oxysterol dendrogenin A increases the activation of migratory dendritic cells by acting through the LXRβ

Julio Bunay*[1], Laly Pucheu, Silia Ayadi, Michel Record, Philippe De Medina, Marc Poirot†, and Sandrine Silvente-Poirot‡

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Background and aim : Dendritic cells (DC) are antigen-presenting cells with the property to initiate immune responses and anti-tumor activity after their maturation and CC chemokine receptor-7 (CCR7)-dependent migration to lymphoid organs. The activation of DC is often reduced in tumors resistant to immunotherapies, such as immune checkpoint inhibitors (ICI), used in the clinic. Hence, the development of novel therapy that restore or enhance DC function would help to enhance ICI response. Dendrogenin A (DDA), is an endogenous oxysterol and a modulator of the Liver-X receptors (LXRα/β), which induces tumor re-differentiation and growth inhibition in various cancers. These effects were shown associated with DC and T cell infiltration into tumors (de Medina et al, Nature Commun. 2013). In addition, the complex DDA/LXRβ in tumor cells increases the secretion of anti-tumor and immunogenic small extracellular vesicles (exosomes) (Record et al, J. Extracell. Vesicle, 2022), indicating that DDA activates an anti-tumor immune response by targeting the LXRβ present in tumors. Since LXR ligands, such as 22R-hydroxycholesterol, were reported to inhibit the maturation and migration of DC as well as anti-tumor immune response (Villablanca, Nat Med, 2010), we characterized, in the present study, the impact of DDA treatment on DC and the mechanism involved.

Methods and Results : Since previous biodistribution assays indicated that DDA accumulates in bone marrow (Segala et al, Nat Commun, 2017), we studied DC derived from bone marrow precursors (BMDC) from mice. We showed that DDA increased the differentiation of BMDC by increasing the population of Ly6C-Cd62l+ DC precursors along with a decrease in macrophage markers. DDA induced the differentiation of BMDC which were enriched in migratory CCR7+ DC and upregulated the transcription of non-canonical transcriptional factors Id2/E2-2a and Stat3. Furthermore, DDA induced the maturation and the activation of migratory DC by increasing the surface expression of CCR7, MHC class II and costimulatory molecules (CD80, CD86) compared to BMDC challenged with the solvent control. This is followed by functional DC maturation shown by increased mRNA levels of cytokines produced during DC maturation. We also showed that the DDA induced the maturation and the activation of migratory DC through LXRβ activation and characterized the potential mechanism involved.

Conclusion : Together, these data show that DDA stimulates the differentiation and maturation of migrating BMDC by acting through the LXRβ. Thus, DDA seems to have a dual effect on anti-tumor immunity by acting on LXRβ present in tumors to release anti-tumor and immunogenic exosomes and also on LXRβ present in BMDC to activate their maturation and migration.

Keywords : DDA, Dendritic cell, activation, immunity, LXR

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Molecular phenotyping of an App knock-in mouse model of Alzheimer's disease with CYP46A1 upregulation

Ljerka Delac*[1], Maria Latorre Leal[1], Francesca Eroli[1], Gabriela Da Cruz Meda[1], Ivan Nalvarte[2], Per Nilsson[1], Ingemar Björkhem[3], and Silvia Maioli[1]

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Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder with a higher prevalence in women compared to men. Recent investigations demonstrate altered cholesterol metabolism in AD and its relation to pathological hallmarks of AD, such as amyloid-beta buildup and neuroinflammation. The CYP46A1 enzyme, central to cerebral cholesterol turnover, and its product, 24 (S)-hydroxycholesterol (24S-OH), show a decrease in the brain levels in different stages of AD. As the activity of CYP46A1 is relevant for the memory and learning processes, we have developed a mouse model of Alzheimer's disease with CYP46A1 upregulation to study its potential neuroprotective effects in a sex-specific manner.

We employed single *App* knock-in mice carrying Swedish, Beyreuther/Iberian, and Arctic mutation (*App NL-G-F*) crossed with mice overexpressing the human CYP46A1 transgene (Cyp46 Tg). Female and male cohorts were aged to 6 months of age. A battery of cognitive and anxiety-like behavioral tests was conducted, followed by cholesterol and oxysterol measurements and immunohistochemical stainings for AD pathology markers.

The results of our study demonstrate that no distinct cognitive deficits in both short- and long-term memory are distinguishable. At six months of age, only male *App NL-G-F* mice exhibit anxiolytic behavior, whereas females do not. Interestingly, increased serum levels of 24S-OH and 27-hydroxycholesterol were not reflected in the brain, the main site of 24S-OH production, as seen previously in Cyp46 Tg animals. Furthermore, quantification of microglia and amyloid-beta deposits has been carried out to examine sex differences and the impact of CYP46A1 overexpression.

These results suggest alterations in cerebral oxysterol metabolism in the presence of triple *App* knock-in mutation and propose Cyp46 Tg x APP NL-G-F as a novel platform to investigate cholesterol perturbations in AD.

Keywords : Alzheimer’s Disease ; Disease Models ; Animal ; Cholesterol 24-Hydroxylase ; 24-hydroxycholesterol ; Sex Differences

∗ Speaker

Development of new synthetic molecules modulating Liver X receptors

Norberta Delporte[1], Aurélie Lagarde[2], Silvère Baron[2], Pierre Chalard[3], Isabelle Thomas[3], and Jean-Marc Lobaccaro*[4]

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Aim. Nuclear receptors for oxysterols LXRa (NR1H3) and LXRb (NR1H2) are two transcription factors that could be activated by natural ligands, usually derived from oxidized derivatives of cholesterol, also known as oxysterols. Both LXRs are associated to the control of numerous physiological functions such as cellular growth, immune response, apoptosis. 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...). Even though activation of LXRb has been associated to trigger lethal autophagy in cancers, to date, only few synthetic compounds are used in human therapy. This is mainly due to the high sequence identity between LXRa and LXRb and the serious side effects observed (transient hypertriglyceridemia and neurological disorders).

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

Results. 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 LXRb transcriptional activity from docking assays. The transfection assays have confirmed the pertinence of this screening.

Conclusions. This screening tool gives hence new opportunities for the study of LXRb modulators and to decipher the molecular characteristics of molecules that could differentiate the transcriptional activity of LXRα vs. LXRb. In vivo studies will be performed soon.

ND & AL equivalent first authors; IT and JMAL equivalent last authors

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Keywords : LXR, synthetic ligands, prostate cancer, screening

∗ Speaker

Role of CYP39A1 deficiency and 24S-hydroxycholesterol in the pathogenesis of pseudoexfoliation syndrome/glaucoma

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Rare damaging mutations in *CYP39A1*, coding for 24S-hydroxycholesterol 7α-hydroxylase, were previously reported to double the risk of pseudoexfoliation (PEX) syndrome and development of PEX glaucoma. Expression of CYP39A1, which is involved in cholesterol clearance by conversion of 24S-hydroxycholesterol (24S-OHC) to downstream intermediates, was significantly reduced in ocular tissues of PEX patients compared to controls. Because the role of CYP39A1 in PEX pathogenesis has not been clarified, we investigated the cellular effects of CYP39A1 deficiency mimicking reduced enzymatic activity.

CYP39A1 mRNA and protein expression was analyzed in ocular tissues of eyes with PEX syndrome/glaucoma and age-matched control eyes. Cultured human nonpigmented ciliary epithelial (hNPE), trabecular meshwork (hTM), retinal pigment epithelial (hRPE), and iris pigment epithelial (hIPE) cells were exposed to 1-25 µM 24-OHC or 2 µM all-trans retinoic acid (ATRA) for 48 hours, or subjected to siRNA-mediated knockdown of CYP39A1. 24-OHC levels were measured in CYP39A1-silenced cell lysates as well as in aqueous humor and serum samples of patients with PEX syndrome, PEX glaucoma without and with *CYP39A1* mutations, and age-matched controls using gas chromatography-mass spectrometry.

Reduced CYP39A1 expression was observed in all ocular tissues of PEX compared to control eyes. Aqueous humor and serum levels of 24S-OHC and 24S-OHC/cholesterol ratios were significantly higher in PEX glaucoma patients without and with *CYP39A1* mutations compared to controls. Silencing CYP39A1 protein expression by ~85% resulted in a ~2-fold accumulation of 24S-OHC within hNPE, hTM and hRPE cells, while silencing in IPE cells by ~92% resulted in a 5-fold cellular accumulation of 24S-OHC. Both CYP39A1 deficiency and exposure to sub-lethal concentrations of 24S-OHC induced generation of free radicals and transcriptional deregulation of several genes related to inflammation (IL6, IL8, NOS2, TNF), oxidative stress (SOD2, NFKB2), barrier function (TJP1-3), matrix metabolism (ELN, LOXL1, LTBP1-2, TGFBR2), cholesterol metabolism (ABCA1, ABCG1, APOC1, LXRA) and retinoic acid signaling (STRA6, ALDH1A1, CRABP2, RARA), which reflect key aspects of PEX pathogenesis. The deleterious effects of 24S-OHC could be partially prevented by physiologic concentrations of ATRA.

The findings suggest that CYP39A1 deficiency and cellular accumulation of 24S-OHC can trigger substantial transcriptional changes and disturbances in cellular homeostasis, highlighting a novel role of CYP39A1 in PEX pathogenesis.

Keywords : ophthalmology, cholesterol metabolism, immunology, Cytochrome P450

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Cerebrotendinous Xanthomatosis : 13-Year Follow-up Case Report of LDL-Apheresis Therapy

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Cerebrotendinous xanthomatosis (CTX) is a rare, inherited autosomal-recessive lipidstorage disorder caused by 27-hydroxylase deficiency. In this study, we report of a 42-year-old man with this disorder who has been treated using chenodeoxycholic acid, simvastatin, and low-density lipoprotein (LDL) apheresis. The LDL apheresis has been performed weekly for the last 13 years. During the first 6 months, the efficacy of LDL-apheresis was monitored using quantitative determination of 7alpha-OH-4-cholesten-3-one in plasma. The clearance efficacy of 7alpha-hydroxy-4-cholesten-3-one from the patient's plasma per LDL-apheresis was sufficient but returned to the initial high levels after seven days. The mean value in plasma before apheresis was 241 ng/mL (1). Here, we present a 13-year follow-up on the long-term effectiveness of LDL-apheresis. The patient has been very adherent to therapy and presents in good condition without significant worsening of symptoms over the last 13 years. Over this whole period, LDL, HDL and total cholesterol were monitored before and after every apheresis treatment. Now, 7alpha-OH-4-cholesten-3-one was again determined for 3 months to reevaluate hepatic synthesis function and to make sure that weekly apheresis treatment is still necessary. The initial levels of 7alpha-OH-4-cholesten-3-one before apheresis were in the same range as 13 years ago (mean 236 ng/mL). There were, however, much less variations of this level in contrast to the beginning of therapy. These results suggest the continued need for effective lipid-lowering treatment. (1) Matysik, S., Orso, E., Black, A., Ahrens, N. & Schmitz, G. (2011). Chem.Phys.Lipids 164, 530-534.

Keywords : Cerebrotendinous xanthomatosis

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Liver X Receptor (LXR) activation is associated with behavioural and neuropathological improvements in Huntington's disease

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Huntington's disease (HD) is a rare neurodegenerative disease characterized by motor, neuropsychiatric and cognitive symptoms, without disease-modifying therapy. HD is associated with several cellular dysfunctions, including cholesterol metabolism deregulation with a decrease of its catabolism by the neuronal enzyme CYP46A1 into 24S-OHC, a natural ligand of the Liver X Receptor (LXR). CYP46A1 restoration in HD mice is neuroprotective and induces upregulation of LXR target genes. We hypothesized that LXR activity may contribute to CYP46A1-mediated neuroprotection since the therapeutic value of LXR has been raised in several neurodegenerative diseases. There are two LXR isoforms, LXRbeta enriched in the brain for cholesterol metabolism and inflammation regulation and LXRalpha mainly expressed in the liver for lipogenesis. The aim of the project is to study the effect of LXR activation on the pathogenesis of a pre-clinical knock-in mouse model of HD, the zQ175 mice. In the first part of our work, an integrated study from mouse behaviour to cellular and molecular features was carried out using the pan LXRalpa/beta synthetic ligand T0901317. LXR activation in primary cultures of HD striatal neurons induced neuroprotection associated with a reduction of mHTT aggregates and restoration of neuronal survival. Treatment of symptomatic zQ175 mice with T0901317 alleviated weight loss and restored short and long-term memory, social interaction and depressive-like behaviour in HD mice. However, T0901317 did not improve mouse motor behaviour. The behavioural effect of LXR activation was associated with transcriptional regulation, with a correction genes involved in pathway strongly altered in HD. In the striatum, T0901317 restored genes involved in striatal neurons identity and function; in the hippocampus, neurotransmission and BDNF signalling pathways were rescued. As expected, T0901317 also induced a strong regulation of cholesterol metabolism genes in both structures. As LXR activation induced restoration of behaviours mainly regulated by hippocampus, we then focused on this brain region. Transcriptional signature was not associated with long-term potentiation regulation in CA1 region of hippocampus. However, the downregulation of *Bdnf* mRNA level in the dentate gyrus of HD hippocampus was significantly improved specifically in neurons, which was associated with a restoration of adult neurogenesis in zQ175 mice. LXR activation also reduced mHTT aggregates in the dentate gyrus, and corrected the downregulation of *ApoE* and the upregulation of pro-inflammatory *Il-1beta* mRNA levels. We reasoned that the effect of T0901317 on behavioural alteration in HD could involve these molecular and cellular regulations, known to be important for neuron survival, cognition, and depressive-like behaviours. Repeated use of these non-isoform-selective synthetic ligands is limited by their effects on lipogenesis, mainly due to LXRalpha activation. Therefore, we now take advantage of a newly synthetized agonist, designed to preferentially target LXRbeta, as illustrated by *in silico* modelling analysis of binding activity. We validated the transcriptional bioactivity of the ligand in primary cultures of striatal neurons and astrocytes, as well as its neuroprotective role in HD cellular model. The next step is to perform a preclinical study to assess its beneficial effect in HD mouse model.

Keywords : Liver X Receptor, Huntington's disease, Behavioural phenotype, Neuropathological alterations, Hippocampus, Striatum

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Primary processes integrating the multiplicity of CYP46A1 activity brain effects

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CYP46A1 catalyzes cholesterol 24-hydroxylation, the major pathway for cholesterol elimination from the brain. Studies on different mouse models identified both neurodegenerative (e.g., Alzheimer's, Huntington's, Niemann-Pick type C1, and prion diseases as well as spinocerebellar ataxia) and non-neurodegenerative (e.g., glioblastoma and depression) brain disorders, which benefited from increased CYP46A1 activity elicited either pharmacologically by low-dose efavirenz or by gene therapy. In all these models, increases in CYP46A1 expression or activity were beneficial and targeted multiple brain processes, some of which were common (e.g., abnormal protein accumulation, memory and motor functions, gene transcription, protein phosphorylation, autophagy, and lysosomal processing) and some were disease specific. Hence, we hypothesized that there are unifying primary processes that integrate a broad range of CYP46A1 targeting outcomes in the brain. We used control and efavirenz-treated 5XFAD mice (an Alzheimer's disease model) and evaluated their brain by different approaches, namely synaptosome characterizations, metabolomic profiling, and unbiased proteomic quantifications of unlabeled, phosphorylated, acetylated, and methylated proteins. As a result, we identified three primary unifying processes - sterol flux through the plasma membranes, acetyl-CoA production, and mevalonate biosynthesis - as well as some of the secondary processes, affected by changes in the identified primary processes. The latter included cholesterol availability in the plasma membranes, protein/histone acetylation, phosphorylation, and methylation, actin cytoskeleton organization, synaptic glutamate release, acetylcholine production, and other. Thus, CYP46A1 plays a central role in maintaining of a variety of brain functions and is a viable target for treatment of different brain disorders. Supported by AG067552.

Keywords : CYP46A1, efavirenz, 24-hydroxycholesterol, brain

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ALOX15B controls macrophage cholesterol homeostasis via lipid peroxidation, ERK1/2 and SREBP2

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Macrophage cholesterol homeostasis is crucial for health and disease and has been linked to the lipid-peroxidizing enzyme arachidonate 15-lipoxygenase type B (ALOX15B). ALOX15B oxygenates free and esterified polyunsaturated fatty acids (PUFAs) to their corresponding lipid hydroperoxides, which normally are reduced to their respective lipid hydroxides. We performed global transcriptome and immunofluorescence analysis of primary human macrophages silenced in ALOX15B and noticed a reduction of nuclear sterol regulatory element-binding protein (SREBP) 2, the master transcription factor of cellular cholesterol biosynthesis. Knockdown of ALOX15B decreased the amount of nuclear SREBP2 and significantly reduced SREBP2-target gene expression. Moreover, the cholesterol biosynthetic intermediates desmosterol and lathosterol as well as the cholesterol-derived oxysterols 25- and 27-hydroxycholesterol were lowered. Confocal microscopy of the lipid peroxidation sensor BODIPY C11 and LC-MS/MS analysis of lipid hydroxides revealed that suppression of ALOX15B reduced lipid peroxidation in primary human macrophages, which attenuated transcription of SREBP2-dependent genes. Western analysis disclosed that lowering lipid peroxidation decreased mitogen-activated protein (MAP) kinase ERK1/2 activation. Concomitantly, ERK1/2 inhibition lowered nuclear and cytosolic SREBP2 and in turn expression of SREBP2-target genes. Low nuclear SREBP2 rendered both, ALOX15B-silenced and ERK1/2-inhibited macrophages refractory to SREBP2 activation upon disruption of the intracellular cholesterol transport by inhibiting the lysosomal NPC intracellular transporter 1 (NPC1). These data suggest a regulatory mechanism controlling macrophage cholesterol homeostasis based on ALOX15B-mediated lipid peroxidation and concomitant ERK1/2 activation.

Keywords : Macrophage, Lipoxygenase, Cholesterol, Lipid Peroxidation, SREBP2

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Unraveling the bioaccessibility of plant sterol oxidation products from plant sterol-enriched rye bread under young adult and senior digestion conditions

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Food enrichment with plant sterols (PS) is a common approach in developing functional foods to reduce the risk of cardiovascular disease [1]. Due to its nutritional properties and increasing popularity, rye bread is an attractive choice for incorporating PS [2]. However, PS can undergo oxidation during baking, raising concerns about potential toxic effects. Therefore, understanding the bioaccessibility of PS oxidation products (POPs) can provide essential information for consumers [3]. The aim of this study was to evaluate POP bioaccessibility from a PS-enriched (1.6 g/100 g) wholemeal rye bread subjected to different digestion conditions. The effect of gastric lipase (GL) and/or cholesterol esterase (CE) under young adult digestion conditions, as well as the adaptation to simulate senior population digestion conditions, were investigated. Bread digestions were performed as follows : i) for young adult conditions [4], INFOGEST method as control digestion (AC), INFOGEST 2.0 method for GL incorporation (A1) and Makran et al. [4] protocol for GL+CE addition (A2) were used; ii) for senior digestion assays[5], digestion with GL+CE was used as control digestion (SC), and adaptations for gastric (S1) and gastric and intestinal (S2) senior conditions were carried out. POPs in bread and bioaccessible fractions (BFs) were analyzed by gas chromatography-mass spectrometry to assess bioaccessibility [6]. In bread, only POPs derivatives from b-sitosterol were observed (mg/100 g) : triol (163.62), a-epoxy (150.31), 7a-hydroxy (143.42), 7-keto (139.74) and 7b-hydroxy (134.94). However, only 7a-hydroxy was detected in BFs from all digestion conditions. In young adult conditions assay, 7a-hydroxy contents were 87.26, 109.42, and 106.97 mg/ 100 g of bread for AC, A1, and A2, respectively. 7a-hydroxy bioaccessibility significantly increased with the addition of GL (A1, 25.45%) and GL+CE (A2, 22.59%) compared to AC. In the digestion assays under senior conditions, 7a-hydroxy content in BF ranged from 125.51 to 148.72 mg/100 g of bread for SC and S1, respectively, while it reached values of 98.40 mg/100 g of bread under S2 conditions. The bioaccessibility of 7a-hydroxy was 25.67% for SC, 30.41% for S1, and 20.12% for S2. Thus, the inclusion of lipid metabolism enzymes, like GL and CE, improved digestion conditions to better mimic the physiological environment. Moreover, the resulting lipolysis products can act as emulsifying agents, potentially facilitating the incorporation of POP into bile salt micelles. However, complete adaptation to senior population conditions reduced POP solubility, likely due to decreased lipolytic effect resulting from reduced enzyme activities. These findings provide valuable insights into the POPs fate under various digestion conditions, giving crucial information for consumers regarding the safety and potential health implications.

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Keywords : bioaccessibility, plant sterol oxidation products, wholemeal rye bread, cholesterol esterase, gastric lipase, senior population

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Chemical synthesis and biochemical properties of cholestane-5α,6β-diol-3-sulfonate, a non-hydrolysable analogue of cholestane-5α,6β-diol-3β-sulfate.

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Cholestane-3β,5α,6β-triol (CT) is a primary metabolite of 5,6-epoxycholesterols (5,6-EC) that is catalyzed by the cholesterol-5,6-epoxide hydrolase (ChEH). CT is a well-known biomarker for Niemann-Pick disease type C (NP-C), a progressive inherited neurodegenerative disease. On the other hand, CT is known to be metabolized by the 11β-hydroxysteroid-dehydrogenase of type 2 (11β-HSD2) into a tumor promoter named oncosterone that stimulates the growth of breast cancer tumors. Sulfation is a major metabolic transformation leading to the production of sulfated oxysterols. The production of cholestane-5α,6β-diol-3β-*O*-sulfate (CDS) has been reported in breast cancer cells. However, no data related to CDS biological properties have been reported so far. These studies have been hampered because sulfate esters of sterols and steroids are rapidly hydrolyzed by steroid sulfatase to give free steroids and sterols. In order to get insight into the biological properties of CDS, we report herein the synthesis and the characterization of cholestane-5α,6β-diol-3β-sulfonate (CDSN), a non-hydrolysable analogue of CDS. We show that CDSN is a potent inhibitor of 11β-HSD2 that blocks oncosterone production on cell lysate. The inhibition of oncosterone biosynthesis of a whole cell assay was observed but resulst from the blocage by CDSN of the uptake of CT in MCF-7 cells. While CDSN inhibits MCF-7 cell proliferation, we found that it potentiates the cytotoxic activity of post-lanosterol cholesterol biosynthesis inhibitors such as tamoxifen and PBPE. This effect was associated with an increase of free sterol accumulation and the appearance of giant multilamellar bodies, a structural feature reminiscent of Type C Niemann-Pick disease cells and consistant with a possible inhibition by CDSN of NPC1. Altogether, our data showed that CDSN is biologically active and that it is a valuable tool to study the biological properties of CDS and more specifically its impact on immunity and viral infection.

Keywords : sterols, oxysterol, oxysterol sulfate, cholesterol, uptake, metabolism, MLB, cancer, NPC1, proliferation, cell death, autophagy

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Evaluation of the plant sterol metabolism during in vitro colonic fermentation of wholemeal rye bread

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Wholemeal rye bread (WRB) is a valuable source of dietary fiber (arabinoxylan, fructan, cellulose, and β-glucan) [1], also promoting beneficial microbial growth (*Lactobacillus* and *Bifidobacterium*) in the colon [2]. Plant sterols (PS) have a low absorption rate (2-3%) and can be transformed by the colonic microbiota into different metabolites [3]. The routes of biotransformation and biological effects of PS metabolites, unlike those from cholesterol, have been scarcely studied [4]. The aim of this study is to evaluate the colonic metabolism of PS during *in vitro* dynamic digestion and fermentation of WRB enriched or not with PS using the simgi® system. The colon compartments (ascending (AC), transverse (TC), and descending (DC)) were inoculated with fecal slurry from one healthy volunteer. After stabilization (13 days), the assay was performed feeding the system with 80g/day of WRB or PS-WRB (containing 0.04 or 1.30g of PS, respectively) during 5 days with a 9-day washout period in between. Sterols and metabolites in breads and fermentation liquids (FL) (after 24, 48, 72, 96 and 120h of fermentation in all colon compartments) were analyzed by Fast-GC-FID. The identified sterols in breads were : campesterol, campestanol, stigmasterol, ß-sitosterol, sitostanol, D5-avenasterol, D5,24-stigmastadienol, D7-stigmastenol, and D7-avenasterol. Total PS content in AC, TC, and DC of WRB ranged between 6.1-10.3, 5.1-18.8, and 1.6-13.4 mg/compartment, respectively. The highest values for campesterol, campestanol and sitostanol were reached at 96-120h in all colon compartments, whereas b-sitosterol, stigmasterol, D5,24-stigmastadienol, D7-stigmastenol and D7-avenasterol increased after 24h remaining relatively constant through fermentation. Neither D5-avenasterol nor formation of sterol metabolites were observed. Total PS content in FL of PS-WRB ranged between 204.5-436.7, 276.5-441.3, 115.6-251.1 mg/compartment in AC, TC, and DC, respectively. In the AC, all sterols reached maximum values at 96h, whereas in TC and DC different trends were observed. The highest campestanol and stigmasterol values were observed at 24h in TC and 96h in DC, while for campesterol, ß-sitosterol and sitostanol the maximum values were reached at 72h in TC and 48-96h in DC. D5-avenasterol achieved its highest content at 120h in TC and 96h in DC and D5,24-stigmastadienol, D7-stigmastenol and D7-avenasterol reached a plateau from 48h onwards. After digestion and fermentation of PS-WRB, only sitostenone was identified from ß-sitosterol biotransformation. In AC and TC, it was determined at all fermentation times with maximum contents between 72 and 120h (0.2, 0.2-0.3 mg/compartment, respectively), while in DC only could be identified at 72 and 96h (0.1 mg/compartment). This study sheds light, for the first time, on the PS biotransformation present in bread by gut microbiota using an *in vitro* dynamic system. Further research can be focused on the biological effects of these metabolites at colonic level.

[1] Åman et al. (2019); [2] Ounnas et al. (2016); [3] Cuevas-Tena et al. (2018); [4] Blanco-Morales et al. (2021).
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Keywords : colonic fermentation, metabolites, phytosterols, rye bread, simgi® system

∗ Speaker

CYP46A1 enzyme : a promising therapeutic target to counteract Alzheimer's disease progression

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Background and rationale : It is now well known that during Alzheimer's disease (AD) progression various oxysterols may act as friend and/or foes. Of note, we previously observed, in autopsy specimens from human AD brains, that CYP46A1 levels decreased in parallel with the reduction of 24-hydroxycholesterol (24-OHC) levels, suggesting a possible association between their reduction and AD progression. Indeed, 24-OHC is produced by the neuron-specific enzyme CYP46A1 and their progressive reduction underlines the progressive neuron loss. To date, the association between CYP46A1 and tau hyperphosphorylation, one feature of AD, is still largely unexplored and controversial. In the present study we aimed to investigate whether maintaining high levels of 24-OHC, by CYP46A1 overexpression, could reduce tau phosphorylation and the accumulation of tau oligomers, thus protecting the neuronal morphology.
Methods : Primary neurons isolated from overexpressing CYP46A1 mice and primary neurons isolated from wild type mice were used. Neurons isolated from wild type mice were incubated with 1 µM 24-OHC to compare the effects exerted by the oxysterol administered exogenously to that produced endogenously due to CYP46A1 overexpression. In order to obtain an AD-like *in vitro* model characterized by tauopathy, some neurons were incubated with 10 nM okadaic acid (OKA), a chemical able to induce tau hyperphosphorylation and consequently its accumulation. Protein levels analysis was performed by Western blotting and immunofluorescence.
Results : Our data show that in all neurons, pre-treated with 24-OHC or overexpressing CYP46A1 and then incubated with OKA, tau hyperphosphorylation was markedly reduced at both residues Thr231 and Ser202/Thr205 ; moreover, the dendritic arborization affected by OKA-treatment was significantly restored, contributing to preserve the organization and stability of the neuronal cytoskeleton. Interestingly, the accumulation of the prefibrillar tau oligomers, the main tau form involved in the spreading of pathology, synaptic impairment, and cognitive decline, was significantly reduced in neurons overexpressing CYP46A1.
Conclusions : These data highlight the importance of maintaining high levels of CYP46A1 in the brain, through genetic or pharmacological stimulation, to avoid 24-OHC loss which can occur in the brain during AD progression and, consequently, to limit hyperphosphorylated tau accumulation. More in-depth investigations are needed to further elucidate the molecular mechanisms underlying the neuroprotective effect by preserving the activity of CYP46A1 enzyme.

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Antiparasitic activity of 25-OH, 27-OH and 7-KC on macrophages infection by Leishmania infantum parasite

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Leishmania parasites are the causative agents of visceral, mucocutaneous or cutaneous leishmaniasis in humans. The macrophage is the predilected host cell of *Leishmania* in which the vector promastigote stage is transformed into amastigote. It is well known that host membrane cholesterol is required for parasite binding, internalization and intracellular multiplication. We have previously shown that the sterol profile of the parasite is modified during its differentiation into amastigote with a shift towards cholesterol at the expense of ergosterol which is the main sterol in the promastigote form. Oxidative stress is increased in macrophages during parasite infection, which may contribute to the generation of oxysterols. Some natural oxysterols such as 25-OH and 27-OH have been shown to exert antiviral activity; in addition, some synthetic oxysterols have demonstrated anti-leishmanial activity. Here, we investigated whether 25-OH, 27-OH, and 7KC could modulate the viability of *Leishmania infantum*, a human cutaneous strain, and its infectivity towards macrophages. The viability of J774 macrophages and the *L. infantum* cutaneous strain MON24 was measured after 20h incubation with increasing concentrations of 25-OH, 27-OH or 7KC. The effect on infectivity was assessed by comparing infection rate and parasite load after 20h of preincubation of J774 macrophages with 10 µM oxysterols, then 24h infection with *L. infantum*. 25-OH, 27-OH, or 7KC had no effect on parasite viability, which remained >90% even at the highest oxysterol concentration of 100 µM. Macrophage viability was not affected for oxysterol concentrations up to 10 µM for 7KC and 100 µM for 25-OH and 27-OH. After preincubation of macrophages with 10 µM oxysterol, both infection rate and parasite load were significantly reduced, with 25-OH exerting the highest inhibition. These results show that exogenous oxysterols can exert anti-leishmanial activity at the level of parasite/macrophage interaction. Further experiments are needed to accurately determine their efficient concentration and to investigate potential mechanisms. Whether oxysterols are produced by macrophages during infection and can play a regulatory role is also worth to be investigated.

Keywords : Parasitic disease, Leishmania, Macrophage

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Importance of oxysterols, phytosterols in nutrition and possible biomarkers of Sepsis-induced brain dysfunction (SIBD)

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Sepsis-induced brain dysfunction (SIBD) is associated with increased mortality and morbidity (e.g. cognitive disabilities). Accurate diagnosis of SIBD remains elusive as current brain injury biomarkers (e.g. NSE, S100ß, GFAP, NFL) are not specific/sensitive, especially at the early stages of SIBD. 24S-hydroxycholesterol (24SOH-Chol) is only produced by neurons ; our early data show a marked increase in septic patient plasma. This holds the potential for SIBD diagnosis and assessment of interventions. We hypothesise that plasma 24S-OH-Chol is a highly predictive and prognostic biomarker of sepsis-induced brain dysfunction. We utilise a well-established analytical strategy using a liquid chromatography tandem mass spectrometry to measure cholesterol metabolites and their precursors in septic patients. We also would like to present an application of this established analytical methodology for characterisation of phytosterols/oxysterols and their oxidation products in baby formula milk.

Keywords : oxysterols, sepsis, baby milk formula

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Synthesis and cellular evaluation of new bi-specific estrogen receptor modulators and ACAT/SOAT inhibitors as anticancer agents

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17β estradiol is a well-known tumor promoter for breast cancer (BC) expressing estrogen receptor which lead to antihormonal therapy for BC which represent the major therapeutic strategy for such BC. On the other hand, cholesteryl esters of fatty acids (CEE) are synthesized by acyl-coA : cholesterol acyl transferase (ACAT/SOAT) and were recently shown to display tumor promoter properties and to strongly accumulate in certain BC suggesting that the inhibition of ACAT could constitute a new anticancer strategy. We previously reported that anti-estrogens such as tamoxifen or faslodex displayed weak inhibitions of ACAT in addition to their ER modulatory activity (de Medina et al, JPET, 2006). We investigated in the present study the synthesis of rationally designed stilbene-based analogues of pure antiestrogens (selective estrogen receptor destructors) in which the structural determinant responsible of both their anti-estrogenicity and of their inhibition of ACAT was modified. We report the production of highly potent new drug candidates with bi-specificity towards ER modulation and ACAT inhibition. We further established that these drugs displayed high cytotoxic potency against ER positive tumor cell lines as well as CEE producing cell lines and were more potent than known antiestrogen and ACAT inhibitors. That kind of bispecific anticancer compounds represent a new class of promising drug which deserve further in vivo anticancer evaluation.

Keywords : cholesteryl fatty acid esters, ER, medicinal chemistry, cancer, faslodex

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Plasma circulating oxysterol levels in patients with metabolic syndrome

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Background : Oxysterols are intermediates of cholesterol metabolism and are generated from cholesterol via either enzymatic or nonenzymatic pathways under oxidative stress conditions. The disruption of cholesterol metabolism, that manifested in altered oxysterol signatures, may have deleterious consequences and is associated with several pathological conditions.
Objectives : Since obesity and metabolic syndrome are associated with altered cholesterol metabolism, we examined how oxysterol levels vary within the plasma. To this end, we screened oxysterol levels in patients affected with metabolic syndrome (MetS) compared to healthy controls.
Methods : Men with metabolic syndrome were enrolled in the study along with age- and sex-matched healthy subjects, used as control group. Liquid chromatography coupled with tandem mass spectrometry was used for determination of levels of non-esterified cholesterol, 24-hydroxycholesterol, 25-hydroxycholesterol, 27-hydroxycholesterol, 7α-hydroxycholesterol, 7β-hydroxycholesterol and 7-ketocholesterol in the plasma samples of the participants. The c-reactive protein, insulin and creatinine levels were measured using standard methods.
Results : 13 patients and 17 healthy controls were enrolled in this study. Among the oxysterols measured, we found that 25-hrydroxysterol levels were consistently decreased in the patients group compared to controls (8.61 µg/mL vs 20.76 µg/mL, p= < 10-3). Meanwhile, MetS patients showed significant increase of 7α-hydroxycholesterol level (19.84 µg/mL vs 12.18 µg/mL, p=0.01). Overall, we did not find any correlation between the biological parameters and variations of oxysterol levels except for creatinine which was positively correlated with 7β -hydroxycholesterol (r=0.76, p=0.002) and with 7-ketocholesterol (r=0.75, p=0.003).
Conclusion : Although the small size of our sample, our findings demonstrates that circulating levels of oxysterols are modified in the plasma of MetS patients, suggesting that disturbed oxysterols levels may be involved in MetS. These data bring new information in the understanding of the pathophysiology of MetS and open new therapeutic approaches.

∗ Speaker

Identification of side chain oxidized sterols as novel liver X receptor agonists with therapeutic potential in the treatment of cardiovascular and neurodegenerative diseases

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The nuclear receptors liver X receptors (LXR α and β) are potential therapeutic targets in cardiovascular and neurodegenerative diseases because of their key role in the regulation of lipid homeostasis and inflammatory processes. Specific oxy(phyto)sterols differentially modulate the transcriptional activity of LXRs providing opportunities to develop compounds with improved therapeutic characteristics. We isolated oxyphytosterols from *Sargassum fusiforme* and synthesized side-chain oxidized sterol derivatives. Five 24-oxidized sterols demonstrated a high potency for LXRα/β activation in luciferase reporter assays and induction of LXR-target genes *APOE*, *ABCA1* and *ABCG1* involved in cellular cholesterol turnover in cultured cells : methyl 3β-hydroxychol-5-en-24-oate (S1), methyl (3β)-3-aldehydeoxychol-5-en-24-oate (S2), 24-ketocholesterol (S6), (3β,22E)-3-hydroxycholesta-5,22-dien-24-one (N10) and fucosterol-24,28 epoxide (N12). These compounds induced *SREBF1c* but not lipogenic *SREBF1c*-target genes such as *SCD1*, *ACACA* and *FASN* in HepG2 cells nor in astrocytoma cells. Moreover, S2 and S6 enhanced cholesterol efflux from HepG2 cells. All five oxysterols induced production of the endogenous LXR agonists 24(S)-hydroxycholesterol by upregulating the CYP46A1, encoding the enzyme converting cholesterol into 24(S)-hydroxycholesterol while S1 and S6 may also act via upregulation of desmosterol production Thus, we identified 5 novel LXR-activating 24-oxidized sterols with a potential for therapeutic applications in neurodegenerative and cardiovascular diseases.

Keywords : oxidized sterols, LXR agonists, cardiovascular disease, Alzheimer's disease, cholesterol efflux

∗ Speaker

Cholenamides and Homocholenamides as Herpetic Antivirals : From Identification by a Library Screening toward Rational Optimization

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Endogenous oxysterols, such as 25-hydroxycholesterol (25-HC) and (25*R*),26-hydroxycholesterol (25*R*,26-HC) have emerged as a broad-spectrum host targeting antivirals. Within an in-house library consisting of more than 100 synthetic oxysterols we recently identified three items, namely *N*,*N*-dimethyl 3β-hydroxy-24a-homochol-5-en-24a-amide (PFM064), *N*,*N*-dimethyl 3β-hydroxychol-5-en-24-amide (PFM067) and (24*R*)-*N*,*N*-dimethyl 24-ethyl-3β-hydroxy-24a-homochol-5-en-24a-amide (PFM069), able to efficiently inhibit late stage replication of herpes simplex virus 2 (HSV-2). In particular, the compounds were found to inhibit both cell-to cell fusion induced by HSV-2 and the production of an intracellular viral progeny by acting in a late stage of HSV-2 replicative cycle. The evidence that all the three antiviral derivatives were characterized by the presence of a terminal *N*,*N*-dimethylamide group at the side chain level, along with the lack of different amide derivatives in our library have prompted us to start a process of chemical modification of the active compounds aimed to define their structure-activity relationship. Hence, a first set of cholenamide and homocholenamide derivatives, characterized by different bulk at the level of the amine portion were designed and synthesized, being the final aim the preparation of a fluorescent analog of PFM64 and/or PFM067 by adding the fluorescent label through a linker to the amine portion of the amide moiety. Although the molecular targets underlying the antiviral properties of our PFM derivatives are to be identified several clues suggest oxysterol-binding protein (OSBP) as a plausible target. The availability of fluorescent oxysterol derivatives retaining the antiviral activity of PFM derivatives could be instrumental for the identification of their molecular target(s).

Keywords : Oxysterols, Antiviral, herpes simplexvirus 2, Oxysterol, binding protein

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Role of the cholesterol-oxysterol-bile acid pathway in maintenance of neuronal health

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High fat high cholesterol diet (HFD) has been implicated in cognitive decline and neurodegeneration, but how this specifically affects neuronal function is poorly understood. Evidence suggests HFD, via communication along the Cholesterol-Oxysterol-Bile acid pathway, may disrupt cholesterol homeostasis in the brain thus interfering with neuronal health. The aim of this research was to understand the relationship between HFD and cholesterol homeostasis. Specifically, focusing on cholesterol conversion to oxysterol 24-Hydroxycholesterol (24-HC) in the brains of rats fed HFD ; intercellular storage of cholesterol in lipid droplets (LD); and effect on ApoE expression in cultured cells when 24-HC metabolism is modulated. We are utilising an enzyme-assisted derivatisation for sterol analysis (EADSA) in combination with liquid chromatography tandem mass spectrometry (LC-MSn) to characterise sterol content in rat brain. Preliminary LC-MS results show a decrease in 24-HC in brain from rats fed HFD compared to controls (*n*=6). Preliminary data in the SH-SY5Y cell model suggests that activating CYP46A1, the enzyme that synthesises 24-HC, with Efavirenz increased LD formation. Activation or inhibition (with Soticlestat) of CYP46A1 increased APoE expression.

Keywords : Oxysterols ; 24-Hydroxycholesterol ; Cholesterol metabolism ; High fat diet ; Neuronal health

∗ Speaker

Combined OCDO/DDA extraction and quantification method for a fast and accurate HPLC-MS analysis

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We recently identified a metabolic deregulation centered on 5,6-epoxycholesterol (5,6-EC) that is linked to breast carcinogenesis. This deregulation relies on a differential metabolism of 5,6-EC when comparing non-malignant tissues and tumors. In normal tissues, our preliminary data showed that 5,6-EC was metabolized into dendrogenin A (DDA), a cationic steroidal alkaloid with tumor suppressive properties, while in breast cancers (BC) DDA levels are below its pharmacologically active concentration. The analyses of 5,6-EC metabolism in BC showed that tumors produce oncosterone (OCDO) in the place of DDA. OCDO is an oncometabolite with tumor promoter properties and is a neutral oxysterol. The determination of the ratio DDA/OCDO from the same patient sample/biopsy would be of high interest in the perspective of large cohort of patients analyses. But the physicochemical properties of DDA and OCDO are very different and so far their dosage were done by LC/MS and GC/MS respectively. The GC/MS analysis of OCDO required a step of derivatization by trimethylsilylation. To further explore 5,6-EC metabolism deregulation on tumors in large cohorts of patients, we have undertaken the setup of a method enabling the quantification of both oxysterols from a single sample without further derivatization. We report, and for the first time, a validated method developed with respect to the EMA and FDA guidelines for quantification of DDA and OCDO appropriate to liquid and solid tissue analysis. A single methanol extraction was applied to tissue homogenates which enable a quantitative recovery of DDA and OCDO. DDA and OCDO were quantified by parallel chromatography using liquid chromatography (HILIC) and reverse phase (RP : C18) respectively. This new method is fast and the sensitivity was found sufficient to analyze tissue samples. This method will be used to routinely analyze small-size samples from large cohorts of patients.

Keywords : DDA, OCDO, breast cancer, LC/MS, HILIC

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Altered brain cholesterol homeostasis in a Down syndrome murine model: could it correlate with early Alzheimer's disease onset?

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Background and rationale. The improvement in the lifespan of individuals with Down syndrome (DS) has created interest in the context of the development of chronic diseases, including Alzheimer's disease (AD). AD is a neurodegenerative disease of complex etiology and DS is considered a genetically determined form of AD. Indeed, individuals with DS develop AD by their fourth decade, ten-twenty years earlier than normal population. Maintenance of brain cholesterol homeostasis is essential for neuronal functioning and brain development. Cholesterol metabolism dysregulation in the brain has been linked to AD development, and hypercholesterolemia is included among risk factors. Interestingly, hypercholesterolemic subjects affected by DS treated with statins exhibit lower risk for AD, as documented for subjects without this condition. Aging, obesity and ApoEε4-associated dyslipidemia are risk factors of AD in both normal and DS population. Besides the overlap between AD and DS, brain cholesterol homeostasis in patients with DS has not been yet reported. In the present study we aimed to : i) perform a systematic analysis of cholesterol, its precursors, and oxysterols of enzymatic and non-enzymatic origin, in the brain of a DS mouse model ; ii) analyze the expression levels of some key enzymes involved in cholesterol homeostasis.

Methods : Brain samples from Ts2Cje or euploid mice, 3 and 12 months old (n=6/group), were used for the analyses. Ts2Cje mice are a well-established murine model of DS characterized by a triple copy of a Robertsonian fusion chromosome carrying the distal end of Chr16 and Chr12. Cholesterol, its precursors (lathosterol, lanosterol, and desmosterol, markers of cholesterol synthesis), and the oxysterols 25-hydroxycholesterol (25-OHC) and 27-hydroxycholesterol (27-OHC) (markers of reverse cholesterol transport), 24(S)-hydroxycholesterol (24-OHC) (brain-specific cholesterol elimination product, marker of brain cholesterol turnover), as well as 7-ketocholesterol (7-KC), 7a-hydroxycholesterol (7a-OHC), and 7b-hydroxycholesterol (7b-OHC) (markers of oxidative stress) were measured by isotope dilution gas chromatography-mass spectrometry (GC-MS). Gene expression was analyzed by real time RT-PCR.

Results : GC-MS analysis pointed out that the levels of cholesterol, cholesterol precursors, and various oxysterols are altered in the brains of Ts2Cje mice, compared to the euploid mice, and in older than younger mice. Gene expression analysis of the main genes involved in cholesterol synthesis and oxidation showed that their levels align with the metabolite trends in almost all cases. For instance, in Ts2Cje mice compared to euploid mice, total cholesterol levels are markedly lower, and the expression of 24-dehydrocholesterol reductase (DHCR24), the key enzyme involved in different steps of cholesterol synthesis, resulted to be significantly reduced ; moreover, the levels of the neuroprotective oxysterol 24-OHC are lower in Ts2Cje mice, as well 24-hydroxilase (CYP46A1) levels.

Conclusions : Given the established role of oxysterols in AD, the altered brain cholesterol homeostasis observed in DS mice suggests a new possible cause of early onset of AD in DS subjects. Further studies are needed to clarify the mechanisms underlying the highlighted differences, and to identify the pathways modulated by oxysterols leading to AD-like neuropathology in DS ; this could help to find new strategies to prevent the early onset of this disabling disease in DS subjects.

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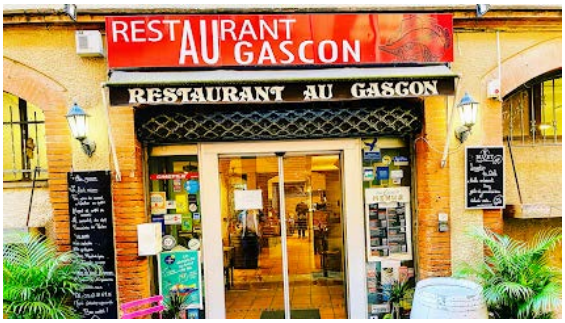
Gala Dinner

Thursday 21rst at 8 p.m.



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