Falk Symposium 175

XXI International Bile Acid Meeting

Bile Acids as Metabolic Integrators and Therapeutics

October 7 – 8, 2010

Freiburg, Germany

Abstracts

Poster Abstracts

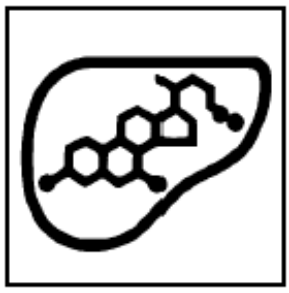
Abstracts of Invited Lectures
Poster Abstracts

Falk Symposium 175

IV Falk Gastro-Conference

XXI International Bile Acid Meeting

BILE ACIDS AS METABOLIC INTEGRATORS AND THERAPEUTICS



Freiburg (Germany)
October 7 – 8, 2010

Scientific Organization:
D. Häussinger, Düsseldorf (Germany)
U. Beuers, Amsterdam (The Netherlands)
A. Stiehl, Heidelberg (Germany)
M. Trauner, Graz (Austria)

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M. Trauner, Graz

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Session I

Metabolism and transport of bile acids

The role of cilia in the regulation of bile flow

Nicholas F. LaRusso, M.D.
Medical Director, Center for Innovation, Charles H. Weinman Professor of Medicine, Biochemistry and Molecular Biology, Distinguished Investigator of The Mayo Foundation, Mayo Clinic, Rochester, MN 55905, USA

Cholangiocytes, the epithelial cells lining intrahepatic bile ducts, are ciliated cells. Each cholangiocyte has a primary cilium consisting of: (i) a microtubule-based axoneme; and (ii) the basal body, centriole-derived, microtubule organizing center from which the axoneme emerges.

Primary cilia in cholangiocytes were described decades ago but their physiologic and pathophysiological significance remained unclear until recently. We now recognize that cholangiocyte cilia extend from the apical plasma membrane into the bile duct lumen and, as such, are ideally positioned to detect changes in bile flow, bile composition, and bile osmolality. These sensory organelles act as cellular antennae that can detect and transmit signals that influence cholangiocyte function. Indeed, recent data show that cholangiocyte primary cilia can activate intracellular signaling pathways (i.e., intracellular calcium and cAMP levels) when they sense modifications in the flow of bile, in the molecular constituents of bile, and in the osmolarity of bile. Their ability to sense and transmit signals depends on the participation of a growing number of ciliary associated proteins (e.g., polycystin 1 and 2, P2y2 receptors, and TrpV 4). Cholangiocyte cilia, in addition to being important in normal biliary physiology, are likely to contribute to the cholangiopathies when their normal structure or function is disturbed. Indeed, the polycystic liver diseases that occur in combination with autosomal dominant and recessive polycystic kidney disease (i.e., ADPKD and ARPKD) are two important examples of such conditions (i.e., cholangiociliopathies). Recent insights into the role of cholangiocyte cilia in cystic liver disease using in vitro and animal models have already resulted in clinical trials that have influenced the management of cystic liver disease. In this presentation, I will discuss the structure and functions of cholangiocyte primary cilia, their role in the cholangiociliopathies, and existing and potential therapeutic approaches.

Posttranslational regulation of Abcc2 through sumoylation

Hiroshi Suzuki[1], Shuichi Sekine[2], Toshiharu Horie[2] and Kousei Ito[1]
[1]Department of Pharmacy, The University of Tokyo Hospital, Faculty of Medicine, The University of Tokyo, Tokyo, Japan
[2]Laboratory of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan

The ATP-binding cassette transporter family C 2 (Abcc2/Mrp2) is a member of efflux transporters involved in the biliary excretion of organic anions from hepatocytes. Abcc2 protein expression or localization is changed by several treatments including oxidative stress, contraceptives, bile duct ligation, osmolarity change, although the molecular mechanism is not fully understood. In the present study, we performed yeast two hybrid screening to identify novel protein(s) which particularly interacts with the linker region of rat Abcc2 located between the N-terminal nucleotide binding domain and the last membrane spanning domain. The screening from human liver cDNA library resulted in the identification of a series of SUMO-related enzymes including Ubiquitin activating enzyme 2 (UBA2), Ubiquitin conjugating enzyme 9 (Ubc9), splicing variants of protein inhibitor of activated STAT (PIAS)x and PIASy, and SUMO-1 itself. These proteins are known to be involved cooperatively in the sequential SUMOylation cycle of the target protein and their substrates; SUMO is activated by SUMO activating enzyme E1 (hetero-dimer of UBA2 and AOS1 [activation of Smt3p]), and then covalently attached to the target lysine residue with the aid of SUMO conjugation enzyme E2 (Ubc9) and SUMO ligase E3 (such as PIASx and PIASy). Ubc9, which is most frequently isolated in our screening (21 clones), is a key enzyme involved in conjugation of SUMO to the lysine residue in the consensus sequence composed of ΨKXE, where Ψ and X represent a hydrophobic and any amino acid, respectively, of the target protein. Indeed, Abcc2 has the consensus sequence in the linker region (IKKE). In yeast experiments, interaction between linker region and those clones were all abolished by substituting the putative consensus site “IKKE” to “IRKE”. In vitro SUMOylation experiments confirmed that the Abcc2 linker was a substrate of Ubc9-mediated SUMOylation. It was also found that the IKKE sequence is the target of SUMOylation, since a mutant with IKKE is substituted by IRKE was not SUMOylated. Furthermore, we demonstrated that Abcc2, endogenously expressed in rat hepatoma derived McARH7777 cells, is SUMOylated. Suppression of endogenous Ubc9 by siRNA resulted in a selective 30% reduction in Abcc2 protein expression in the post-nuclear supernatant, whereas subcellular localization of Abcc2 confirmed by semi-quantitative immunofluorescence analysis was minimally affected. Collectively, it was demonstrated that Abcc2 protein expression, but not its localization, can be positively regulated by SUMOylation system.

Role of the organic solute transporter Ostα-Ostβ in bile acid homeostasis

Ned Ballatori
Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY, USA

Organic solute transporter alpha-beta (Ostα-Ostβ) is a heteromeric bile acid and sterol transporter that is localized to the basolateral membrane of cells involved in the transport of these compounds, including epithelial cells of the small intestine, kidney, liver, testis, adrenal gland and other steroidogenic tissues. Of significance, Ostα-Ostβ appears to be the primary bile acid efflux transporter on the basolateral membrane of intestinal cells, and is therefore essential for bile acid homeostasis and the enterohepatic circulation. The transporter is composed of a predicted 340-amino acid, 7-transmembrane (TM) domain protein (Ostα) and a putative 128-amino acid, single-TM domain polypeptide (Ostβ). Heterodimerization of the two subunits increases the stability of the individual proteins, facilitates their post-translational modifications, and is required for delivery of the functional transport complex to the plasma membrane. Ostα-Ostβ substrates include bile acids, certain steroids (estrone 3-sulfate, dehydroepiandrosterone 3-sulfate, and digoxin), and prostaglandin E_2. Transport occurs by a facilitated diffusion mechanism, and thus Ostα-Ostβ can mediate cellular efflux or uptake depending on that substrate's electrochemical gradient. Expression of both *Ost* genes is positively regulated by bile acids through the bile acid-activated farnesoid X receptor, Fxr, and hepatic expression is upregulated in cholestasis in both humans and rodents, indicating a hepatoprotective role. Recent studies in Ostα-deficient mice provide compelling evidence for a role of Ostα-Ostβ in bile acid homeostasis. These mice display a marked defect in intestinal bile acid and conjugated steroid absorption; a decrease in bile acid pool size and serum bile acid levels; altered intestinal, hepatic and renal disposition of known substrates of the transporter; and altered serum triglyceride, cholesterol, and glucose levels. Ostα-deficient mice are also protected from liver injury in obstructive cholestasis through adaptive responses in both the kidney and liver that enhance clearance of bile acids into urine and through detoxification pathways. Taken together, these observations indicate that Ostα-Ostβ is essential for bile acid and sterol disposition, and for the enterohepatic circulation. Current studies are defining the mechanisms by which the transporter regulates bile acid levels and are examining its potential contribution to lipid homeostasis.

Inactivation of organic solute transporter-alpha (Osta) alters bile acid homeostasis via the FXR-FGF15 pathway

Paul A. Dawson, Tian Lan, Jamie Haywood, Anuradha Rao
Department of Internal Medicine, Section on Gastroenterology, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA

Introduction: Mutations in apical sodium-dependent bile acid transporter (SLC10A2) block intestinal bile acid absorption, resulting in a compensatory increase in hepatic bile acid synthesis. Inactivation of basolateral membrane bile acid transporter (OSTa-OSTb) also results in impaired intestinal bile acid absorption, but hepatic bile acid synthesis was paradoxically repressed.
We hypothesize that this dramatic phenotype difference resulted from ileal trapping of bile acids that act via FXR to induce expression of FGF15/19 expression. In order to test this hypothesis, we investigated whether blocking FXR signalling would reverse the Cyp7a1 phenotype in Osta null mice.

Methods: The corresponding null mice were crossbred to generate OstaFxr double-null mice. All experiments compared wild type, Osta, Fxr, and OstaFxr null littermates. Analysis of the in vivo phenotype included measurements of bile acid fecal excretion, pool size, and composition. Intestinal cholesterol absorption, hepatic lipid content, hepatic and intestinal gene and protein expression were also examined.

Results: OstaFxr null mice exhibited increased bile acid fecal excretion and pool size, and decreased bile acid pool hydrophobicity, as compared to Osta null mice. Inactivation of Fxr reversed the increase in ileal FGF15 expression, and this was associated with a significant increase in hepatic Cyp7a1 expression. Intestinal cholesterol absorption was dramatically reduced in Osta null mice, and this was reversed by inactivation of Fxr.

Discussion/Conclusion: Inactivation of Fxr largely unmasked the bile acid malabsorption phenotype and corrected the bile acid homeostasis defect in Osta null mice, suggesting that inappropriate activation of the FXR-FGF15 pathway underlies this phenotype.

Herbert Falk Lecture

Herbert Falk: A vital force in renascence of bile acid research and bile acid therapy

Alan F. Hofmann, M.D.
University of California, San Diego, CA, USA

Herbert Falk died on the 8th of August, 2008 after a long illness. It was his vision that initiated the Bile Acid Meetings and introduced chenodeoxycholic acid (CDCA) for the dissolution of cholesterol gallstones and ursodeoxycholic acid (UDCA) for the successful treatment of cholestatic liver disease.

The first Bile Acid Meeting was a small workshop held in the hospital in 1970. Great interest in the topic was evident at that small meeting and led to a much larger meeting in 1972 whose scope included both basic and clinical aspects of bile acids. These meetings have continued at annual or biennial intervals, the present meeting being the 21st. Always, the program has included discussions of the most fundamental aspects of bile acid biosynthesis and metabolism, as well as clinical applications of bile acid therapy. The meetings have featured brief presentations, ample time for discussion, and imaginative social programs. They have always been flawlessly organized.

From a scientific standpoint, there has been enormous progress in understanding the chemistry and biology of bile acids. Progress has included cloning of the transporters and identification of disease conditions associated with their impaired function, as well as discovery of their nuclear receptors and the G-coupled receptor TGR5. Herbert Falk established the Windaus Prize in 1978, and the prize has been given to individuals whose contributions moved the field forward. The majority of meetings have occurred in Freiburg, but occasionally the venue has moved to Switzerland, the Netherlands, Sweden, and the United States.

For the small number of scientists working in the field of bile acids, the Bile Acid meetings have been marvelous, rewarding experiences. We must all be grateful to Herbert Falk's vision in establishing the Falk Foundation that has so generously sponsored these meetings. And we must also express our gratitude to his widow, Ursula Falk, who has continued this worthy tradition.

Session II

Receptor regulation by bile acids

Bile acid signaling, a journey from the nucleus to the membrane

Kristina Schoonjans, Johan Auwerx
Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland

Over the last 5 years, the field of bile acid (BA) research has undergone a considerable evolution (Thomas et al., Nat Rev Drug Discov, 2007). Besides their well-established roles in dietary lipid absorption and cholesterol homeostasis, it has recently become clear that BAs are also biological signaling molecules. The first indication in this direction came from the fact that BAs control their own synthesis in the liver through a feedback inhibitory pathway involving the nuclear receptors farnesoid X receptor (FXR) and short heterodimeric partner (SHP) (Lu et al., Mol Cell, 2000). In addition, we have demonstrated that BAs decrease the hepatic production of triglycerides and very low density lipoproteins (VLDL) via the activation of the same nuclear receptor signaling pathway (Watanabe et al., JCI, 2004). The fact that bile acids could signal beyond the enterohepatic axis came from our work that demonstrated that BAs increase energy expenditure systemically by increasing triiodothyronine levels in rodent brown adipose tissue and human skeletal muscle, thereby preventing obesity and insulin resistance (Watanabe et al., Nature, 2006). This effect is mediated by TGR5, a G protein-coupled receptor, which upon activation by BAs stimulates cAMP production and type 2 deiodinase enzyme activity. These observations build a strong case that BAs have effects beyond the enterohepatic axis and function as systemic metabolic integrators. Collectively, these data demonstrate that the TGR5 locus is a critical region to maintain energy homeostasis and identify TGR5 as an attractive and potential target for the treatment of obesity and associated disorders.

TGR5 in the biliary tree

Verena Keitel, Dieter Häussinger
Clinic for Gastroenterology, Hepatology and Infectiology, Heinrich-Heine-University, Düsseldorf, Germany

TGR5 (Gpbar-1) is a G-protein coupled, membrane-bound bile acid receptor, which is expressed in many rodent and human tissues, including liver. The role of TGR5 in bile acid signalling is mainly elusive. However, recent data suggest that activation of TGR5 by bile acids may regulate glucose homeostasis (1, 2), increase energy expenditure in brown adipose tissue (3), modulate liver microcirculation (4), suppress cytokine secretion from macrophages (5, 6) and protect against bile acid-induced apoptosis in the liver (4).

Bile acids have been shown to affect cholangiocyte secretion, proliferation and survival (7). The underlying signalling events are still only partially understood.

TGR5 has been detected in the apical membrane domain of biliary epithelial cells (6, 8). In isolated cholangiocytes and gallbladder epithelial cells TGR5 was localized in the primary cilium, a sensory organelle, which protrudes from the apical membrane into the bile duct/gallbladder lumen. Activation of TGR5 in gallbladder epithelial cells led to an increase in intracellular cAMP, activation of the cAMP-regulated chloride channel cystic fibrosis conductance regulator CFTR (ABCC7) and subsequent chloride secretion (8).

Cholangiocyte proliferation was stimulated in the presence of bile acids or a TGR5 agonist. This effect was significantly lower in cells derived from TGR5 knockout mice, suggesting a role for TGR5 in bile acid-mediated cholangiocyte proliferation. Activation of TGR5 with a specific agonist significantly reduced CD95 ligand-dependent apoptosis in isolated wildtype cholangiocytes as measured by TUNEL. Furthermore, activation of TGR5 significantly increased CD95 receptor serine phosphorylation, indicating that TGR5 has anti-apoptotic properties.

In cholangiocytes TGR5 may function as a bile acid sensor coupling biliary bile acid concentrations to ductular bile formation and bile flow. Furthermore, activation of TGR5 may promote proliferation and protect cholangiocytes from bile acid-induced cell injury.

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Fibroblast growth factor 19: A message from the intestine

Peter L.M. Jansen, Vassilis Triantis and Frank Schaap
Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, The Netherlands

Fibroblast growth factors are involved in embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. FGF19, FGF21 and FGF23, are FGF family members with metabolic functions. FGF19 is synthesized in the terminal ileum upon activation of FXR by bile salts (1). FGF19 is secreted into the portal circulation. In human serum FGF19 peaks 2–4 hours after a fatty meal (2). In the liver it activates the FGF receptor, FGFR4. This receptor on the hepatocyte plasma membrane exists in two molecular forms: a highly glycosylated and a core glycosylated form. The highly glycolsylated form is the active protein. The balance between the active and the inactive form is determined by *β*-klotho, a protein that facilitates the proteosomal degradation of the inactive form (3).

In rodents, the FGF19 homologue Fgf15 is formed in the intestine. In humans, FGF19 is also formed in the liver but only under cholestatic conditions. In patients with extrahepatic cholestasis due to pancreatic head carcinoma, FGF19mRNA in liver tissue is greatly elevated while CYP7A1mRNA is reduced (4). What triggers hepatic FGF19 synthesis in the liver during cholestasis is not known but one may speculate that also in the liver FGF19 synthesis is stimulated by bile salts with FXR as intermediary. Why this does not occur in rodents is unknown.

FGF19 has a regulatory role in metabolism as it not only causes the down-regulation of CYP7A1expression but also suppresses the expression of fatty acid synthase and phosphoenolpyruvate carboxykinase, rate determining enzymes in the biosynthesis of triglycerides and carbohydrates. This suggests that in normal physiology, FGF19 from the intestine plays a role in the fine tuning of bile salt, lipid and carbohydrate metabolism in the liver.

FGF19 may play a role in the pathophysiology of non-alcoholic fatty liver (NAFL). In patients with NAFL and normal insulin sensitivity (HOMA < 2.5), FGF19 in serum is elevated after a fatty meal and the CYP7A1 metabolite C4 is reduced. However, in patients with NAFL and insulin resistance (HOMA > 2.5) C4 is not reduced after a fatty meal despite a normal FGF19 serum response. This suggests that in patients with NAFL and insulin resistance there might also be resistance to the action of FGF19. If these findings can be confirmed for triglyceride synthesis, resistance to FGF19 may contribute to the pathogenesis of NAFL.

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The role of Foxa2 in bile acid homeostasis and bile duct development

Klaus H. Kaestner, Ph.D.
Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

Bile acids are potent detergents that are synthesized by the liver. They are crucial for the absorption of lipophilic nutrients. In addition, synthesis of bile acids is the predominant mechanism for the excretion of excess cholesterol. Dysregulation of bile acid homeostasis leads to cholestatic liver disease and induces cellular stresses, including ER stress. We show using global location analysis of Foxa2 occupancy in liver chromatin and cell-type specific gene ablation that the winged helix transcription factor Foxa2 is required for normal bile acid homeostasis. As suggested by the location analysis, deletion of Foxa2 in hepatocytes in *Foxa2*$^{loxP/loxP}$*Alfp.Cre* mice leads to decreased transcription of genes encoding bile acid transporters on both the basolateral and canalicular membranes, resulting in cholestasis and increased plasma bile acid levels. Foxa2-deficient mice are strikingly sensitive to a diet containing cholic acid, which results in toxic accumulation of hepatic bile salts, ER stress, and liver injury.

Deletion of both Foxa2 and the closely related Foxa1 gene in the embryonic liver causes hyperplasia of the biliary tree. Abnormal bile duct formation in Foxa1/2-deficient liver is, at least in part, due to the activation of interleukin-6 (IL-6) expression, a proliferative signal for cholangiocytes. The glucocorticoid receptor (GR) is a negative regulator of IL-6 transcription. In the absence of Foxa1/2, GR fails to bind to the IL-6 promoter, causing enhanced IL-6 expression. Thus, after liver specification, Foxa1 and Foxa2 are required for normal bile duct development. Our data suggest that Foxa1/2 function as terminators of bile duct expansion in the adult liver via inhibiting IL-6 expression.

Role of nuclear receptors in the biliary epithelium

Nicolas Chignard[1, 2]
[1]UPMC Univ. Paris 06, Centre de Recherche Saint Antoine, UMRS_938, Paris, F-75005, France
[2]Inserm, Centre de Recherche Saint Antoine, UMRS_938, Paris, F-75012, France

The biliary epithelium is organized as a single layer of biliary epithelial cells (i.e. cholangiocytes) lining the biliary tree. Biliary epithelial cells have three major biological functions: protection, secretion and proliferation. These functions are controlled by nuclear receptors.

The main role of the biliary tree is to convey bile from the liver to the duodenum. Bile is a complex fluid containing steroids, endotoxins and xenobiotics. Biliary epithelial cells by forming a tight epithelium confer passive protection to the liver and avoid the spilling of toxics into the hepatic parenchyma. Active protection against toxics, such as endotoxins, can be elicited by the vitamin D receptor (VDR) or the farnesoid X receptor (FXR). VDR or FXR are activated by vitamin D and bile salts to increase the expression of cathelicidin, an antimicrobial peptide with endotoxin neutralizing properties (1, 2). VDR and FXR activation may thus avoid inflammation of the biliary epithelium, which is otherwise constantly exposed to inflammatory stimuli. Anti-inflammatory activities may also be triggered by PPARα and γ, which have been shown to inhibit the effect of LPS and TNFα in biliary epithelial cells (3, 4).

Biliary epithelial cells are responsible for 30–40% of bile volume in humans, while representing 3–5% of the liver cells, highlighting that secretion is one of their primary function. The regulation of biliary epithelial cell secretions mainly arises from circulating factors (5). Luminal factors, such as bile salts, may also elicit fluid secretion. Bile salts control biliary epithelial cell fluid secretion by classical intracellular pathways (6, 7), membrane receptors (8) and nuclear receptors (9–11). FXR or the glucocorticoid receptor (GR) has been shown to increase the expression of gene encoding membrane bound proteins that participate to biliary epithelial cell secretion (9–11).

Biliary epithelial cells are quiescent cells able to proliferate in pathophysiological settings. Biliary epithelial cell proliferation is controlled by neuropeptides or hormones. Progesterone increases biliary epithelial cells proliferation by acting on the progesterone receptor (PR) (12). Moreover, inhibition of estrogen receptor (ER) signalling decreases pathological biliary epithelial cell proliferation, while having no effect in normal liver (13). Although ERα and β display a low expression in biliary epithelial cells of the normal liver, their expression is increased in cholangiopathies (13, 14). Thus, nuclear receptors control biliary epithelial cell proliferation in pathological settings.

Taken together these observations suggest that nuclear receptors are involved in the control of biliary epithelial cell biology. A better delineation of the specific biliary epithelial cell functions controlled by nuclear receptors may shed light on potential therapeutic molecular targets of cholangiopathies.

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Intestinal specific regulation of nuclear bile acid receptor FXR by the caudal-related homeobox 2 (CDX2) transcription factor: Relevance for colorectal cancer

Salvatore Modica[1], Annalisa Morgano[1], Elena Bellafante[1], Lorena Salvatore[1], Stefania Murzilli[1], Jean Noel Freund[2], Antonio Moschetta[1,3]
[1]Laboratory of Lipid Metabolism and Cancer, Department of Translational Pharmacology, Consorzio Mario Negri Sud, 66030 Santa Maria Imbaro (CH), Italy
[2]INSERM, University Luis Pasteur of Strasbourg, France
[3]Clinica Medica "A. Murri"', University of Bari, Policlinico, 70124 Bari, Italy

We identified CDX2 as a transcriptional activator of FXR in normal intestine and tumors.

Introduction: FXR is a BA-responsive nuclear receptor highly expressed in the enterohepatic system where it regulates the expression of genes involved in BA metabolism and detoxification. Although several intestinal FXR target genes have been identified, the mechanisms behind the transcriptional regulation of FXR in the intestine remain elusive.

Methods: *In silico* analysis, reporter assays and EMSA were performed on the human FXR promoter. Then, the transcriptional regulation of FXR in the intestine was investigated using complementary human, murine and cellular models of intestinal tumorigenesis.

Results: Putative binding sites for CDX2 were identified on the human FXR promoter. Indeed, intestinal FXR expression was reduced in $CDX2^{-/+}$ mice. Since CDX2 is a downstream mediator of the APC *(adenomatous polyposis coli)* protein, a gatekeeper for colorectal cancer (CRC) development, CDX2 and FXR expression were reduced in both human and murine intestinal tumors carrying inactivating APC mutations. Oppositely, FXR expression was retained in human intestinal tumors that still presented CDX2 expression. Furthermore, ablation of CDX2 expression during *in vitro* enterocyte differentiation resulted in blunted FXR expression. Then, transduction of colorectal cancer cells via CDX2 adenovirus and treatment with FXR synthetic ligand resulted in over-expression of FXR and induction of its target genes. Finally, reporter assays and EMSA showed a direct regulation of FXR by CDX2.

Discussion/Conclusion: We disclosed for the first time a transcriptional mechanism regulating the intestinal expression of FXR; this involves the APC-CDX2 pathway. Epidemiological studies and animal experiments link high levels of BAs to CRC progression. Indeed, APC-CDX2 mutations would account for reduced FXR expression with consequent exposure to chronic high levels of BAs that would further promote CRC progression.

Session III

Pleiotropic actions of bile acids

The effect of TLCS and CD95L on chloride currents in primary cultured hepatocytes

Silvia Dossena, Sara Funke, Margherita Tamplenizza, Charity Nofziger and Markus Paulmichl
Institute of Pharmacology and Toxicology, Paracelsus Medical University, Strubergasse 21, A-5020 Salzburg, Austria

In hepatocytes, proapoptotic stimuli such as the CD95 ligand (CD95L) and the hydrophobic bile acids TLCS (taurolithocholylsulfate) lead to an increase in the cytosolic chloride concentration ($[Cl^-]_i$) and a subsequent acidification of the endosomal compartment; ultimately resulting in activation of the acidic sphingomyelinase. The neutral bile acid TC (taurocholate) and the anti-apoptotic bile acid TUDC (tauroursodeoxycholate) do not increase $[Cl^-]_i$. Furthermore, the two latter compounds do not lead to an acidification of the endosomal compartment and therefore do not activate apoptosis. The upstream events leading to the increased $[Cl^-]$i are still unknown.
We wondered if the pro-apoptotic bile acids increase $[Cl^-]_i$ by inhibiting chloride channels, which otherwise allow chloride to exit the cell. Therefore, the intent of the study was to characterize the effect of different bile acids on chloride currents in primary cultured hepatocytes.
Chloride currents, with the exception of calcium activated chloride currents and CFTR dependent chloride currents, were measured in rat hepatocytes in primary culture (1–2 days after the isolation procedure) by the patch clamp technique in whole cell configuration.
Under isotonic conditions, hepatocytes in primary culture show an outwardly rectifying chloride current resembling the chloride current seen in many other cells after reducing the extracellular osmolality. Accordingly, reducing the extracellular osmolality leads to a further increase of the currents observed under isotonic conditions, while increasing the extracellular osmolality leads to a current decrease. A detailed biophysical characterization of these currents revealed the biophysical signature known for the swelling activated chloride current ICl_{swell}, i.e. outward rectification and potential-dependent inactivation at potentials more positive than +40 mV.
The addition of the pro-apoptotic bile acid TLCS to the extracellular solution (final concentration: 50 µM) rapidly leads to a reduction of the amplitude of the chloride current under isotonic, as well as under hypotonic conditions. This current inhibition occurs at physiological membrane potential (-20 to -60 mV), as well at positive membrane potentials, reaches values up to 50% (at +80 mV) and is fully reversible. Moreover, the potential-dependent inactivation of the current is strongly accelerated by TLCS. Both current inhibition (EC_{50} = 31.7 µM) and acceleration of current inactivation (EC_{50} = 1.79 µM), are dose-dependent effects.
Upon the addition of the anti-apoptotic bile acid TUDC to the extracellular isotonic solution, an inhibition of the current at positive potentials was measured, although to a lesser extent with respect to TLCS. TUDC also accelerates the potential-dependent inactivation of the outward chloride currents at holding potentials more positive than +40 mV. At negative (physiological) membrane potentials, however, TUDC did not cause any modification of the chloride current.
The neutral bile acid TC did not show any effect on the inward or outward chloride currents.

In addition, TLCS, TUDC and TC were added to the pipette filling solution (the intracellular solution in whole cell configuration), for testing a possible effects from the intracellular side; in this experimental set-up no modification of the chloride currents were observed. This last result points to the fact that the transport of the bile acids into the intracellular compartment is not required to induce the inhibitory effect.
In contrast to bile acids, the death ligand CD95L activates the chloride current measured under isotonic as well hypotonic conditions; as expected for a receptor-binding ligand, CD95L affects the current only when signal transduction could occur, i.e. only after a long-term preincubation (30 min or more) of intact cells.

Our results show that only the pro-apoptotic bile acid TLCS was able to reduce, in our experimental setting, the chloride efflux from the cell at negative membrane potentials, supporting the above mentioned working-hypothesis. The other pro-apoptotic stimuli e.g. CD95L leads to a current activation, which under depolarized conditions would lead again to an intracellular chloride increase. Therefore, we conclude that the bile acid and CD95L-induced increase of the cytosolic chloride concentration occur via independent mechanisms that finally lead to the same apoptotic pathway.

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Bile acid signalling in the fetal tissues: Implications for intrahepatic cholestasis of pregnancy

Catherine Williamson
Professor of Obstetric Medicine, Institute of Reproductive and Developmental Biology, Imperial College London, Du Cane Road, London W12 0NN, UK

The fetus is capable of synthesising bile acids from early in gestation. In uncomplicated pregnancy there is a feto-maternal gradient for bile acids consistent with maternal excretion of bile acids of fetal origin. Some biliary bile acid transporters are not expressed in the placenta, e.g. ABCB11/BSEP. However there is evidence for ATP-dependent and independent bile acid transport in human and rodent placentas. In cholestatic pregnancy the feto-maternal serum bile acid gradient is reversed. Bile acids of maternal origin are increased in the fetal compartment and it is likely that they are responsible for the fetal complications of intrahepatic cholestasis of pregnancy (ICP). These include preterm labour, fetal anoxia and intrauterine death.

Rodent data have demonstrated placental pathology in the presence of raised maternal serum bile acids. Our group is evaluating bile acid-induced pathology in human placentas and using *ex vivo* models and trophoblast cell lines, and is evaluating the role of specific drugs in protecting against damage. Furthermore, our studies using rodent models demonstrate that maternal cholestasis influences bile and cholesterol transport in the placenta, and in the fetus it causes alterations in bile acid homeostasis and cholesterol metabolism. *In vitro* models using rodent and human fetal cardiomyocytes have also demonstrated that administration of taurocholate causes arrhythmia. Bile acid-induced arrhythmia is prevented by pre-incubation with ursodeoxycholic acid.

Regulation of bile acid synthesis by fat-soluble vitamins A and D

Daniel R. Schmidt, Steven A. Kliewer, and David J. Mangelsdorf
Departments of Pharmacology, Molecular Biology and the Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX, USA 75390

Bile acids are required for proper absorption of dietary lipids, including fat-soluble vitamins. Here, we show that the dietary vitamins A and D inhibit bile acid synthesis by repressing hepatic expression of the rate-limiting enzyme, CYP7A1. Receptors for vitamin A and D induced expression of *Fgf15*, an intestine-derived hormone that acts on liver to inhibit *Cyp7a1*. These effects were mediated through distinct cis-acting response elements in the promoter and intron of *Fgf15*. Interestingly, *trans*-activation of both response elements appears to be required to maintain basal *Fgf15* expression levels in vivo. Furthermore, while induction of *Fgf15* by vitamin D is mediated through its receptor (VDR), the induction of *Fgf15* by vitamin A is mediated through the RXR/FXR heterodimer and is independent of bile acids. This latter finding supports a novel role for the RXR/FXR heterodimer as a dietary sensor for vitamin A. Notably, vitamin A treatment reversed the effects of the bile acid sequestrant cholestyramine on *Fgf15*, *Shp*, and *Cyp7a1* expression, suggesting a potential therapeutic benefit of vitamin A under conditions of bile acid malabsorption. These results reveal an unexpected link between the intake of fat-soluble vitamins A and D and bile acid metabolism, which may have evolved as a means for these dietary vitamins to regulate their own absorption.

Pleiotropic effects of bile acids: Role of biliary HCO_3^- secretion

Ulrich Beuers, Simon Hohenester, Lucas J. Maillette de Buy Wenniger, Andreas E. Kremer, Peter L.M. Jansen, Ronald P.J. Oude Elferink
Department of Gastroenterology and Hepatology & Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, University of Amsterdam, The Netherlands

The pathogenesis of chronic cholestatic liver diseases such as primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and other fibrosing cholangiopathies remains enigmatic[1]. This lecture focuses on the hypothesis that biliary HCO_3^- secretion in humans serves to maintain an alkaline pH near the apical surface of hepatocytes and cholangiocytes to prevent the uncontrolled membrane permeation of protonated glycine-conjugated bile acids[2]. Functional impairment of this biliary HCO_3^- umbrella or its regulation may lead to enhanced vulnerability of cholangiocytes and periportal hepatocytes towards the attack of apolar hydrophobic bile acids. An intact interplay of hepatocellular and cholangiocellular ATP secretion, ATP/P2Y- and bile salt/TGR5-mediated Cl^-/HCO_3^- exchange and HCO_3^- secretion, and alkaline phosphatase-mediated ATP breakdown may guarantee a stable biliary HCO_3^- umbrella under physiological conditions. Genetic and acquired functional defects leading to destabilization of the biliary HCO_3^- umbrella may contribute to development and progression of various forms of fibrosing/sclerosing cholangitis[2].

Our hypothesis needs confirmation by experimental studies both *in vitro* and *in vivo*. Confirmation of the concept of a biliary HCO_3^- umbrella would have clinical impact both in further unravelling the pathogenesis of chronic fibrosing cholangiopathies and in developing therapeutic strategies which would focus on strengthening the biliary HCO_3^- umbrella in fibrosing cholangiopathies beyond the effects observed with UDCA so far.

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The molecular mechanism of cholestatic pruritus

Ronald P.J. Oude Elferink, Andreas E. Kremer, Job J.W.W. Martens, Ulrich Beuers
Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, The Netherlands

In many systemic disorders pruritus (itch) represents an important clinical problem that imposes considerable burden to the patient. Pruritus is also a frequent symptom in patients with cholestatic liver diseases. Pruritus may be mild and tolerable, but it can also be excruciating, causing sleep deprivation or even suicidal ideations. In rare cases, intractable pruritus may become a primary indication for liver transplantation. The molecular mechanism of itch signal transduction is largely unclear. In the past, both enhanced serum levels of bile salts and μ-opioids have been implicated in the etiology of cholestatic pruritus. However, neither correlations between itch intensity and bile salt or opioid levels nor a causative link have ever been established. In the last decade more information has become available on potential mechanisms that lead to itch perception. We have attempted to identify factors that play a role in cholestatic itch perception. During cholestasis compounds accumulate in plasma that are normally excreted into bile. We have based our experiments on the hypothesis hypothesis that these compounds act as direct or indirect pruritogens by affecting receptor mediated signalling in itch fibers. Along the lines of this hypothesis we have screened plasma samples of a large group of patients with various cholestatic conditions; mainly women with intrahepatic cholestasis of pregnancy (ICP) and primary biliary cirrhosis (PBC) (Kremer et al. Gastroenterology. 2010; Epub ahead of print). In a screen we tested the capacity of plasma samples to activate neuroblastoma cells. The readout of this screen was the potency of samples to induce an increase in intracellular calcium concentration (as measured by ratiometric fluorescence). Quite strikingly, we found that samples from itchy cholestatic patients caused a significantly higher activation than samples from non-itchy cholestatic patients and from healthy controls. Analysis of these plasma samples revealed lysophosphatidic acid (LPA) as the active compound. LPA is a very potent signalling lipid molecule that can activate cells through various LPA receptors. Subsequently, we could demonstrate that plasma levels of the enzyme autotaxin are strongly increased in cholestatic patients with pruritus. Autotaxin is the enzyme that converts lysophosphatidylcholine (LPC) into LPA. In fact, there was a highly significant correlation between the ATX level in plasma and the severity of itch (as indicated by patients on a visual analogue scale). This is a striking finding as autotaxin has never been connected to itch perception thus far. On the other hand it is thought to play a role in tumour metastasis and wound healing but more recently it has also been found to mediate neuropathic pain. We have also shown that LPA, when injected intradermally, causes itch in mice.

On the basis of our results we hypothesize that during cholestasis expression of autotaxin is induced and gives rise to increased local formation of LPA near unmyelinated nerve ending of itch fibers. LPA then activates these neurons through one of the LPA receptors. Currently, we are investigating by what mechanism plasma autotaxin is elevated; this could potentially be caused by enhanced expression and/or by reduced clearance of the enzyme.

Adolf Windaus Prize Lecture

Bile acid signaling and the control of metabolism

Kristina Schoonjans, Johan Auwerx
Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland

Over the last 5 years, the field of bile acid (BA) research has undergone a considerable evolution (Thomas et al., Nat Rev Drug Discov, 2007). Besides their well-established roles in dietary lipid absorption and cholesterol homeostasis, it has recently become clear that BAs are also biological signaling molecules. The first indication in this direction came from the fact that BAs control their own synthesis in the liver through a feedback inhibitory pathway involving the nuclear receptors farnesoid X receptor (FXR) and short heterodimeric partner (SHP) (Lu et al., Mol Cell, 2000). In addition, we have demonstrated that BAs decrease the hepatic production of triglycerides and very low density lipoproteins (VLDL) via the activation of the same nuclear receptor signaling pathway (Watanabe et al., JCI, 2004). The fact that bile acids could signal beyond the entero-hepatic axis came from our work that demonstrated that BAs increase energy expenditure systemically by increasing triiodothyronine levels in rodent brown adipose tissue and human skeletal muscle, thereby preventing obesity and insulin resistance (Watanabe et al., Nature, 2006). This effect is mediated by TGR5, a G protein-coupled receptor, which upon activation by BAs stimulates cAMP production and type 2 deiodinase enzyme activity. These observations build a strong case that BAs have effects beyond the enterohepatic axis and function as systemic metabolic integrators.

In enteroendocrine cells, TGR5 has been associated with BA-mediated secretion of glucagon-like peptide 1 (GLP-1). GLP-1 is an incretin with potent antidiabetic effects, due to its ability to enhance pancreatic β-cell function. Although the link of TGR5 in BA-mediated GLP-1 secretion has been established *in vitro*, no evidence exists at present that this is also important *in vivo*. We therefore hypothesized that TGR5, in addition to its role in energy balance, may also have a role in the control of glucose homeostasis. To evaluate the role of TGR5 in glucose metabolism and its contribution to type 2 diabetes (T2D), we generated both mice that are overexpressing TGR5 by BAC transgenesis (TG1-TGR5 mice) and mice with a germline deficiency of the TGR5 gene (TGR5-KO mice), and performed a comprehensive metabolic profiling on both control (CT), TG1-TGR5, and TGR5-KO cohorts fed a normal chow (CD) or a high-fat (HFD) diet. Our results demonstrate that TG1-TGR5 mice are protected against diet-induced glucose intolerance, whereas TGR5-KO mice are glucose intolerant under these conditions. The improved glucose tolerance observed in HFD challenged TG1-TGR5 mice cannot be attributed to a decrease in body fat mass, but is rather the result of improved insulin release. We further show that this effect is mediated by GLP-1, which is increased after an oral challenge with glucose or a high glucose/high lipid meal. In the pancreas, β-cell function of TG1-TGR5 mice is improved compared to those derived from control littermates. Most of these beneficial metabolic effects seen in the TG1-TGR5 transgenic mice are recapitulated upon the administration of 6α ethyl, 23(S)-methyl cholic acid, a semi-synthetic BA that selectively and potently activates TGR5, and are lost in TGR5-KO mice (Thomas et al., Cell Metab, 2009).

Collectively, these data demonstrate that the TGR5 locus is a critical region to maintain normal pancreatic β-cell function and identify TGR5 as an attractive and potential target for the treatment of T2D.

Session IV

Clinical relevance of genetic variants

Genetic variants in cholestatic liver disease: An update

Frank Lammert
Klinik für Innere Medizin II, Universitätsklinikum des Saarlandes, Homburg/Saar, Germany

Investigations into the molecular mechanisms of cholestasis have revealed intricate and intriguing details of bile salt metabolism as well as its regulatory mechanisms in health and disease. Extensive investigation into genotype-phenotype correlations in monogenic diseases such as progressive familial and benign recurrent intrahepatic cholestasis facilitate diagnostics and improve the risk assessment of hepatobiliary transporter gene variants in bile pathophysiology.

While the comparatively easy targets in monogenic cholestasis have been identified for some time now, progress in complex liver disease is rather laborious but steady. We will present recent results on an "oligogenic" disease, intrahepatic cholestasis of pregnancy (ICP). Genetic investigations into ICP have served as a useful approach to prove the contribution of *ABCB4, ABCB11* and *FXR* variants on cholestatic phenotypes in distinct subgroups of cases.

Genomewide association scans are the next step in gathering information about common contributors towards polygenic (multifactorial) cholestatic diseases. The latest results for primary biliary cirrhosis (PBC) and primary sclerosing cholangitis have confirmed the contribution of HLA for both and interleukin 12 and its receptor genotypes for PBC in particular.

New determinants of bile salt metabolism affecting feedback loops within the liver or the enterohepatic circulation are presently under investigation for their contribution towards complex cholestatic syndromes. We will present an update on the potential roles of genetic variants in *TGR5* and *FGF19* and its dedicated receptor, *FGFR4*.

TGR5 sequence variation in primary sclerosing cholangitis

Johannes R. Hov[1], Verena Keitel[2], Dieter Häussinger[2], Tom H. Karlsen[1]
[1]Norwegian PSC Research Center, Clinic for Specialized Medicine and Surgery, Oslo University Hospital Rikshospitalet, Oslo, Norway
[2]Clinic for Gastroenterology, Hepatology and Infectiology, Heinrich-Heine-University, Düsseldorf, Germany

The function of TGR5, the G protein-coupled bile acid receptor 1 (GPBAR1), has been linked to inflammatory pathways as well as bile homeostasis. The *TGR5* gene localizes within a region at chromosome 2q35 which has been implicated in susceptibility to primary sclerosing cholangitis (PSC) and ulcerative colitis (UC). Establishing causality for a susceptibility gene in complex diseases like PSC and UC includes the systematic collection of statistical, genetic and functional evidence. To initiate this process for *TGR5*, we performed complete resequencing of the gene in 267 PSC patients and 276 healthy controls. Six nonsynonymous mutations were identified (four in patients only, one in both healthy controls and one patient and one in a healthy control only). In addition, we detected 16 novel single-nucleotide polymorphisms. We developed an *in silico* protein model to predict the impact from the nonsynonymous variants on the TGR5 domains. We also introduced mutated *TGR5* constructs into HEK293 and MDCK cells, and five of the nonsynonymous mutations were found to reduce or abolish different aspects of TGR5 expression and function as observed by confocal microscopy, flow cytometry and a cAMP-sensitive luciferase assay. Fine-mapping of the previously reported PSC and UC associated locus at chromosome 2q35 in large case-control panels revealed strong linkage disequilibrium, precluding the identification of a single causative variant. The strongest *TGR5* associations were found for single-nucleotide polymorphism rs11554825, which associated with both PSC (odds ratio = 1.14, 95% confidence interval: 1.03–1.26, p = 0.010) and UC (odds ratio = 1.19, 95% confidence interval: 1.11–1.27, $p = 8.5 \times 10^{-7}$). Concluding the status of the work, we have identified novel nonsynonymous variants critically influencing the function of TGR5, and which yield important insight into the importance of various structural domains of the receptor. While rs11554825 was statistically associated with both PSC and UC, further functional studies are required to establish the biological basis of this association and to conclusively define what role TGR5 may play in these closely related conditions.

Treatment of primary biliary cirrhosis with UDCA and fibrates

Raoul Poupon
UPMC Liver Center, INSERM, UMRS 893, AP-Hôpitaux de Paris, Hôpital St-Antoine, Paris, France

Fibrates derivatives have a 40-year history in the management of dyslipidemia. Their use led to the discovery of peroxysome proliferator-activated receptors (PPARs). PPARα, the target of synthetic hypolipidemic fibrates is highly expressed in the liver, as well as in the T and B cells of the immune system. Ligands for PPARα are now recognised to have therapeutic activity in several rodent models for human inflammatory and autoimmune diseases such as experimental autoimmune encephalomyelitis, inflammatory bowel disease and arthritis.

Their parsimonious use for the treatment of primary biliary cirrhosis (PBC) came initially from reports showing they can induce paradoxical hypercholesterolemia, cholesterol supersaturation of bile, intrahepatic and choledocholithiasis in patients with PBC and acute or chronic liver diseases associated with autoimmune features. In patients with dyslipidemia, fibrates markedly reduce serum alkaline phosphatase activity. This observation was the basis for studies from Japan of fibrates in PBC. In the last decade, the discovery of a wide range of biological and hepatoprotective effects of PPARα ligands have boosted clinical studies in patients with PBC. In this talk I review the effects of fibrates in PBC according to the published reports and my own experience. In brief, fibrates improve not only serum alkaline phosphatase but also an array of markers of cholestasis including GGT, 5' nucleotidase, glutamate dehydrogenase, transaminases and serum bile acids levels in patients already treated with UDCA. Specific immune features of PBC, IgM levels and AMA titers are also reduced during fibrates administration suggesting that the anticholestatic effect of fibrates might be mediated through an immune and antiinfmmatory mechanism. The long-term potential therapeutic effects of fibrates in PBC as well as their PPARα dependent and independent mechanism of action will be discussed.

Genetic variants of the bile salt export pump: Inducers and modifiers of liver diseases

Ralf Kubitz[1], Christoph Brinkmayer[1], Abdurrahman Sagir[1], Diran Herebian[2], Dieter Häussinger[1]
[1]Department of Gastroenterology, Hepatology and Infectiology, University Hospital Düsseldorf, Germany
[2]Department of Pediatrics, University Hospital Düsseldorf, Germany

Introduction: The bile salt export pump (BSEP, ABCB11) is the major transporter protein for the excretion of bile salts into bile. Mutations and genetic polymorphisms may affect the expression of BSEP with consequences for bile salt homeostasis.

Methods: We have sequenced the ABCB11 gene in 33 patients (26 children, 7 adults) with suspected inherited cholestasis and analysed the V444A polymorphism in 353 patients with hepatitis C infection and in 366 controls.

Results: 17 patients with suspected inherited cholestasis had no significant variants in the ABCB11 gene, whereas in the remaining 16 patients, 12 known and 15 new mutations were detected. The new mutations included 1 nonsense, 8 missense, 3 frame shift and 2 splice site mutations. Furthermore, a whole triplet deletion leading to a single amino acid deletion at the junction of ninth transmembrane domain and the sixth intracellular loop was identified. Biliary profile of bile salts was almost unaffected by this mutation. However, a missense-mutation in the twelfth transmembrane domain close to the transporter pore was associated to an almost complete exclusion of conjugated chenodeoxycholate from bile.
Not only rare mutations, but also the common polymorphism V444A (valine to alanine at amino acid position 444) may modify the course of liver diseases. We tested the influence of V444A on the success rate of interferon/ribavirin treatment in patients with HCV-infection. In a cohort of 353 HCV patients, 282 were infected with HCV genotype 1 or 4. Of these patients, homozygous carriers of the wild-type polymorphism (V/V) had an end of treatment response (ETR) in 84% and a sustained virological response (SVR) in 62%. Patients with one or two polymorphic alleles (V/A or A/A) had statistically significant lower success rates with an ETR in 66% ($p < 0.05$) and a SVR in only 33% ($p = 0.001$). Multivariate regression analysis revealed that V444A was an independent predictive factor for therapy response.

Discussion/Conclusion: In conclusion, BSEP is an important modifier of liver diseases. This may be attributed to its central role in bile salt homeostasis in connection with the numerous biological effects of bile salts.

Genetic variation in biliary transporters as a susceptibility factor for cholangiocarcinoma

Christopher A. Wadsworth[1], Peter H. Dixon[2], Jason H. Wong[1], Michael H. Chapman[3], Siobhan C. McKay[4], Amar Sharif[1], Duncan R. Spalding[4], Stephen P. Pereira[3], Howard Thomas[1], Simon D. Taylor-Robinson[1], John Whittaker[5], Catherine Williamson[2], Shahid A. Khan[1]
[1]Department of Hepatology and Gastroenterology, Imperial College London, London, UK
[2]Institute of Reproductive and Developmental Biology, Imperial College London, London, UK
[3]Institute of Hepatology, University College London Medical School, London, UK
[4]Department of Hepatobiliary Surgery, Imperial College London, London, UK
[5]London School of Hygiene and Tropical Medicine, University of London, London, UK

Introduction: Cholangiocarcinoma (CC) is increasing in incidence globally but its pathogenesis remains poorly understood. Chronic inflammation of the bile duct and cholestasis are major risk factors but most cases in the West are sporadic. Genetic polymorphisms in biliary transporter proteins have been implicated in benign biliary disease and, in the case of progressive familial cholestasis, have been associated with childhood onset of CC. A recent case-control study of a single nucleotide polymorphism c.3972C>T (rs3740066) in *ABCC2*, reported an association with CC. In the current study, five biologically plausible candidate genes were investigated; *ABCB11* (BSEP); *ABCB4* (MDR3); *ABCC2* (MRP2); *ATP8B1* (FIC1) and *NR1H4* (FXR).

Methods: Germline DNA was collected from 172 Caucasian individuals with confirmed CC. A control cohort of 256 healthy Caucasian patients was included in the analysis. 73 SNPs were selected using the HapMap database in Haploview 4.1 (build 22; MAF > 0.05, pair-wise comparisons only) to capture the majority of common genetic variation around the five candidate loci. Genotyping was undertaken with a competitive allele-specific PCR-based robotic genotyping system. Confirmation of Hardy-Weinberg equilibrium and Cochran-Armitage trend testing were performed using PLINK v1.07. Haplotype frequencies were compared using haplo.stats v1.4.4.

Results: All 73 SNPs were in Hardy-Weinberg Equilibrium. Four SNPs in *ABCB11* were associated with altered susceptibility to CC, including the V444A polymorphism (c.1331T>C, rs2287622, $p < 0.007$) but these associations did not retain statistical significance after Bonferroni correction for multiple testing. Haplotype analysis of the genotyped SNPs in *ATP8B1* identified significant differences in frequencies between cases and controls (global p-value 0.005). None of the SNPs in *ABCC2*, including rs3740066, showed association with CC. Haplotype analysis in *ABCC2* failed to detect significant association.

Discussion/Conclusion: This is the largest study to date of biliary transporter polymorphisms as susceptibility factors for CC. The previously reported association between SNP rs3740066 in *ABCC2* and CC was not replicated. Haplotypes in *ATP8B1* demonstrated a significant difference between CC and control groups. There was also a trend towards significant association of V444A with CC. V444A has been strongly implicated in other cholestatic diseases. Given the biological plausibility of polymorphisms in *ABCB11* and *ATP8B1* as risk modifiers for CC, further study in a validation cohort is required.

Session V

Therapeutic potential of bile acids

Targeting nuclear bile acid receptors for liver disease

Michael Trauner, M.D.
Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, A-1090 Vienna, Austria
E-Mail: michael.trauner@meduniwien.ac.at

Nuclear receptors (NRs) are transcription factors which are activated by natural or synthetic ligands and control embryonic development, metabolism and reproduction. In liver, NRs play a key role in the transcriptional control of critical steps of a wide range of hepatic functions ranging from hepatic lipid and glucose metabolism to bile formation, phase I/II metabolism of endo and xenobiotics such as bile acids and drugs. Apart from these metabolic roles, NRs may also play a key role in the control of hepatic inflammation, fibrogenesis, replication of hepatotrophic viruses, liver regeneration and carcinogenesis. As such NRs are key for understanding the pathogenesis of several liver and extrahepatic disorders and therefore represent attractive drug targets.

Since bile acids (BAs) are potentially cytotoxic, a tight control of their concentration is necessary. NRs are critically involved in regulation of bile formation and BA homeostasis under physiological and pathological conditions. Cholestasis results in intrahepatic accumulation of potentially cytotoxic BA which cause liver damage ultimately leading to biliary fibrosis and cirrhosis. Cholestatic liver injury is counteracted by a variety of adaptive hepatoprotective mechanisms including alterations in bile acid transport, synthesis and detoxification. The underlying molecular mechanisms are mediated mainly at a transcriptional level via a complex network involving NRs including the farnesoid X receptor (FXR; NR1H4), pregnane X receptor (PXR; NR1I2), constitutive androstane receptor (CAR; NR1I3), vitamin D receptor (VDR; NR1I2) and liver X receptor (LXR; NR1H3) which are activated by BAs and other biliary constituents and target overlapping, although not identical, sets of genes involved in the defense against BAs. However, these intrinsic adaptive mechanisms do not suffice to overcome cholestatic liver injury damage since NRs themselves undergo a dramatic reduction under inflammatory and cholestatic conditions.

Since the intrinsic adaptive response to BAs cannot fully prevent liver injury in cholestasis, therapeutic targeting of these receptors via specific and potent agonists may further enhance the hepatic defense against toxic BAs. Activation of these receptors results in repression of bile acid synthesis, induction of phase I and II bile acid hydroxylation and conjugation and stimulation of alternative BA export while at the same time limiting hepatocellular BA import. Furthermore, the use of NR ligands may not only influence bile acid transport and metabolism but may also target biliary lipid composition (counteracting intrinsic bile acid toxicity), hepatic fibrogenesis and inflammation as key mechanisms involved in the progression of chronic biliary disorders such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). Many drugs already used to treat cholestasis and its complications such as pruritus (e.g., ursodeoxycholic acid [UDCA], rifampicin, fibrates) may act via activation of nuclear receptors (FXR, PXR, peroxisomal proliferator-activated receptor [PPARalpha]). Some of UDCA's effects can be attributed to activation of nuclear receptors since UDCA is a (weak) ligand for FXR, PXR and glucocortocid receptor (GR; NR3C1). Conversely, UDCA may also exert FXR antagonistic properties by changing the bile acid pool composition and reducing the relative amounts of stronger FXR ligands such as CDCA and CA. Interestingly, the

combination of UDCA and the GR ligand dexamethasone but not UDCA or dexamethasone alone increase anion exchanger 2 (AE2) expression and function via interaction of HNF1 and GR on an *AE2* alternate promoter. These findings may contribute to the beneficial effects of the combination of glucocorticoids and UDCA in patients with PBC, since AE2 expression is reduced in PBC and respective knockout mice develp features of PBC (including formation of AMA). More specific and potent nuclear receptor ligands (e.g. for FXR) are currently being developed clinically. As such, a clinical phase II study has revealed promising effects of 6-ECDCA in PBC patients with incomplete response to UDCA. Recent experimental data indicate that a dual FXR/TGR5 agonist is able to reverse liver injury in a mouse model of sclerosing cholangitis and hepatic fibrosis. These findings suggest, FXR/TGR5 agonists could constitute a new avenue for treatment of human chronic cholangiopathies. *Nor*UDCA, a side chain-shortened homologue of UDCA with convincing therapeutic effects in animal models of cholestasis, is currently prepared for clinical development. Its therapeutic effects include robust choleretic, adaptive, anti-inflammatory and anti-fibrotic (in part transcriptional) effects, but so far no classic BA-activated NR has been identified as target of *nor*UDCA.

Beside their well established role in the regulation of dietary lipid absorption and cholesterol homeostasis, BAs may also play a key role as endocrine signaling molecules that coordinate hepatic lipid homeostasis through NRs and thus might represent a promising therapeutic treatment strategy for non-alcoholic fatty liver disease (NAFLD) and arteriosclerosis. The flux of reabsorbed BA undergoing an enterohepatic circulation, arriving in the liver with the co-absorbed nutrients (e.g. glucose, lipids) provides a signal that coordinates hepatic triglyceride (TG), glucose and energy homeostasis. As signalling molecules with systemic endocrine functions, BA can activate protein kinase A and C, mitogen-activated protein kinase pathways, are ligands for a G-protein-coupled BA receptor (TGR5/Gpbar-1), and activate NRs such as FXR. As such, FXR and its downstream targets play a key role in the control of hepatic *de novo* lipogenesis, very low density lioprotein-TG export and plasma TG turnover. BA-activated FXR and signal transduction pathways are also involved in the regulation of hepatic gluconeogenesis, glycogen synthesis and as insulin sensitivity. Via TGR5 BA are able to stimulate glucagon-like perptide-1 secretion in the small intestine and energy expenditure in brown adipose tissue and skeletal muscle. Dysregulation of BA transport and impaired BA receptor signalling may contribute to the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Thus, BA transport and BA-controlled NRs and signalling pathways are valid drug targets for treatment of NAFLD. As such FXR and/or TGR5 ligands have shown promising results in animal models of NAFLD and clinical pilot studies. Despite being a poor FXR and TGR5 ligand, UDCA improves hepatic ER stress and insulin sensitivity. Notably, *nor*UDCA, a side chain-shortened homologue of UDCA, improves fatty liver and atherosclerosis in Western diet-fed in ApoE$^{-/-}$ mice. Collectively, these findings suggest that BAs and targeting their receptors/signalling pathways may represent a promising approach to treat NAFLD and closely linked disorders such as obesity, diabetes, dyslipidemia and arteriosclerosis.

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Role of the anion exchanger 2 for the pathogenesis and treatment of PBC

Juan F. Medina, M.D., Ph.D.
Division of Gene Therapy and Hepatology-Liver Unit, School of Medicine, Clinic & CIMA University of Navarra, and Ciberehd, Pamplona, Spain

In the liver, primary generation of the bile-salt independent bile flow involves vectorial ion transport through the canaliculus and the basolateral membrane of the hepatocytes. Parallel hydroionic fluxes through the apical and basolateral membrane of the bile duct epithelial cells (cholangiocytes) are required for the fluidization and alkalinization of bile while proceeding along the biliary tract, a process that is normally stimulated by secretin. Biliary bicarbonate secretion occurs through an apical electroneutral Na^+-independent Cl^-/HCO_3^- anion exchange (AE activity). This Cl^-/HCO_3^- exchange is facilitated by the outside to the inside transmembrane gradient of Cl^- that results from the efflux of Cl^- to the lumen mediated by the apical cAMP-dependent (CFTR) and Ca^{2+}-dependent Cl^- channels. The essential anion exchanger involved in biliary bicarbonate secretion is AE2/SLC4A2, a membrane protein which has also been recognized to be relevant for the regulation of the intracellular pH (pH_i) in several cell types. In the human liver, AE2 immunoreactivity was localized at the lumenal membrane of bile duct epithelial cells (mainly in small and medium size bile ducts) and also at the canalicular membrane of hepatocytes, particularly those of the periportal area. Studies of *in situ* hybridization indicate that the level of *AE2* gene expression is higher in cholangiocytes than in hepatocytes. Ae2 gene silencing in rat cholangiocytes and experiments performed with $Ae_{2a,b}^{-/-}$ mouse cholangiocytes have confirmed that AE2 is the only mediator of Cl^-/HCO_3^- exchange in bile duct cells.

Previously, we reported that the expression of AE2 mRNA is diminished in liver biopsies and peripheral mononuclear cells from patients with PBC. Also, immunohistochemical studies indicated that the expression of the AE2 protein is decreased in the bile ducts and hepatocytes in PBC livers. Moreover, we found that bile duct cells isolated from PBC patients and cultured for a few passages, exhibit defective Na^+-independent Cl^-/HCO_3^- exchange. Interestingly, positron emission tomography (PET) studies showed that PBC patients, even at the early stages of the disease, fail to secrete bicarbonate to bile in response to secretin, a defect that can be partially reversed after several months of treatment with UDCA. Altogether, these findings sustained our hypothesis that dysfunctions related to AE2 might have a role for the pathogenesis of PBC. Inadequate AE2 function in lymphocytes would disturb pHi regulation in these cells and alter immune homeostasis leading to autoimmunity. On the other hand, reduced AE2 in cholangiocytes could cause cholestasis and oxidative stress of bile duct cells. Cholangiocyte changes, together with altered immune homeostasis, could favor the development of antimitochondrial antibodies and the autoimmune attack to biliary ducts. Our recent findings that $Ae2_{a,b}$-deficient mice indeed display most of these features strongly support the notion that AE2 abnormalities may be involved in the pathogenesis of PBC.

New treatment strategies for PSC

Keith D. Lindor, M.D.
Mayo Clinic; Rochester, MN, USA

Primary sclerosing cholangitis (PSC) is a progressive liver disease without adequate treatment. Similar conditions such as IgG4 associated disease seem to respond to immunosuppressive therapy, but conventional immunosuppressive therapy has not been effective in patients with PSC. Furthermore, bile acids have not been effective in doses of 13–15 mg/kg/day or 28–30 mg/kg/day in PSC patients. Because the pathogenesis of PSC is not well understood, specific therapies designed to alter the pathogenesis are not available.

There are a number of potentially pathogenetic mechanisms that can be targeted for therapy. These include: bacterial infection, abnormal receptor function, enhanced apoptosis, immunologic abnormalities and increased fibrosis. Animal models suggest a link between bacterial overgrowth, lipopolysaccharides from the bacterial cell walls and stimulation of endogenous tumor necrosis factor formation which can be inhibited by pentoxifylline. Disruption of each of these steps in an animal model prevented the development of biliary tract lesions. Unfortunately, in humans treated with pentoxifylline little effect was seen. Antibiotics such as metronidazole, vancomycin, tetracycline and azithromycin in small studies show biochemical improvement. Abnormalities in the cystic fibrosis transporter receptor have been described in some patients with PSC. Docosahexanoic acid has been used for treating patients with such a defect in cystic fibrosis, and in a small pilot study led to some improvement in liver biochemistries.

There has been more interest recently in the role of nuclear receptors. They are involved in multiple steps in cellular metabolism. An FXR agonist, 6-ethylchenodeoxycholic acid, in pilot studies of patients with PBC led to substantial biochemical improvement, and this drug is ripe for testing in patients with PSC. Apoptosis is another potential target, although the evidence for a role of enhanced apoptosis in PSC is weak. Minocylcine reduces inducible nitric oxide synthesis and may inhibit apoptosis. In patients with PSC this led to biochemical improvement. Immunologic abnormalities have been considered to be potentially important. Attempts to treat PSC with mycophenolate mofetil and tacrolimus have been largely unsuccessful. Budesonide has shown a limited benefit but perhaps more so in patients with elevated IgG4 levels.

In the initial animal model, tumor necrosis factor appeared to play an important role. Anti-TNF agents such as silymarin, pentoxifylline, etanercept and infliximab have been tested in patients with PSC. Silymarin led to a minor biochemical improvement. Pentoxifylline, as noted before had virtually no benefit, and parenteral anti-TNF agents have had little effect. PSC is characterized by marked fibrosis. Antifibrogenic approaches have been considered. Pirfenidone was one such agent which was unsuccessful. Other potential options include modulation of the renin angiotensin system as well as use of rapamycin, but data from this approach are scant. Bile acids have been tested perhaps as widely but initial doses used in PBC led to only biochemical improvement. Higher doses of ursodeoxycholic acid (28–30 mg/kg/day) actually seemed to be associated with a worsening outcome. Intermediate doses have been tested in an underpowered study where biochemical improvement was seen and

trends towards enhanced survival were found, but only about 2/3 of the anticipated enrollment occurred.

At this time, there are multiple potential therapeutic avenues to explore for patients with PSC, and it is hoped in the not too distant future one of these will lead to identification of a proven therapy for this disease.

Ursodeoxycholic acid improves liver enzymes and LDL-cholesterol in morbidly obese patients

Hanns-Ulrich Marschall[1,3], Martin Wagner[4], Gernot Zollner[4], Peter Fickert[4], Cord Lackner[5], Anders Thorell[2], Michael Trauner[4,6]
Karolinska Institutet, Departments of [1]Medicine at Karolinska University Hospital Huddinge and [2]Surgery at Ersta Hospital, Stockholm, Sweden; [3]Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Sweden; [4]Department of Medicine and [5]Institute of Pathology at Medical University of Graz, Austria; and [6]Medical University of Vienna, Austria

Introduction: Ursodeoxycholic acid (UDCA) was shown to improve insulin resistance and steatosis in mice. The efficacy and possible modes of action of UDCA treatment in human non-alcoholic fatty liver disease (NAFLD) have been debated. Since bile acids (BA) have recently been identified as major integrators of hepatic fatty acid (FA) and triglyceride (TG) metabolism we hypothesized that UDCA may exert antilipogenic effects through modulation of hepatic BA transport and metabolism. We aimed to explore potential mechanism(s) of UDCA action in patients with morbid obesity awaiting bariatric surgery.

Methods: Forty morbidly obese patients were randomized to UDCA (20mg/kg/day in three weeks before surgery) or no treatment (controls). Serum liver functions tests, lipids, bile acids and markers of insulin resistence/diabetes (OGTT, HOMA) were obtained before and after treatment. During surgery, biopsies were taken from the liver and from subcutaneous and omental fat for histology and subsequent gene expression analysis.

Results: Treatment and controls groups were well matched by gender (female, 68.4 vs. 77.7%), age (42.8 ± 12.3 vs. 38.5 ± 10.1 years), BMI (41.9 ± 4.6 vs. 40.6 ± 3.9 kg/m^2), HOMA (5.1 ± 2.5 vs. 6.6 ± 3.9; normal; n = 2 vs. n = 3)) and NASH scores (no, 29.7 vs. 35.1%; borderline, 10.8 vs. 10.8%; definite, 10.8 vs. 2.7%). UDCA significantly ($p < 0.05$) decreased ALT, AST, gGT, total- and LDL-cholesterol and expanded the BA pool 10.6 ± 7.6-fold (≤ 55.3 mmol/L), unrelated to NASH scores, and without formation of toxic metabolites. However, UDCA did not significantly improve HOMA IR (6.6 ± 3.9 before vs. 5.8 ± 3.1 after treatment; $p = 0.15$) nor altered hepatic mRNA expression of major BA and FA metabolism genes and inflammatory mediators.

Discussion/Conclusion: Despite their enormous BMI and insulin resistance, our morbidly obese patients had a surprisingly low incidence of advanced NAFLD/NASH. UDCA improved liver enzymes but not HOMA or major metabolism and inflammation genes.

List of Chairpersons, Speakers and Scientific Organizers

Prof. Dr. J. Auwerx
École Polytechnique
Fédéral de Lausanne
SV-IBI1-LISP-NCEM1
Bâtiment AI, Station 15
1015 Lausanne
Switzerland

N. Ballatori, Ph.D.
Professor of Medicine
University of Rochester
School of Medicine & Dentistry
Dept. of Environmental Medicine
601 Elmwood Ave.
Rochester, NY 14642
USA

Prof. Dr. U. Beuers
Univ. van Amsterdam, Tytgat Institute
for Liver & Intestinal Research
Department of Gastroenterology
& Hepatology, B1-244.2
Meibergdreef 9
1105 AZ Amsterdam
The Netherlands

Prof. Dr. I. Björkhem
Karolinska University
Hospital Huddinge
Department of Clinical Chemistry
141 86 Stockholm
Sweden

Dr. N. Chignard
Université Pierre et Marie Curie
Site Saint Antoine
Faculté de Médecine
27, rue Chaligny
75012 Paris
France

P.A. Dawson, Ph.D.
Professor of Medicine
Wake Forest University
Bowman Gray School of Medicine
Department of Medicine
Medical Center Boulevard
Winston-Salem, NC 27157
USA

Prof. Dr. D. Häussinger
Gastroenterologie/Hepatologie
Universitätsklinikum Düsseldorf
Moorenstr. 5
40225 Düsseldorf
Germany

A.F. Hofmann, M.D., Ph.D.
Prof. of Medicine (Emeritus)
5870 Cactus Way
La Jolla, CA 92037
USA

Prof. Dr. P.L.M. Jansen
Univ. van Amsterdam, Tytgat Institute
for Liver & Intestinal Research
Department of Gastroenterology
and Hepatology
Meibergdreef 9
1105 AZ Amsterdam
The Netherlands

Dr. T.H. Karlsen
University of Oslo
Rikshospitalet
Insitute of Immunology
0027 Oslo
Norway

K.H. Kaestner, M.D.
Professor of Genetics
University of Pennsylvania
School of Medicine
Inst. for Diabetes & Obesity
415 Curie Boulevard
Philadelphia, PA 19104
USA

Dr. V. Keitel
Gastroenterologie/Hepatologie
Universitätsklinikum Düsseldorf
Moorenstr. 5
40225 Düsseldorf
Germany

Prof. Dr. D. Keppler
Tumorbiochemie
Deutsches Krebsforschungszentrum
Im Neuenheimer Feld 280
69120 Heidelberg
Germany

Prof. Dr. R. Kubitz
Gastroenterologie/Hepatologie
Universitätsklinikum Düsseldorf
Moorenstr. 5
40225 Düsseldorf
Germany

Prof. Dr. F. Lammert
Klinik für Innere Medizin II
Universitätsklinikum
des Saarlandes
Kirrberger Straße
66424 Homburg/Saar
Germany

N.F. LaRusso, M.D.
Prof. of Medicine & Biochemistry
Mayo Clinic
Gastroenterology Unit, Box 0001
200 First Street SW
Rochester, MN 55905
USA

Prof. Dr. U. Leuschner
Interdisziplinäres Facharztzentrum
Stresemannallee 3
60596 Frankfurt
Germany

K.D. Lindor, M.D.
Professor of Medicine
Mayo Clinic
Gastroenterology Unit, Box 0001
200 First Street SW
Rochester, MN 55905
USA

D.J. Mangelsdorf, Ph.D.
Professor of Pharmacology
University of Texas
Southwestern Medical Center
Department of Pharmacology
6001 Forest Park Road
Dallas, TX 75390
USA

Prof. Dr. Dr. H.-U. Marschall
University of Göteborg
Sahlgrenska Sjukhuset
Department of Internal Medicine
Division of Hepatology
413 45 Göteborg
Sweden

Prof. Dr. J.F. Medina
Clinica Universitaria Pamplona
Terapia Génica y Hepatologia
Avda. Pio XII 55
31008 Pamplona
Spain

Dr. A. Moschetta
Università di Bari
Consorzio Mario Negri Sud
Via Nazionale 8/A
66030 Sta. Maria Imbaro (Chieti)
Italy

Prof. Dr. R.P.J. Oude Elferink
Univ. van Amsterdam, Tytgat Institute
for Liver & Intestinal Research
Afd. Maag-, Darm- en Leverziekten
Meibergdreef 69–71
1105 BK Amsterdam
The Netherlands

Prof. Dr. M. Paulmichl
Paracelsus Med. Universität
Institut für Pharmakologie
und Toxikologie
Strubergasse 21
5020 Salzburg
Austria

Prof. Dr. G. Paumgartner
Medizinische Klinik II
Klinikum der Universität
München - Großhadern
Marchioninistr. 15
81377 München
Germany

Prof. Dr. R. Poupon
Hôpital Saint-Antoine
Service d'Hépato-Gastro-Entérologie
184, rue du Faubourg St. Antoine
75571 Paris
France

Dr. K. Schoonjans
École Polytechnique
Fédéral de Lausanne
SV-IBI1-LISP-NCEM1
Bâtiment AI, Station 15
1015 Lausanne
Switzerland

Prof. Dr. J. Sjövall
Karolinska Institutet
Department of Medical
Biochemistry and Biophysics
171 77 Stockholm
Sweden

Prof. Dr. A. Stiehl
Langgewann 17
69121 Heidelberg
Germany

Prof. Dr. H. Suzuki
University of Tokyo
School of Pharmaceutical Sc.
Department of Pharmacy
7-3-1, Hongo, Bunkyo-ku
Tokyo 113-0033
Japan

Prof. Dr. M. Trauner
Medizinische Universität Wien
Klinische Abteilung für
Gastroenterologie & Hepatologie
Währinger Gürtel 18–20
1090 Wien
Austria

Dr. C.A. Wadsworth
Imperial College London
Hammersmith Hospital Campus
Department of Hepatology
& Gastroenterology
Du Cane Road
London W12 0HS
Great Britain

Prof. Dr. C. Williamson
Imperial College London
Hammersmith Hospital Campus
Dept. of Surgery
Du Cane Road
London W12 0HS
Great Britain

POSTER ABSTRACTS

Organic anion-transporting polypeptide 1b2 (Oatp1b2) is important for the hepatic uptake of unconjugated bile acids: Studies in Oatp1b2-null mice

Iván L. Csanaky, Hong Lu, Youcai Zhang, Kenichiro Ogura, Supratim Choudhuri, Curtis D. Klaassen
Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS, USA

Introduction: The members of organic anion transporting polypeptide 1b family (Oatp1b2 in rodents and OATP1B1/1B3 in human) are liver specific transporters that transport various chemicals into the liver. However, the role of the Oatp1b family in the hepatic uptake of bile acids (BAs) into liver is unknown.

Methods: Oatp1b2-null mice were developed in our laboratory. The concentration of BAs in plasma, liver, and bile of Oatp1b2-null and WT mice were determined by UPLC-MS/MS. The mRNA expression of genes in liver and ileum samples were determined by Quantigene Plex 2.0 Technology. Plasma elimination studies of BAs were done on anaesthetised renal-pedicle-ligated Oatp1b2-null and WT mice.

Results: It was first determined that expression of other hepatic transporters was not altered in livers of the Oatp1b2-null mice, suggesting that the mice did not compensate for the loss of Oatp1b2. However, the mRNA of Cyp7a1 was 70% lower in the Oatp1b2-null mice. Increased expression of fibroblast growth factor (Fgf) 15 in intestine of Oatp1b2-null mice might be responsible for decreased hepatic expression of Cyp7a1 in Oatp1b2-null mice. The hepatic concentration and biliary excretion of conjugated and unconjugated BAs were essentially the same in Oatp1b2-null and WT mice. The serum concentration of taurine (T) conjugated BAs was essentially the same in the two genotypes. In contrast, the serum concentrations of unconjugated BAs were 3 to 45 times higher in Oatp1b2-null than WT mice. After i.v. administration of cholate (CA) to Oatp1b2-null mice, its clearance was 50% lower than in WT mice, but the clearance of taurocholate (TCA) was similar in the two genotypes.

Conclusion: This study indicates that Oatp1b2 has a major role in hepatic uptake of unconjugated BAs.

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Adaptive mechanisms in organic solute transporter-alpha (Osta) null mice

Anuradha Rao[1], Tian Lan[1], Jamie Haywood[1], Gary D. Kruh[2], Paul A. Dawson[1]
[1]Department of Internal Medicine, Section on Gastroenterology, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA
[2]Department of Medicine and Cancer Center, University of Illinois at Chicago, Chicago, IL 60612, USA

Introduction: Inactivation of the basolateral membrane bile acid transporter, Osta-Ostb results in impaired intestinal bile acid absorption and altered bile acid metabolism. To further understand the function role of Osta-Ostb, we have used single and double knockout mouse models to identify the adaptive mechanisms engaged following disruption of the Osta gene.

Methods: The corresponding null mice were crossbred to generate OstaMrp3 or OstFxr double-null mice. All experiments compared wild type and corresponding single or double knockout mouse littermates. Analysis of in the vivo phenotype included measurements of intestinal morphology and histology, bile acid intestinal content, fecal excretion, pool size, and composition. Hepatic and intestinal mRNA and protein expression were also examined.

Results: Osta but not Asbt, Mrp3, or Fxr null mice exhibit significant intestinal lengthening, and increased villous hypertrophy and hyperplasia. The enterocyte microvilli were also significantly blunted in Osta versus wild type mice. Inactivation of Fxr in Osta null mice failed to reverse the intestinal hypertrophy and hyperplasia. Notably, the intestinal morphological changes in Osta null mice were not associated with evidence of inflammation or ER stress. Intestinal Mrp3 mRNA and protein expression was significantly increased in Osta null mice. However, the intestinal phenotype and bile acid metabolism were similar in Osta and OstaMrp3 null mice. The intestinal bile acid content of ileal enterocytes was not increased in Osta or OstaMrp3 null mice as a result of a paradoxical decrease in hepatic bile acid synthesis and decreased intestinal expression of Asbt mRNA and protein.

Discussion/Conclusion: Inactivation of Osta results in an intestinal adaptation response that does not appear to involved bile acid signalling via Fxr. Major protective mechanisms include the down-regulation of hepatic bile acid synthesis and reduced intestinal apical membrane bile acid uptake. Induction of Mrp3 expression plays only a minor protective role.

Ezetimibe induced stimulation of fecal neutral sterol excretion in mice depends on abcg5/abcg8 function

Lily Jakulj[1], Maud N. Vissers[1], Renze Boverhof[4], Cindy P. van Roomen[2], Carlos Vrins[2], Cindy Kunne[3], John J.P. Kastelein[1], Frans Stellaard[4], Albert K. Groen[4]
Departments of [1]Vascular Medicine and [2]Medical Biochemistry, [3]AMC Liver Center, University of Amsterdam, Amsterdam, The Netherlands; [4]Center for Liver, Digestive and Metabolic Diseases, University Hospital Groningen, Groningen, The Netherlands

Introduction: Ezetimibe, a cholesterol absorption inhibitor has been shown to increase fecal neutral sterol (FNS) excretion in mice, which cannot be solely attributed to inhibition of cholesterol absorption alone. Moreover, according to recent reports, ezetimibe might promote reverse cholesterol transport, although the underlying mechanisms are unknown. Therefore, we investigated whether these effects are mediated via abcg8.

Methods: Abcg8$^{-/-}$ C57BL/6J mice (abcg8$^{-/-}$) and C57BL/6J mice (WT) were fed a control diet with or without ezetimibe 10 mg/kg/d for a period of 14 days. Cholesterol balance was assessed by measuring FNS excretion, biliary cholesterol secretion and dietary cholesterol intake. In order to enhance cholesterol absorption and biliary excretion, experiments were repeated in the same mouse strains with a 0.5% cholate-enriched diet with or without ezetimibe 10 mg/kg/d.

Results: Ezetimibe increased FNS excretion 1.5-fold and 2.7-fold in abcg8$^{-/-}$ and WT mice, respectively (15.1 ± 1.9 versus 10.1 ± 1.6 µmol/100 g BW and 22.9 ± 2.2 versus 8.4 ± 2.7 µmol/100 g BW, respectively), without significantly affecting biliary cholesterol excretion.
Daily FNS excretion exceeded the sum of dietary cholesterol intake and biliary excretion with 6.4 µmol/100 g BW and 5.4 µmol/100 g BW in abcg8$^{-/-}$ and WT mice respectively, indicating an alternative origin of the FNS. Ezetimibe treatment enhanced this 'extra' FNS excretion by 1.9-fold in abcg8$^{-/-}$ mice and 3.5-fold in WT mice, respectively. With the cholate-enriched diet, these ezetimibe-induced increases were 8-fold in abcg8$^{-/-}$ and 20-fold in WT mice respectively.

Discussion/Conclusion: Our results indicate that the ezetimibe-induced stimlulation of FNS excretion requires intact abcg8 function. As this increase was not accompanied by enhanced biliary sterol excretion, intestinal rather than hepatic abcg8 might be involved in this ezetimibe-induced augmentation of FNS loss, possibly through stimulation of direct trans-intestinal cholesterol excretion (TICE).

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Fish and lizards: Living with an apparent mutation in the bile salt biosynthesis pathway

L.R. Hagey[1], M.D. Krasowski[2], and A.F. Hofmann[1]
[1]University of California, San Diego, CA, USA
[2]University of Iowa Hospitals and Clinics, Iowa City, IA, USA

Since the most ancient vertebrates (jawless fish) have 5α-bile salts, and since both cyprinoid fish and lizards are considered ancient species, it has long been assumed that their 5α-bile salts indicates the persistence of an ancient pathway for bile acid biosynthesis. The problem with this assumption is that the vertebrate precursors of these species have predominantly 5β-bile salts.

In mammals, the conversion of cholesterol to 5β-bile acids involves 3 enzymes that saturate the steroid nucleus and epimerize the 3β-hydroxy group of cholesterol. The first enzyme 3β-hydroxy-Δ^5-C_{27} steroid oxidoreductase (HSD3B7) simultaneously isomerizes the double bond from $\Delta^{5,6}$ to $\Delta^{4,5}$ and dehydrogenates the 3β-hydroxy group to a 3-oxo group, thereby forming a 3-oxo-$\Delta^{4,5}$ intermediate. The second enzyme Δ^4-3-oxosteroid-5β-reductase (AKR1D1) stereospecifically reduces the double bond at $\Delta^{4,5}$ to form a 5β-(A/B *cis*) 3-oxo- bile acid. The third enzyme, 3α-hydroxy steroid dehydrogenase (AKR1C4) reduces the 3-oxo group to a 3α-hydroxy group. (The AKR1D series and the AKR1C series are the result of a gene duplication event and differ by only a few amino acids). Based on structural studies, the reductase AKRD1 normally only accepts bile salts via head-on entry and this specificity of substrate binding is determined by only a one or two amino acid difference that precludes tail-first entry. We propose that AKRD1 is mutated in lizards and Cyprinoid fish, resulting in a change that allows a tail-first entry of the 3-oxo-Δ^4 intermediate, which is then reduced to the 5α-configuration. 5α-bile salts probably function almost as well as 5β-bile salts, provided they are conjugated with taurine, and consequently, there has been little selection pressure to eliminate the mutation.

Clathrin adaptor protein complex 2 (AP2)-dependent internalization of bile salt export pump (BSEP/ABCB11)

Hisamitsu Hayashi, Kaori Inamura, Kensuke Aida, and Yuichi Sugiyama
Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan

Introduction: BSEP mediates the biliary excretion of bile acids in an ATP-dependent manner. Reduced BSEP expression at the canalicular membrane can lead to BSEP dysfunction, ultimately inducing or deteriorating intrahepatic cholestasis. Although the rapid internalization and subsequent degradation of BSEP has been suggested to be responsible for the reduced BSEP expression under cholestatic conditions, the underlying molecular mechanism has not yet been clarified. Our current study explored the molecular mechanism of BSEP internalization. We specifically focused on AP2, which helps clathrin-mediated internalization by recruiting targeted protein into clathrin coated pits, since BSEP has several potential recognition sites for AP2 in its cytosolic region.

Methods: 3 x FLAG-BSEP-expressing HeLa cells and rat liver were used to investigate the involvement of AP2 in BSEP internalization.

Results: Studies using immunostaining and co-immunoprecipitation showed the colocalization of BSEP with α-subunit of AP2 (AP2α) on the plasma membrane in 3 x FLAG-BSEP-expressing HeLa cells and the canalicular membrane in rat hepatocytes as well as interaction between BSEP and AP2α, suggesting the plasma membrane as a potential site of interaction. Biotinylation study demonstrated that 3 x FLAG-BSEP expression at the cell surface was markedly enhanced in clathrin heavy chain knockdown HeLa cells and AP2 function-deficient HeLa cells, which were constructed by siRNA-mediated depletion of AP2α. In addition, the internalization rate of 3 x FLAG-BSEP was significantly slower in AP2 function-deficient HeLa cells than in control cells by almost 85%.

Discussion/Conclusion: AP2 mediates clathrin-mediated internalization of BSEP through the direct interaction with BSEP, and thereby negatively modulates BSEP expression at the cell surface.

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Faecal bile acid pattern in interleukin-10 deficient and wildtype mice

Sylvia Keller[1], Steffen Wohlgemuth[2], Gunnar Loh[2], Romy Kertscher[1], Michael Blaut[2], Gerhard Jahreis[1]
[1]Friedrich Schiller University, Institute of Nutrition, Dornburger Str. 24, 07743 Jena, Germany
[2]German Institute of Human Nutrition, Department of Gastrointestinal Microbiology, Arthur-Scheunert-Allee 114–116, 14558 Nuthetal, Germany

Introduction: Due to a correlation between immune-mediated inflammatory bowel diseases and the intestinal microbiota, the faecal bile acid (BA) pattern in interleukin-10 (IL10) deficient and wildtype (WT) mice was analysed.

Methods: The faeces of five 129/SvEv interleukin-$10^{-/-}$ deficient mice and wildtype mice were analysed for the faecal dry matter and the faecal BA pattern. On the basis of the concentrations of cholic acid (CA), deoxycholic acid (DCA) and 12keto-deoxycholic acid (12keto-DCA), the conversion rate from primary to secondary BAs was determined.

Results: IL10-deficient mice had a significantly lower faecal dry matter compared with WT mice (33.0 ± 4.5 vs. 42.2 ± 0.7%, $p < 0.05$). The total BA concentration in fresh faeces was significantly lower in IL10-deficient mice compared with WT mice (0.11 ± 0.03 vs. 0.22 ± 0.05 mg/g fresh faeces, $p < 0.05$). However, the total BA concentration in the faecal dry matter did not differ between both mouse models. The microbial CA conversion was significantly reduced in IL10-deficient mice compared with WT mice (42 ± 20 vs. 82 ± 3%, $p < 0.05$).

Discussion/Conclusion: In comparison with faeces of WT mice, the faeces of IL10-deficient mice is characterised by a lower faecal dry matter and a diminished microbial conversion of CA in their secondary metabolites DCA and 12keto-DCA. The differences in the faecal BA pattern of IL10-deficient mice might be caused by a colonic dysfunction in the removal of water combined with an imbalanced intestinal microbiota.

Effect of mitochondrial genome depletion in bile acid-induced regulation of ABC transporters in Hepa1-6 mouse hepatoma cells

R.I.R. Macias[1], M.J. Perez[2], E. Gonzalez-Sanchez[1], A. Gonzalez-Loyola[1], R. Rosales[1], M.R. Romero[1], M.A. Serrano[1], J.J.G. Marin[1]
[1]Laboratory of Experimental Hepatology and Drug Targeting, CIBERehd, University of Salamanca. 37007 Salamanca, Spain
[2]Research Unit, University Hospital, Salamanca, Spain

Introduction/Aims: Mitochondria is involved in the toxicity of several compounds and in the control of defence mechanisms against chemical stress and induction of apoptosis. The role of the genoma of these organelles in the control of ABC proteins expression in response to bile acids and acetaminophen (APAP) was investigated.

Methods/Results: Wild type (WT) and Rho Hepa1-6 mouse hepatoma cells with steady-state depletion in mtDNA (-70% as revealed by 16S/18S rRNA ratio) were used. Basal reactive oxygen species (ROS) generation and spontaneous apoptosis were decreased in Rho cells, which were more resistant to glycochenodeoxycholic acid (GCDCA)- and APAP-induced cell death. As compared with WT cells, in Rho cells, GCDCA and Fas were weaker activator of apoptosis whereas GCDCA and APAP were weaker stimulators of ROS production. Mdr1 was significantly up-regulated in Rho cells. In WT but not in Rho cells, treatment with GCDCA enhanced Mdr1 expression. In contrast, basal expression of Mrp1 and Mrp4 was similar in WT and Rho cells, whereas, only in WT cells GCDCA and APAP induced up-regulation of both ABC proteins together with Shp and Nrf2, but not Fxr or Pxr. Increased expression of Nrf2 was accompanied by enhanced translocation into the nucleus. Under glycoursodeoxycholic acid treatment no change in any of the parameters assayed was observed.

Discussion/Conclusion: The ability of toxic bile acids and APAP to induce up-regulation of certain ABC proteins depends on the integrity of mitochondria genome, which affects ROS production, induction of apoptosis and signalling pathways involving Shp and Nrf2.

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New fluorescent bile acid tools for probing bile acid trafficking

Ferenc Májer, Ruchika Sharma, John F. Gilmer
School of Pharmacy and Pharmaceutical Sciences, Trinity College, Dublin 2, Ireland

Introduction: Deoxycholic acid (DCA) is a putative promoter of intestinal carcinogenesis. Conversely, ursodeoxycholic acid (UDCA) is cytoprotective and is used for treatment of hepatic inflammatory diseases. Our group is interested in the contrasting roles these bile acids play in the pathogenesis of oesophageal cancer. To this end we have made a variety of observations regarding bile acid induced cytotoxicity, activation of the glucocorticoid receptor by UDCA and induction of Golgi fragmentation by DCA (1). A puzzling feature of UDCA and DCA is that they have interacting effects when introduced into cell suspensions. It would be useful in this context to know more about their relative distribution intracellularly.
The purpose of this project was to synthesise and characterise fluorescent UDCA and DCA derivatives that could be used to determine the intracellular distribution of DCA and UDCA in support of mechanistic investigations. A requirement of such analogs would be cellular stability, similarity in chemical character to the bile acids they are intended to mimic and lack of cytotoxicity.

Methods: We have designed and synthesised A-ring modified bile acids incorporating Dansyl- and NBD fluorescent derivatives at C-3(N-alpha and N-beta). We have also prepared a non acidic 24-NBD UDCA analog.
The novel fluorescent compounds were assessed in esophageal (HET-1A) and hepatic (HUH-7) cell lines using a combination of GE IN Cell Analyser 1000 and confocal microscopy. Competition experiments were performed using the native bile acids and the temperature dependence of uptake was assessed. Compound stability was assessed following incubation in cell suspensions by a validated HPLC method. Cytotoxicity was assessed using the MTT assay.

Results: The Dansyl and NBD compounds were stable. The NBD compounds were rapidly taken up by both cell types. The NBD compounds associated with intracellular vesicles (0.1–10 μM) the appearance of which was not structure dependent. The Dansyl derivatives were also rapidly taken up but were diffuse in the cell. Their distribution differed to the NBD compounds and was structure dependent. The Dansyl compounds were found generally close to the nucleus. The UDCA analogs partitioned into the nucleus whereas the DCA analogs tended not to. In the esophageal cell line, pre-treatment with DCA attenuated UDCA nuclear entry.

Discussion/Conclusion: The presentation will focus on the distribution of these novel fluorescent analogs in these cell types and their potential in mechanistic studies involving DCA and UDCA.

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Isolation, identification and comparative study of bile acids in the capuchinbird *(Perissocephalus tricolor)* and bare-throated bellbird *(Procnias nudicollis)*

Shoujiro Ogawa[1], Yuuki Adachi[1], Takashi Iida[1], Mizuho Une[2], Nariyasu Mano[3], Junichi Goto[3], Matthew D. Krasowski[4], Lee R Hagey[5], Alan F. Hofmann[5]
[1]Department of Chemistry, College of Humanities and Sciences, Nihon University, Tokyo, Japan
[2]Faculty of Pharmaceutical Sciences, Hiroshima International University, Kure, Japan
[3]Department of Pharmaceutical Sciences, Tohoku University Hospital, Sendai, Japan
[4]Department of Pathology, University of Iowa Hospital and Clinics, Iowa City, IA, USA
[5]Department of Medicine, University of California, San Diego, CA, USA

Introduction: C_{24} and C_{27} bile acids, together with C_{27} bile alcohols, are the predominant metabolites of cholesterol in most vertebrates. The species differences in the bile acid and bile alcohol metabolism of vertebrates are of particular interest from the view point of their physiological functions, as well as phylogenetic significance. We report here the isolation and identification of major, unique bile acids present in the gallbladder bile of two birds, the capuchinbird *(Perissocephalus tricolor)* and bare-throated bellbird *(Procnias nudicollis)*, both of which are in the Cotingigidae family.

Methods: The bile of the vertebrates was applied to a Sep-Pak tC_{18} cartridge and then individual bile acids were isolated by C_{18} reversed-phase HPLC with an ELSD. The structures of the isolated components were elucidated by LC-MS/MS with an ESI probe and 2D-NMR techniques.

Results and Discussion:

Capuchin bird: HPLC analysis of the bile acid fraction obtained from the gallbladder bile of the capuchin bird showed four major peaks, which were designated as compounds A (67%), B (4%), C (14%) and D (4%). Based on the *m/z* values of the deprotonated molecules in the LC-ESI-MS, peak A and peak B were estimated to be trihydroxylated C_{27} bile acid taurine conjugates and peaks C and D to be dihydroxylated C_{27} bile acid taurine conjugates.

Bare-throated bellbird: HPLC analysis of the bile acid fraction obtained from the gallbladder bile of the bellbird showed three major peaks, which were corresponded with retention times of the peaks A (4%), C (80%), and D (15%) in the capuchin bird's bile. Further confirmatory evidence for the detailed structures of these compounds is now being conducted.

Src kinase Fyn mediates hyperosmolarity-induced Mrp2- and Bsep-retrieval from the canalicular membrane

Miriam Cantore*, Martin Becker*, Roland Reinehr and Dieter Häussinger
(*shared first authorship)
Clinic for Gastroenterology, Hepatology and Infectiology; Heinrich-Heine-University Düsseldorf, Germany

Introduction: In perfused rat liver, hyperosmolarity induces cholestasis (Häussinger et al., Biochem J 1992) due to Mrp2- (Kubitz et al., Gastroenterology 1997) and Bsep- retrieval (Schmitt et al., Hepatology 2001) from the canalicular membrane. The underlying molecular mechanisms remained unclear.

Methods: Rat livers were perfused with either normo- (305 mosmol/L) or hyperosmotic medium (385 mosmol/L). Liver tissue was obtained for immunohistochemical Mrp2- and Bsep-staining and analzyzed automatically for transporter localization. In addition, activation of Src-kinases c-Src, Fyn and Yes was determined by use of Western blot analysis.

Results: Hyperosmolarity induced within 30 min an activating phosphorylation of the Src-kinases Fyn and Yes in perfused rat liver, while no activation of c-Src occurred. In addition, hyperosmolarity led within 30 min to a significant retrieval of Mrp2 and Bsep from the canalicular membrane. Both, hyperosmolarity-induced transporter retrieval and Src-kinase activation was sensitive to N-acetylcysteine (NAC; 10 mmol/L), apocynin (300 µmol/L) and SU6656 (1 µmol/L) indicating an involvement of NADPH-oxidase-driven reactive oxygen species (ROS) formation. Since Src-kinase inhibitor SU6656 blocks Yes and Fyn, PP-2, another Src-kinase inhibitor which blocks Fyn and c-Src only, was used in order to determine whether Fyn and/or Yes are involved. PP-2 (250 nmol/L) inhibited hyperosmolarity-induced Fyn phosphorylation as well as transporter retrieval from the canalicular membrane, whereas Yes activation still occurred under these conditions. This data suggest an involvement of Fyn, but not Yes, in this setting.

Discussion/Conclusion: In perfused rat liver, hyperosmolarity induces NADPH-oxidase-driven ROS formation which mediates a Fyn-dependent retrieval of Mrp2 and Bsep from the canalicular membrane.

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Investigation of bile acid circadian rhythm using liquid chromatography-tandem mass spectrometry

Carine Steiner[1], Ines Burkard[1], Arnold von Eckardstein[1], Katharina Rentsch[1]
[1]Institute for Clinical Chemistry, University Hospital Zürich, Rämistr. 100, 8091 Zürich, Switzerland

Introduction: It is known that bile acid levels in human undergo diurnal variation. However, most previous studies report quantification of total or unconjugated bile acids. In order to evaluate the effect of concentration changes due to circadian rhythm between samples from various patients taken at different sampling times, the circadian profile for the 15 major human bile acids was described in 4 healthy volunteers during 24 hours. Because bile acids undergo enterohepatic circulation, assessment of the bile acid precursor 7alpha-hydroxy-4-cholesten-3-one (C4) may be used in some studies to reflect bile acid biosynthesis and therefore to differentiate between newly synthesised bile acids and bile acids reabsorbed from the intestine. The circadian rhythm of C4 was also described in all 4 volunteers.

Methods: The 15 major human bile acids (cholic acid, chenodeoxycholic acid, deoxycholic acid, lithocholic acid and ursodeoxycholic acid with their glycine- and taurine-conjugates) as well as their precursor C4 were quantified in serum using two methods based on liquid chromatography-tandem mass spectrometry. This technique allows sensitive quantification of compounds in the micromolar range and sufficient specificity in order to differentiate between the isomers present in the bile acid pool.

Results: Concentration profiles of unconjugated bile acids showed considerable interindividual variation with maximum concentrations in the early morning. In contrast, the concentrations of the glycine- and taurine-conjugates seemed to depend on food intake, which is in agreement with previously reported data. C4 concentrations showed a maximum in the late evening.

Discussion/Conclusion: Our methods enabled the description of the differentiated circadian rhythm for all of the referred analytes. These results allowed us to define the time interval with the least intraindividual fluctuations, which was set between 4 pm and 7 pm. All blood samples should ideally be collected within this time frame in order to allow relevant comparisons between sample concentrations.

Dynamics of the enterohepatic circulation of bile acids in mice

F. Stellaard[1], M.Y.M. van der Wulp[2,3], H. Adema[1], R. Boverhof[1], A.K. Groen[1,2]
Departments of [1]Laboratory Medicine and [2]Pediatrics, University Medical Center Groningen, University of Groningen, The Netherlands and [3]TI Food and Nutrition, Wageningen, The Netherlands

Introduction: Extensive research on bile acid metabolism is performed in genetically modified mouse models, mainly focused on gene and protein expression levels of enzymes and transporters involved in bile acid (BA) metabolism. The genes involved are regulated by the flux of BA through the enterohepatic circulation (EHC). However, little is known about the movement of BA through the EHC in the mouse. Therefore, we aimed to study BA transit through the EHC in fed and fasted mice.

Methods: 2,2,4,4-D_4-cholic acid (D_4-CA) was intravenously administered to male C57BL/6J mice in the fed and 24 h fasted states (7 am). The progression of D_4-CA was monitored in compartments of the EHC (gallbladder, liver, small intestine in 3 parts, cecum, colon) 5, 15, 30 and 60 minutes after administration (N = 3 per time point).

Results: After 5 minutes conjugated D_4-CA molecules were already present in the gallbladder and proximal third of the small intestine. After 60 minutes the majority was located in the mid and distal thirds. No D_4-DCA appearance could be detected in the cecum or colon within 60 min. After 5 minutes unconjugated D_4-CA was present in the lumen of all three segments and in the mucosa of the small intestine, suggesting direct transport from blood to the intestinal lumen. In the fasted state gallbladder weight was 4x heavier than in the fed state. Compared to the gallbladder, the intestinal lumen contained more D_4-CA in both the fed and fasted state. After 60 min the liver still contained 30% of the D_4-CA amount present after 5 min.

Discussion/Conclusion: In C57BL/6J mice gallbladder storage of bile acids is incomplete. Hepatic transport determines the transit of BA molecules through the EHC. Transintestinal transport of unconjugated CA appears possible. EHC transit lasts more then 1 hour.

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The circadian rhythm of bile acid synthesis in man is eradicated by chronic but not by single day treatment with cholestyramine. Importance of bile acids for circadian regulation of CYP7A1

S. Straniero, B. Sjöberg, B. Angelin, M. Rudling
Department of Endocrinology, Metabolism & Diabetes, Karolinska Institute M63, Karolinska Univ Hospital, Huddinge, 141 86 Stockholm, Sweden

Introduction: Human BA synthesis has a diurnal rhythm with two peaks at 1 pm and 10 pm while cholesterol synthesis has a diurnal rhythm peaking at 3 am. We evaluated if resin-induced interference with BAs in the enterohepatic circulation may be important for the circadian rhythm for BA synthesis.

Methods: Normal subjects were sampled during a 33 h period (day 0 and 1) 4 g of cholestyramine was taken with each meal day 0 (4 g x 4). On day 3 and 4 fasting morning samples were taken. The effect of chronic resin treatment was made during resin treatment after 3 weeks of pretreatment.

Results: Single day resin treatment d 0 induced BA synthesis 4-fold day 1 whereas the circadian rhythm of BA synthesis persisted day 1. In contrast, chronic on going resin treatment diminished the diurnal rhythm of BA synthesis.

Discussion/Conclusion: The resin-induced increase of BA synthesis per se does not alter the circadian rhythm of hepatic BA synthesis. However, when resin should be present in the small intestine, the normal 1 pm peak in BA synthesis is diminished. Overall the results suggest that circulating BAs should be an important driving force for the circadian rhythm of BA synthesis.

Folding defects in ATP8B1 associated with hereditary cholestasis are ameliorated by 4-phenylbutyrate

Lieke M. van der Velden[1], Janneke M. Stapelbroek[2], Elmar Krieger[3], Peter V. van den Berghe[1], Ruud Berger[1], Patricia M. Verhulst[4], Joost C. Holthuis[4], Roderick H. Houwen[2], Leo W. Klomp[1*], Stan F. van de Graaf[1*]
[1]Department of Metabolic and Endocrine Diseases, UMC Utrecht, The Netherlands
[2]Department of Pediatric Gastroenterology, UMC Utrecht, The Netherlands
[3]Centre for Molecular and Biomolecular Informatics, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, The Netherlands
[4]Department of Membrane Enzymology, Bijvoet Center and Institute of Biomembranes, Utrecht University, The Netherlands

Introduction: ATP8B1 deficiency is a severe and clinically highly variable hereditary disorder that is primarily characterized by intrahepatic cholestasis. It presents either as a progressive (progressive familial intrahepatic cholestasis type 1; PFIC1) or intermittent (benign recurrent intrahepatic cholestasis type 1; BRIC1) disease. ATP8B1 deficiency is caused by autosomal recessive mutations the gene encoding ATP8B1, a putative aminophospholipid-translocating P-type ATPase. The exact pathogenesis of the disease is elusive, and no effective pharmacological therapy is currently available. Here, the molecular consequences of six distinct *ATP8B1* missense mutations (p.L127P, p.G308V, p.D454G, p.D554N, p.I661T, p.G1040R) and one nonsense mutation (p.R1164X) associated with PFIC1 and/or BRIC1 on ATP8B1 expression were systematically characterized.

Methods: ATP8B1 expression was quantified by RT-PCR and Western blot analysis. ATP8B1 localization was determined by immunofluorescence in U2OS cells and plasma membrane abundance quantified by cell surface biotinylation.

Results: Except for the p.L127P mutation, all mutations resulted in markedly reduced ATP8B1 protein expression, whereas mRNA expression was unaffected. Five out of seven mutations resulted in (partial) retention of ATP8B1 in the endoplasmic reticulum (ER). Reduced protein expression was partially restored by culturing the cells at 30°C and by treatment with proteasomal inhibitors, indicating protein misfolding and subsequent proteosomal degradation. Protein misfolding was corroborated by predicting the consequences of most mutations onto a homology model of ATP8B1. Treatment with 4-phenylbutyrate (4-PBA), a clinically approved pharmacological chaperone, partially restored defects in expression and localization of ATP8B1 G308V, D454G, D554N and in particular I661T, the most frequently identified mutation in BRIC1.

Discussion/Conclusion: A surprisingly large proportion of *ATP8B1* mutations resulted in aberrant folding and decreased expression at the plasma membrane. These effects were partially restored by treatment with 4-PBA. We propose that treatment with pharmacological chaperones may represent an effective therapeutic strategy to ameliorate the recurrent attacks of cholestasis in BRIC1 patients.

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Oligomerization of the human liver Na^+-dependent taurocholate cotransporting protein NTCP provides a novel mechanism to regulate bile salt uptake

Stan F.J. van de Graaf[1], Joachim Geyer[2], Rianne A.M Bouwmeester[1], Klaas-Nico Faber[3], Leo W.J. Klomp[1]
[1]Department of Metabolic and Endocrine Diseases, UMC Utrecht, The Netherlands
[2]Institute of Pharmacology and Toxicology, Justus Liebig University of Giessen, Germany
[3]Department of Gastroenterology and Hepatology, UMC Groningen, The Netherlands

Introduction: The Na^+ taurocholate cotransporting protein (NTCP) is the primary mediator of bile salt absorption from the portal circulation into the hepatocyte. NTCP (or SLC10A1) is part of the SLC10A protein family, consisting of seven members. SLC10A2 mediates the majority of intestinal bile salt reabsorption. With the exception of SLC10A6, which transports steroid sulfates, the substrates and physiological functions of the other SLC10A family members are unknown. The aim of the present study was to investigate the interdependence of SLC10A proteins in the regulation of bile salt uptake.

Methods: Protein interactions were determined by co-immunoprecipiation, chemical cross-linking and by Fluorescence Resonance Energy Transfer (FRET) using ACP-labeled NTCP subunits. Bile salt uptake was quantified using ^3H-labeled taurocholate. Protein localization was determined by immunofluorescence and plasma membrane abundance quantified by cell surface biotinylation

Results: Interaction between individual NTCP subunits was investigated by co-immunoprecipitation of HA- and FLAG-tagged NTCP subunits, which revealed the oligomeric nature of NTCP. Chemical cross-linking and immunoblot analysis of rat hepatocyte membranes demonstrated a preference for dimeric complexes. NTCP lacking the carboxyl-terminus was poorly targeted to the plasma membrane and was retained in the endoplasmic reticulum (ER). Furthermore, co-expression of this truncated NTCP retained wild-type NTCP in the ER in a dominant fashion, suggesting that oligomerization occurs early in the secretory pathway. FRET results further demonstrated that the oligomerization persists at the plasma membrane. Next, immunoprecipitations using lysates of cells co-expressing NTCP and SLC10A family members demonstrated that NTCP can form heteromeric complexes with several of its family members. Importantly, expression of SLC10A6 or SLC10A7 resulted in a significant reduction of NTCP-mediated taurocholate uptake.

Discussion/Conclusion: These data demonstrate that NTCP adopts a dimeric structure architecture. Furthermore, NTCP hetero-oligomerization with SLC10A family members provides a putative novel regulatory mechanism to fine-tune bile salt uptake.

Heteromeric interactions required for abundance and subcellular localization of human CDC50 proteins and class 1 P_4 ATPases

Lieke M. van der Velden, Catharina G.K. Wichers, Adriana E.D. van Breevoort, Ruud Berger, Leo W.J. Klomp and Stan F.J. van de Graaf
Department of Metabolic and Endocrine Diseases, UMC Utrecht, and Netherlands Metabolomics Centre, Utrecht, The Netherlands

Introduction: Members of the P_4 family of P-type ATPases (P_4 ATPases) are believed to function as phospholipid flippases in complex with CDC50 proteins. Mutations in the human class 1 P_4 ATPase gene *ATP8B1* cause a severe syndrome characterized by impaired bile flow (intrahepatic cholestasis) often leading to end-stage liver failure in childhood. This suggests that loss of ATP8B1 function cannot be compensated for by other P_4 ATPase family members. To adress this in more detail, we determined the specificity of human class 1 P_4 ATPases interactions with CDC50 proteins and the functional consequences of these interactions on protein abundance and localization of both protein classes.

Methods: Interaction between CDC50 proteins and P_4-ATPases was determined by co-immunoprecipitation. Protein expression was quantified Western blot analysis. P_4-ATPase localization was determined by immunofluorescence in U2OS cells and plasma membrane abundance quantified by cell surface biotinylation.

Results: ATP8B1 and ATP8B2 co-immunoprecipitated with CDC50A and CDC50B, while ATP8B4 and ATP8A1 only associated with CDC50A. ATP8B1 shifts from ER to the plasma membrane upon coexpression of CDC50A or CDC50B. ATP8A1 protein translocated from the ER to the Golgi and plasma membrane upon coexpression of CDC50A, but not CDC50B. ATP8B2 and ATP8B4 already displayed partial plasma membrane localization in the absence of CDC50 coexpression but displayed a large increase in plasma membrane abundance upon coexpression of CDC50A. ATP8B3 did not bind CDC50A and CDC50B and was invariably present in the ER.

Discussion/Conclusion: Our data shows that interactions between CDC50 proteins and class 1 P_4 ATPases are essential for ER exit and stability of both subunits. Furthermore, the subcellular localization of the complex is determined by the P_4-ATPase, not the CDC50 protein. The interactions of CDC50A and CDC50B with multiple members of the human P_4 ATPase family suggest that these proteins perform broader functions in human physiology than thus far assumed.

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Development of a FRET-based sensor for intracellular bile salt detection

Misha V. Golynskiy[1], Saskia W. van Mil[2], Leo W. Klomp[2], Stan F. van de Graaf[2], Maarten Merkx[1]
[1]Laboratory of Chemical Biology, Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands
[2]Department of Metabolic and Endocrine Diseases, UMC Utrecht, The Netherlands

Introduction: The nuclear receptor FXR regulates transcription of proteins involved in bile acid, lipid and cholesterol homeostasis in response to changes in intracellular bile acid concentration. In this study, we used FXR to engineer a single-chain fluorescent biosensor based on Förster Resonance Energy Transfer (FRET) to detect bile salts and synthetic FXR ligands. Wide applicability of this sensor in bile salt-related research is anticipated, e.g. high-throughput screening for novel agonists of FXR, bile salt dynamics in subcellular compartments, and bile salt detection in serum or urine of cholestatic patients.

Methods: The biosensor employs the ligand-dependent interaction between the ligand-binding domain of FXR and an LXXLL motif from the coactivator NCOA2 as input and the energy transfer between Cerulean and Citrine fluorescent proteins as signal output.

Results: Binding of the purified sensor protein to cholic and chenodeoxycholic acids (CA and CDCA), natural ligands of FXR, as well as to GW4064, a synthetic agonist of FXR resulted in a robust change in emission ratio, readily detectable by fluorescence microscopy and FACS. The sensor binds both natural and synthetic ligands, with micromolar and submicromolar affinities, respectively. We next transfected the sensor and controls (non-binding mutants) into HEK293 and U2OS cells and verified the expression of the sensor in different cell organelles using different localization signals. Furthermore, we demonstrate that the sensor, but not the non-binding mutants, is capable of intracellular binding of CDCA and GW4064 in a dose- and time-dependent manner. Finally, we showed that this sensor can be used to report on the functionality of bile acid transporter proteins by monitoring uptake of glyco-CDCA via the bile salt importer NTCP.

Discussion/Conclusion: This study implies that the FXR-based bile salt sensor is suitable not only for high-throughput screening of FXR ligands, but also for intracellular characterization of functionally-compromised mutant variants of transport proteins involved in bile-salt homeostasis. We expect that this sensor will have future applications for studying bile salt-related pathologies.

Abcb11 (Bsep) overexpression in mice affects the entero-hepatic circulation of bile salts

Henkjan J. Verkade[1], Rick Havinga[1], Renze Boverhof[1], Richard M. Green[2], Anne Henkel[2]
[1]Pediatric Hepatology, Beatrix Children's Hospital, University Medical Center Groningen, The Netherlands
[2]Department of Medicine, Division of Hepatology, Northwestern University, Chicago, IL, USA

Introduction: The Bile Salt Export Pump (Abcb11) is the predominant canalicular transport protein for biliary bile salt (BS) secretion. Genetically inactive Abcb11 results in interruption of the enterohepatic circulation (EHC) of BS, causing Progressive Familial Intrahepatic Cholestasis type 2. It is unclear whether overexpression of Abcb11 also affects the EHC.

Aim: To determine the effect of Abcb11 overexpression on the EHC in mice.

Methods: We used a stable isotope dilution technique in transgenic mice overexpressing hepatic Abcb11 (TTR-Abcb11) and C57BL/6J controls to determine the pool size, fractional turnover rate (FTR) and synthesis rate of the primary BS cholate (CA) under steady state conditions without interruption of the EHC. Afterwards, the gallbladder was cannulated to determine the bile flow, BS composition, and the biliary secretion rates of CA, total BS, phospholipid and cholesterol. The combined data allowed for calculation of the CA cycling time and the fraction of CA lost per cycle. Hepatic and intestinal gene expression was determined by quantitative PCR.

Results: Abcb11 overexpression did not significantly affect bile flow or biliary secretion rates of total BS, CA, phospholipid, or cholesterol. Abcb11 overexpression slightly increased the contribution of CA (73%; +5%) and deoxycholate (5%, +3%) at the expense of chenodeoxycholate (3%, -1%) and β-muricholate (7%, -5%; each $p < 0.05$). The pool size of CA was similar in TTR-Abcb11 and controls (22.5 ± 5.9 vs 27.7 ± 6.7 µmol/100 g BW, NS), as was the cycling time (6.1 ± 3.1 vs. 6.1 ± 1.8 h, NS). Yet, TTR-Abcb11 mice had a strongly decreased FTR (-41%, $p < 0.001$) and synthesis rate (-28%, $p < 0.01$) of CA. Abcb11 overexpression decreased the fraction of CA that was lost per cycle of the EHC (7 ± 3% vs 12 ± 6% in controls; $p < 0.05$). Hepatic expression of CYP7a1, the rate-limiting enzyme for bile acid synthesis, was suppressed > 50% in TTR-Abcb11 mice (0.5 ± 0.4 vs 1.1 ± 0.5 in controls; $p < 0.05$). Ileal expression of FGF15, the primary mediator of bile salt feedback inhibition of CYP7a1, was increased in TTR-Abcb11 mice (8.3 ± 4.4 vs 1.1 ± 0.5 in controls; $p < 0.05$). Jejunal expression of FGF15 was > 10-fold higher in TTR-Abcb11 mice (1.2 ± 1.3 vs 0.1 ± 0.1 in controls; $p < 0.05$). Abcb11 overexpression did not alter intestinal expression of ASBT or IBABP.

Discussion/Conclusion: Abcb11 overexpression in mice profoundly increases the conservation of bile salts within the enterohepatic circulation. Present data provide strong evidence for the existence of feed-forward communication between hepatic expression of a bile salt transport protein and the intestine.

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Endocytosis of sodium taurocholate cotransporting polypeptide (Ntcp) as a protective mechanism against toxic bile acid levels

Katrin Weissenberger, Claudia Stross, Angelika Helmer, Dieter Häussinger, Ralf Kubitz
Department of Gastroenterology, Hepatology and Infectiology, University Hospital Düsseldorf, Germany

Introduction: The sodium taurocholate cotransporting polypeptide (Ntcp) is the main basolateral uptake transporter for conjugated bile salts from portal blood. Ntcp regulates serum as well as intracellular bile salt concentrations in hepatocytes. During cholestasis Ntcp is down-regulated, thereby reducing bile salt uptake which may protect hepatocytes from toxic bile salt concentrations.

Methods: Ntcp was fused to an intracellular green fluorescent protein additionally to the FLAG peptide on its extracellular domain and stably expressed in the human hepatoma cell line HepG2 and it was shown recently that PKC induces endocytosis of Ntcp. Localization of Ntcp and cell responses to bile salts were evaluated by flow cytometry, immunofluorescence and live cell imaging.

Results: Taurolithocholate (TLC), taurodeoxycholate (TDC), taurochenodeoxycholate (TCDC), taurolithocholic acid-3 sulfate (TLCS), deoxycholate (DC) and glycochenodeoxycholate (GCDC) lead to a dose and time dependent increase in cell death in Ntcp-transfected HepG2-cells. Wild-type HepG2-cells are almost resistant to TLCS and GCDC-induced cell damage, suggesting that their effects are mediated by Ntcp-dependent uptake. Accordingly, induction of Ntcp endocytosis by a 60 minutes preincubation with the PKC activator PMA abolishes the adverse effects of TLCS and GCDC in a BIM I-sensitive manner. PMA-induced Ntcp endocytosis is associated with a reduced number of damaged cells and with preserved cell morphology.

Discussion/Conclusion: Our findings demonstrate that short-term endocytosis of Ntcp protects hepatocytes from bile salt induced cell damage by reducing bile acid uptake.

PKC mediated endocytosis and subsequent lysosomal degradation of the sodium taurocholate cotransporting polypeptide (Ntcp)

Claudia Stross, Katrin Weissenberger, Angelika Helmer, Boris Görg,
Dieter Häussinger, Ralf Kubitz
Department of Gastroenterology, Hepatology and Infectiology, University Hospital Düsseldorf, Germany

Introduction: The sodium taurocholate cotransporting polypeptide (Ntcp) is the major uptake system for conjugated bile salts in the liver. It is constitutively expressed in hepatocytes but its expression can be influenced by exposure to e.g. bile salts and proinflammatory stimuli. At the posttransciptional level, exocytosis of Ntcp stored in intracellular vesicles, has been shown and proposed to adjust transport capacity to varying serum bile acid concentrations.

Methods: Ntcp was cloned with a N-terminal FLAG- and a C-terminal EGFP-tag. Stably transfected HepG2 cells were used to study Ntcp endocytosis via flow cytometry, TIRF (total internal reflection microscopy), immunofluorescence and Western blot analyses.

Results: We could show vesicular retrieval of Ntcp in response to activation of protein kinase C (PKC). This process is selective, not affecting the Na^+/K^+-ATPase, and is not caveolin mediated. Phorbol-12-myristate-13-acetate (PMA) as well as the classical (c)PKC selective activator thymeleatoxin (TTX) lead to a 30–40% decrease in membrane expression. Furthermore, the cPKC selective inhibitors Gö6976 and Ruboxistaurin abolish PMA induced endocytosis of Ntcp. After prolonged PKC activation (2–4 h) endocytosed Ntcp colocalizes with the lysosomal marker Lamp-1. In line with a lysosomal targeting an approximately 26% reduction in protein levels is measured 4 h after PMA stimulation which is sensitive to bafilomycin A1, an inhibitor of the vesicular type H^+-ATPase.

Discussion/Conclusion: Activation of classical PKCs induces endocytosis of Ntcp and subsequent lysosomal degradation. This mechanism may adjust cellular transport capacity on a long- and short-term scale and may protect hepatocytes from toxic intracellular bile salt concentrations.

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A functional klotho-beta polymorphism associates with colonic transit in health and in functional gastrointestinal disorders

Banny S. Wong, Michael Camilleri, Paula J. Carlson, Maria E. Guicciardi,
Duane Burton, Sanna McKinzie, Alan R. Zinsmeister, Gregory J. Gores
Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA

Introduction: Klotho-beta (KLB) is involved in FGF19-mediated negative regulation of bile acid (BA) synthesis by hepatocytes. $KLB^{-/-}$ mice show elevated BA synthesis and excretion. BA diarrhea has been reported in 20–50% of functional diarrhea or IBS with diarrhea (IBS-D) patients. We aimed to determine whether single nucleotide polymorphisms (SNPs) in KLB and 6 other genes involved in BA homeostasis are associated with GI functions and symptoms in health and functional gastrointestinal disorders (FGID) in humans, and if so, the functional significance of such SNP(s).

Methods: Fifteen nonsynonymous or tag SNPs within or near coding sequences of candidate genes were chosen based on 9% minimum minor allele frequency. 465 FGID and 231 healthy subjects were genotyped. Associations between genotypes and gut physiology measured by validated methods were examined using ANCOVA with false discovery rate (FDR) adjustment for multiple comparisons. Functional analysis of the allelic variants of SNP rs17618244 in KLB involved cycloheximide protein stability assay via transient transfection into HEK293 cells.

Results: KLB SNP rs17618244 (Arg728Gln) significantly associated with colonic transit. In dominant genetic modeling, minor A allele (Gln728), compared to major G allele (Arg728), associated with delayed colonic transit ($p = 0.0033$ for geometric center at 24 hours (GC24), $p = 0.0018$ for GC48 [FDR $p < 0.05$ for both]). This delay was present in all subgroups and remained significant in the healthy and IBS-D subgroups. No associations were found in other SNPs tested. Protein stability assay revealed KLB Gln728's half life to be significantly longer than KLB Arg728.

Discussion/Conclusion: KLB Gln728 is associated with increased protein stability and delayed colonic transit in health and FGID relative to KLB Arg728. KLB Gln728 protein's relative longevity may promote FGF19-mediated suppression of BA synthesis in a dominant fashion. The mechanism by which this KLB variant affects colonic transit and susceptibility to colonic dysmotility deserves further study.

Increased degradation of cholesterol via the alternate pathway of bile acid biosynthesis in primary hypercholesterolemia

M. Bertolotti, M. Del Puppo*, C. Gabbi, C. Anzivino, L. Carulli, M. Galli-Kienle*, N. Carulli
Dipartimento di Medicina, Endocrinologia, Metabolismo e Geriatria, Università di Modena e Reggio Emilia, Modena, Italy, and *Dipartimento di Medicina Sperimentale, Università degli Studi di Milano Bicocca, Monza, Italy

Introduction: The first step in the alternate biosynthetic pathway of bile acid synthesis is represented by cholesterol 27-hydroxylation (27-OH), which takes place both in liver and in extrahepatic tissues. The physiological relevance of such pathway is unknown, even if it is believed to play a role in peripheral degradation of cholesterol. Aim of the present study was to investigate the rates of cholesterol 27-hydroxylation *in vivo* in patients with primary hypercholesterolemia, and to assess the effects induced by treatment with statin drugs.

Methods: Seven patients with primary hypercholesterolemia underwent determination of 27-OH rates *in vivo* by i.v. infusion of deuterated 27-hydroxycholesterol. Isotope enrichment was assayed by GC-MS, allowing to calculate the rate of 27-OH. The data were compared with those obtained in 4 normocholesterolemic controls. In some patients the infusions were repeated during treatment with atorvastatin or rosuvastatin.

Results: The rates of 27-hydroxylation were significantly higher in untreated hypercholesterolemic patients, compared with controls (8.7 ± 2.7 mg/h vs 3.7 ± 1.2 mg/h, mean ± SD, $p < 0.01$). After treatment with statins, hydroxylation rates dropped by nearly 50% along with a drastic reduction in plasma total and LDL-cholesterol, so that the ratio between 27-hydroxylation rates and plasma cholesterol was unchanged. No difference was detectable between the two statins. Linear regression analysis showed a correlation trend between plasma cholesterol and 27-hydroxylation rates.

Discussion/Conclusion: Primary hypercholesterolemia associates with increased rates of cholesterol 27-hydroxylation, which tend to normalize during hypocholesterolemic treatment with statins. The correlation between plasma cholesterol levels and 27-hydroxylation support the view that the latter may act as a compensatory mechanism in a condition of larger plasma cholesterol pool. A regulatory role for hepatic and extrahepatic nuclear receptors seems reasonable. These data might encourage novel pharmacological approaches for the management of hypercholesterolemia and the prevention of atherosclerosis.

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Analysis of glutathione conjugates of bile acids in rat bile by LC/ESI-MS/MS

Shigeo Ikegawa[1], Naohiro Hori[1], Takashi Iida[2], Kuniko Mitamura[1]
[1]Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1 Kowakae, Higashi-Osaka, 577-8502, Japan
[2]Department of Chemistry, College of Humanities & Sciences, Nihon University, 3-25-40 Sakurajousui, Setagaya-ku, Tokyo 156-8550, Japan

Introduction: We have previously shown the biotransformation of reactive acyl-linked metabolites of bile acids (BAs) into S-acyl glutathione (GSH) conjugate in vitro and in vivo in rat. In the present studies, we examined BA-GSHs in the bile of intact rats by a sensitive LC/ESI-MS/MS method.

Methods: To a 100 µl aliquot of bile sample acidified with AcOH was added ([2,2,4,4-d_4]-deoxycholyl)-*S*-glutathione as an internal standard, and the mixture was deproteineized with MeCN (200 µl). After centrifugation at 1500 g for 5 min, the supernatant diluted with water was subjected to a solid-phase extraction. Subsequent analysis of the extracts was carried out by means of LC/ESI-MS/MS in the negative- and positive-ion modes by monitoring characteristic transition ions of the analytes.

Results: The product ion mass chromatogram monitored with the transition of $[M-H]^-$ → $[GSH-H]^-$ ions of the monohydroxy-, dihydroxy-, trihydroxy- and monohydroxyoxo-BAs showed the peaks corresponded to GSH conjuates of muricholic, cholic, deoxy-cholic, hyodexycholic, lithocholic, and 12-oxolithocholic acid. The CID spectra of the peaks associated with these peaks definitely identified by comparison of retention time and CID spectra with those of the reference compounds. Such conjugates were present at low concentrations as compared to amino acid conjugates.

Discussion/Conclusion: This is the first report on the quantitation of GSH conjugates of BAs in the bile of rat. Additional studies are required to define the significance of biliary excretion of these conjugates and the secretary process which may be mediated by distinct active carrier-mediated transport system in a polarized fashion in the hepatocytes.

Analysis of the portal solutes in course of ileal intraluminal bile acid infusion using a new model of in situ perfused rat intestine

M. Montagnani[1], M. Tsivian[2], F. Neri[2], L. Puviani[2], I. Mantovani[1], P. Nanni[3], A. Marangoni[4], M. Pariali[5], P. Simoni[1], R. Fato[6], C. Bergamini[6], S. Leoni[6], G. Lenaz[6], R. Aldini[7]
[1]Department of Internal Medicine and Gastroenterology, [2]Department of General Surgery and Transplantations, [3]Department of Chemical Pharmaceutics, [4]DMCSS, [5]Department of Oncoemathology and Pathology, [6]Department of Biochemistry "G. Moruzzi", [7]SMETEC Department, University of Bologna, Bologna, Italy

Introduction: Due to the importance of the intestinal transport in pharmacological studies and the emerging role of the intestinal signalling activity in the gut-liver axis, we have developed a new simple method to investigate the intestinal transport by means of a cell free and serum free mesenteric perfusion system. In particular, we focused on FGF15 detection in the portal venous system after bile acid infusion into the ileal segment.

Methods: After ligation of the subdiaphragmatic aorta, splenic and pyloric veins, the abdominal aorta and portal vein were cannulated. The abdominal aorta was then infused with Krebs-Ringer solution and the effluent from the portal vein was collected. The terminal ileum was infused with tauroursodeoxycholate (TUDCA) and its absorption was assessed by its recovery in the portal vein. The oxygen consumption of isolated enterocytes was measured. Histology studies were also performed. Liquid chromatography and mass spectrometry analysis were performed both on gel bands digestion products and on portal outflow samples, in order to evaluate if negligible amounts of FGF15 (up to fmoles), not detectable by western blot, were present.

Results: The intestinal preparation proved to be stable for at least one hour. TUDCA absorption was efficient and the intestinal morphology and oxygen consumption of isolated enterocytes were normal. Despite accurate analysis, we didn't find FGF15 in the portal venous system.

Discussion/Conclusion: The method proved to be reliable for studying the active bile acid absorption and possibly useful for the identification in the portal circulation of substances produced by enterocytes in response to the absorption of different molecules. Since FGF15 was not recovered, we suggest that in the rat it has mainly an autocrine activity.

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The mouse bile acid metabolome

Youcai Zhang, Curtis D. Klaassen*
Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA

Introduction: Various bile acid (BA)-supplemented diets have been fed to rodents. Individual BAs vary markedly in their physiological and pathological responses *in vivo*. Therefore, it is important to know the effect of feeding BAs on individual BA composition and concentrations in the liver.

Methods: An improved UPLC-MS/MS method was established for the simultaneous analysis of various BAs, and applied to investigate liver BA metabolism in C57BL/6 mice fed various diets supplemented with 1% cholic acid (CA), 0.3% deoxycholic acid (DCA), 0.3% chenodeoxycholic acid (CDCA), 0.3% lithocholic acid (LCA), or 3% ursodeoxycholic acid (UDCA).

Results: Mouse livers have a remarkable ability to maintain BA homeostasis. Feeding CA, DCA, CDCA, and LCA had little effect on total BAs in livers, whereas feeding UDCA markedly increased total BAs, which was due to the higher dose of UDCA compared to other BAs. The BA profiles in mouse livers were similar between feeding CA and DCA, as well as between feeding CDCA and LCA. Feeding CA suppressed both the classic and alternative pathways of BA biosynthesis, whereas feeding CDCA mainly suppressed the classic pathway. Gender differences of BA composition in mouse livers were observed after feeding CA, DCA, CDCA, and LCA, but they were not prominent after feeding UDCA. Sulfation of CA and CDCA was found at the 7-OH position, and increased by feeding CA or CDCA more in male than female mice. In contrast, sulfation of LCA and TLCA was female predominant, and increased by feeding UDCA and LCA. Glucuronidation of BAs was a minor BA metabolic pathway in mice, which was only detected after feeding UDCA.

Discussion/Conclusion: The present systematic study on liver BA metabolism and synthesis will aid in interpreting BA-mediated gene regulation, as well as hepato-toxicity and therapeutic uses of various BAs.

Sulphated progesterone metabolites attenuate FXR function

Shadi Abu-Hayyeh[1], Georgia Papacleovoulou[1], Mehreen Tahir[1], Malcolm Parker[2], Catherine Williamson[1]
[1]Maternal and Fetal Disease Group, [2]Molecular Endocrinology Laboratory, Institute of Reproductive and Developmental Biology, Imperial College London, Du Cane Road, London W12 0NN, UK

Introduction: Intrahepatic cholestasis of pregnancy (ICP) symptom severity broadly correlates with the gestational period when serum reproductive hormone levels are at their highest. Total sulphated progesterone metabolite (P4-S) levels are reported to be ≈22 μM in third trimester normal pregnancy and 65 μM in ICP. We aimed to investigate if ICP levels of P4-S can interfere with the function of the hepatic bile acid nuclear receptor FXR, and thus potentially explain cholestasis in ICP.

Methods: P4-S FXR agonist studies: 0–200 μM P4-S were screened against a FXR-reporter system and primary human hepatocytes in culture ± FXR siRNA to identify P4-S FXR agonists.
P4-S antagonism of FXR activity was assessed by co-incubating 100 μM CDCA and increasing P4-S concentrations to study FXR induction/activation profiles in: i) FXR reporter assays, ii) FXR-ligand binding domain (LBD) SRC1 cofactor recruitment assays, iii) Fao cells, studying endogenous Bsep/Shp expression levels.

Results: FXR agonism: The P4-S compounds epiallopregnanolone-sulphate, epipregnanolone-sulphate and epiallo-pregnanediol 3-sulphate activated the FXR-reporter assay system 2-, 1.5- and 2-fold respectively, transactivating FXR in a dose-dependent manner. Primary human hepatocytes ± FXR siRNA treated with 50 μM epiallopregnanolone-sulphate resulted in a FXR-dependent 2- and 1.5-fold increase in SHP and BSEP expression and a 10-fold repression of CYP7A1 expression.
FXR antagonism: Increasing epiallopregnanolone-sulphate/epipregnanolone-sulphate/epiallo-pregnanediol 3-sulphate concentration against 100 μM CDCA dose reduced CDCA activation/induction of FXR reporter assay system as well as SRC1 peptide cofactor recruitment to the FXR-LBD in a dose dependent manner. Epiallopregnanolone-sulphate IC50 values ranged from 37–74 μM in these assays. Epiallopregnanolone-sulphate antagonism of CDCA-activated endogenous FXR targets was confirmed in Fao cells; CDCA induction of Shp and Bsep expression were both attenuated in a dose dependent manner.

Conclusion: ICP levels of P4-S have weak FXR agonist activity and more importantly antagonistic activity, resulting in reduced induction of FXR bile acid target genes which may explain bile acid dysregulation in ICP.

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Histone H3K4 trimethylation (H3K4me3) at the promoter loci mediated by MLL3 as part of the ASCOM (ASC-2 containing) complex is critical for activation of bile acid transporter genes by nuclear receptors and is downregulated in a murine model of experimental cholestasis

M. Ananthanarayanan[1], Yanfeng Li[1], S. Surapureddi[2], N. Balasubramaniyan[1], J.A. Goldstein[2], Frederick J. Suchy[1]
[1]Department of Pediatrics, Mount Sinai School of Medicine, New York, NY
[2]Laboratory of Pharmacology, NIEHS, RTP, NC, USA

Introduction: Role of epigenetic modifications in normal physiology and how alteration of epigenetic signature(s) contributes to bile acid transporter dowregulation in cholestasis is unknown. Using a microarray we identified decreased expression of MLL3, a H3K4 methyl transferase at 1 and 3 days of post-CBDL in mice. Further studies using siRNA-mediated silencing, protein-protein interaction and Chromatin Immunoprecipitation (ChIP) analysis revealed that H3K4me3 of transporter promoters by MLL3 as part of ASCOM complex is essential for activation of BSEP, NTCP and MRP2 by nuclear receptors. Downregulation of this epigenetic mark leads to decreased transporter expression in experimental cholestasis.

Methods: cDNAs from total RNA of 1 and 3 day sham and CBDL livers were hybridized to a customized microarray of chromatin-modifying gene primers and quantitated by real-time PCR. Specific siRNAs against MLL3, MLL4 and nuclear receptor coactivator 6 (NCOA6, ASC-2) were used for silencing and effects on BSEP and NTCP mRNAs were investigated. FXR and NCOA6 interactions were studied. ChIP analysis of recruitment of NCOA6, MLL3 and H3K4me3 to transporter genes was assessed in HepG2 cells and mouse livers.

Results: Microarray analysis revealed that 1- and 3 days post-CBDL, MLL3 message levels were downregulated 9.1 and 2.1-fold respectively. However, mRNA levels for NCOA6 that recruits other proteins including MLL3 into the ASCOM complex was not altered during CBDL. Silencing of NCoA6 by siRNA treatment led to 55%, 40% and 50% decrease in BSEP, NTCP and NCOA6 message levels and 96% decrease in NCoA6 protein. Similarly MLL3 siRNA knockdown led to 24%, 40% and 55% decrease in BSEP, NTCP and MLL3 mRNAs and 77% decrease in MLL3 protein level. Human BSEP promoter transactivation by FXR/RXR was enhanced in a dose-dependent fashion by NCOA6 full-length cDNA coexpression in HepG2 cells. GST-pull down assays showed that Domain 3 and 5 of NCOA6 (containing LXXLL motifs) interacted with FXR with domain 5 interaction being increased by CDCA. *In vivo* ChIP assays in HepG2 cells revealed recruitment of ASCOM complex to FXRE in the BSEP promoter and GRE in the NTCP promoter. ChIP analysis of 3-day sham and CBDL mice demonstrated significantly diminished recruitment of ASCOM complex components and H3K4me3 to Bsep and Mrp2 promoter FXREs in cholestatic livers compared to sham controls.

Discussion/Conclusion: Taken together, these data show that the ‘H3K4me3’ epigenetic mark is essential to activation of BSEP, NTCP and MRP2 genes by nuclear receptors and is downregulated in cholestasis

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Nuclear receptors and obstructive cholestasis in humans. Increased hepatic expression of short heterodimer partner: A compensatory mechanism?

M. Bertolotti, C. Gabbi, C. Anzivino, E. Baldelli, E. Meschiari, M.R. Odoardi, L. Carulli, N. Carulli
University of Modena and Reggio Emilia, Modena, Italy

Introduction: Cholestasis associates with important changes in the expression of hepatobiliary transporters in liver tissue. Little is known on the role of hepatic nuclear receptors. Aim of the present study was to study the changes induced by obstructive cholestasis on hepatic expression of biliary lipid transporters, as related to the main nuclear receptors involved in sterol metabolism, in humans.

Methods: Eight subjects with obstructive cholestasis undergoing abdominal surgery were investigated, and compared with 22 untreated subjects operated for non-cholestatic conditions. Liver biopsies were collected; expression of the main nuclear receptors involved in transcriptional regulation of bile acid synthesis or transport and of biliary transporters was analyzed by real time RT-PCR using 18 S as reference gene.

Results: Hepatic expression of cholesterol 7α-hydroxylase (CYP7A1), the limiting enzyme of bile acid synthesis, was not significantly different in the two groups. Among the different nuclear receptors, short heterodimer partner (SHP), a target for the bile acid receptor FXR and a *sensor* of hepatic bile acid content, was significantly ($p < 0.01$) more expressed in cholestatic patients, and so were several genes coding for canalicular biliary lipid transporters, such as ABCB4 and ATP8B1 ($p < 0.05$). The basolateral organic anion transporting polypeptide A (SLCO1A2) was significantly ($p < 0.01$) suppressed. The expression of SHP was directly correlated with a number of canalicular transporters and inversely correlated with SLCO1A2.

Discussion/Conclusion: In human obstructive cholestasis, significant alterations in hepatic expression of nuclear receptors and hepatobiliary transporters take place. These alterations seem to be mediated by increased expression of SHP and appear to be finalized to stimulate extrusion of biliary lipid out of the liver and to inhibit further uptake of organic anions. Such findings might provide insight into the patho-physiology of cholestasis and hopefully suggest novel molecular targets for its management.

Modulation of FXR activity via O-linked N-acetylglucosaminylation upon refined sugar feeding in mice

Marije Boesjes[1], Hilde Herrema[1], Jurre Hageman[1], Folkert Kuipers[1,2], Albert K. Groen[1,2]
Laboratories of [1]Pediatrics and [2]Laboratory Medicine, Center for Liver Digestive and Metabolic Diseases, University Medical Center Groningen, Groningen, The Netherlands

Introduction: Dietary refined sugar intake has been shown to decrease bile salt synthesis via yet unknown mechanisms. It was the aim of this study to determine whether and to what extent FXR, the bile salt-activated nuclear receptor, is involved in this phenomenon.

Methods: Male C57Bl6/J or intestinal specific FXR knock-out mice were fed a normal chow or a semi synthetic diet in which the carbohydrate fraction consisted solely of dextrose for 2 weeks. After the diet intervention, mice were terminated at 7AM and 7PM. Upon termination plasma and hepatic parameters as well as intestinal and hepatic gene expression patterns were measured. Bile acid kinetic parameters where examined by stable isotope dilution technique. O-linked N-acetylglucosaminylated (O-GlcNac) FXR was immunoprecipitated from FXR transfected HEK293 cells *in vitro* and detected on Western blot.

Results: Biliary (-33%) and fecal bile salt (-35%) secretion were decreased in dextrose-fed mice compared to chow fed controls. Primary bile salt synthesis was massively (-50%) decreased contributing to a 40% smaller pool size observed in dextrose-fed mice. Interesting, despite anticipated low bile salt levels in the ileum of dextrose-fed mice, ileal expression of the FXR target genes *Shp* and *Fgf15* were significantly increased, particularly in the fed state (7AM), but expression of FXR itself was not altered. Dextrose feeding had no effect on *Shp* and *Fgf15* expression in intestinal FXR knock-out mice. We therefore investigated possible posttranslational modification of FXR induced by dextrose feeding. Preliminary results of *in vitro* studies indicated that FXR is posttranslationally regulated by O-GlcNac, a well known glucose sensing mechanism.

Discussion/Conclusion: Simple carbohydrate feeding suppresses bile salt synthesis, possibly by the upregulation of intestinal *Fgf15* expression via activation of FXR. Since intestinal bile salt concentrations were reduced we postulate that transcriptional activity of FXR was increased via posttranslational O-GlcNac of the protein.

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NHERF-1 knockout mice express low levels of the apical sodium-dependent bile acid transporter (Asbt) in ileum and kidney and are protected from cholestasis induced by bile duct ligation

M. Li, A. Mennone, C.J. Soroka, E.J. Weinman, J.L. Boyer
Liver Center, Yale University School of Medicine, New Haven, CT, USA

Introduction: The Na^+/H^+ exchanger regulatory factor 1 (NHERF-1/EBP50) plays a critical role in protein expression in polarized epithelia including the canalicular expression and function of Mrp2 (Li M et al. J Biol Chem. 2010; Epub ahead of print). Here we examined if bile acid transporters and homeostasis was affected in the intestine and kidney of NHERF-$1^{-/-}$ mice under basal and cholestatic conditions.

Methods: Whole cell lysates, membrane-enriched fractions and total RNA were isolated from liver, ileum and kidneys of wild-type and NHERF-$1^{-/-}$ mice. Expression of bile acid transporters in sham and 7 day bile duct ligated (BDL) NHERF-$1^{-/-}$ mice and WT mice was determined by Western blotting and real-time PCR. Liver histology, bile acid levels in liver, serum and feces and bile acid pool size were determined.

Results: In the ileum of NHERF-$1^{-/-}$ mice, Asbt was significantly reduced in both whole cell lysates and membrane-enriched fractions to ~60% ($p < 0.05$) and ~40% ($p < 0.01$), respectively. Ileal Fgf15 mRNA was reduced to ~25% of the wild-type ($p < 0.01$) whereas fecal bile acid excretion was increased 3-fold ($p < 0.01$). However, serum and liver bile acid concentrations and bile acid pool size remained normal and the expression levels of liver Fxr, Shp, Fgfr4 and Cyp7a1 mRNA were not significantly changed. Renal Asbt protein was also reduced in the knockout animals as were the apical transporters, Mrp2 and Mrp4. Following bile duct ligation, both hepatic necrosis and serum aminotransferases were significantly decreased in the knockout animals compared with the wild-type mice.

Discussion/Conclusion: These findings indicate that NHERF-1 is an important determinant of the expression of apical transporters that regulate bile acid transport and homeostasis in ileum and kidney, changes that have protective effects in cholestatic liver injury. Extrahepatic determinants of bile salt homeostasis may be potential therapeutic targets in cholestasis.

Different roles of LXRα and LXRβ in the regulation of intestinal cholesterol absorption by altering bile acid composition in mice

Xiao-Li Hu[1], Knut Steffensen[2], Zhao-Yan Jiang[1], Paolo Parini[1], Jan-Åke Gustafsson[2], Mats Gåfvels[1] and Gösta Eggertsen[1]
[1]Division of Clinical Chemistry, Department of Laboratory Medicine, [2]Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Stockholm, Sweden

Introduction: Bile acids (BA) are critical for the intestinal cholesterol absorption by facilitating micellar formation, and alterations of BA composition and pool size will affect the cholesterol uptake. The liver X receptors (LXRs) also modulate cholesterol absorption, yet the individual role of LXRα and LXRβ remains unclear. Our study aims to define individual functions of LXRα and LXRβ on cholesterol absorption and bile acid metabolism.

Methods: Wild type (WT), LXRα$^{-/-}$ or LXRβ$^{-/-}$ mice were used. Cholic acid (CA) and the LXR agonist GW3965 was given for 7 and 4 days, respectively.

Results: Under chow diet, no significant changes were observed between the different genotypes. However, when fed 0.2% cholesterol diet, LXRβ$^{-/-}$ mice showed significant lower cholesterol absorption compared to the WT animals. Biliary CA fraction and the ratio between CA and β-muricholate (β-MCA) of LXRβ$^{-/-}$ mice also displayed significant reductions. Treatment of 0.2% cholesterol and GW3965 lead to a significantly higher cholesterol absorption in LXRα$^{-/-}$ mice. The result was in accordance with an elevated CA/β-MCA ratio in the LXRα$^{-/-}$ mice, while much lower levels were found in the LXRβ$^{-/-}$ and WT animals. No major differences were seen in the gene expression of several intestinal cholesterol transporters. Interestingly, the differences in cholesterol absorption between LXRα$^{-/-}$ and LXRβ$^{-/-}$ mice were completely eliminated by adding 0.05% CA to 0.2% cholesterol diet, demonstrating the strong correlation between altered bile acid composition and intestinal cholesterol absorption. Analysis of liver Cyp8b1 gene expression showed a reduction in WT and LXRβ$^{-/-}$ mice with 0.2% cholesterol ± GW3965, which was consistent with the reduced CA fraction.

Discussion/Conclusion: Our data indicate that LXRα and LXRβ are able to regulate the bile acid composition in different ways when stimulated by endogenous or synthetic ligands, causing alterations in the intestinal cholesterol absorption.

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Role of the TGR5 C-terminus for localization and function

Lina Spomer[1], Johannes R. Hov[2], Tom H. Karlsen[2], Verena Keitel[1],
Dieter Häussinger[1]
[1]Klinik für Gastroenterologie, Hepatologie und Infektiologie, Heinrich-Heine-Universität, Moorenstr. 5, 40225 Düsseldorf, Germany
[2]Norwegian PSC Research Center, Clinic for Specialized Medicine and Surgery, Oslo University Hospital Rikshospitalet, Oslo, Norway

Introduction: TGR5 (Gpbar-1) is a plasma membrane-bound bile acid receptor, which is coupled to a stimulatory G-protein. The structure of TGR5 is unknown and it is unclear which amino acids are important for receptor membrane localization and function. Aim of the present study was to identify TGR5 gene variations and to analyze their impact on receptor localization and function.

Methods: TGR5 variations were introduced into TGR5-cDNA constructs by site-directed mutagenesis. Impact on receptor localization was determined by immuno-fluorescence microscopy and FACS analysis. TGR5 function was analyzed through cotransfection of HEK293 cells with TGR5 and a cAMP sensitive luciferase reporter gene.

Results: Several nonsynonymous mutations were identified. While most mutations led to an amino acid exchange, one mutation resulted in a premature stop codon (Q296X) and the deletion of the 35 C-terminal amino acids. Introduction of this variation into the TGR5-YFP-cDNA construct resulted in a truncated protein, as demonstrated by the absence of YFP-fluorescence. However, using a N-terminal FLAG-tagged TGR5-cDNA construct (FLAG-TGR5-Q296X-YFP), the mutant protein could be detected in the endoplasmic reticulum of transfected cells. Q296X completely abolished TGR5 function as measured by luciferase activity. These findings indicate that the C-terminal tail of TGR5 is required for normal surface expression and function. To confirm these findings we generated further truncation mutations (Q300X, S310X). Both Q300X and S310X resulted in a truncated protein, however, TGR5 activation by bile acids was unaffected by the absence of the last 30 or 20 amino acids, respectively.

Discussion/Conclusion: Deletion of 35 C-terminal residues of the TGR5 cyto-plasmic tail resulted in a retention of the mutated protein within the endoplasmic reticulum and complete loss of function. Truncation of the C-terminal 30 amino acids did not affect receptor activation by bile acids, indicating that these residues are not required for TGR5 cell surface expression and function.

Bile acids regulate bile acid synthetic enzymes (Cyp7a1 and 8b1) but not hepatic transporters (Ntcp or Bsep)

Curtis D. Klaassen, Pei-zhen Song
Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS, USA

Introduction: Bile acids (BAs) are known to regulate their own homeostasis, however, it is not clear whether both BA synthetic enzymes and transporters are regulated by BAs.

Methods: Mice were fed five individual BAs (CA, CDCA, DCA, LCA, and UDCA) at various concentrations (0.01 to 3.0%) or 2% resin in their diets for one week. Messenger RNAs of hepatic and enteric genes involved in BA homeostasis were quantified using QuantiGene Plex assay.

Results: The mRNA expression of SHP increased in liver of mice fed all concentrations of BAs. All concentrations of the BAs markedly decreased the mRNA of the key BA-synthetic enzymes Cyp7a1 and Cyp8b1, and increased the cholesterol transporter Abcg5/g8 in liver. FGF15 in ileum was up-regulated by CA and DCA at all concentrations, and by CDCA and LCA at concentrations higher than 0.1% in the diet. In contrast, feeding BAs did not alter the major BA-uptake and efflux transporters Ntcp and Bsep. After resin feeding, the mRNA of BA-biosynthetic enzymes Cyp7a1 and Cyp8b1 were increased, but SHP was not decreased. Neither the sinusoidal BA uptake transporter Ntcp nor the efflux transporter Bsep on the canalicular membrane were markedly altered by feeding the resin. The sinusoidal efflux transporters, Mrp3 and 4, were increased by the resin. Resin feeding increased the intestinal uptake transporter Asbt, whereas it markedly decreased the expression of FGF15 mRNA in ileum.

Conclusion: Feeding BAs produces a decrease in Cyp7a1 and Cyp8b1, but has no effect on the BA transporters (Ntcp and Bsep) in liver. Feeding the resin, increased Cyp7a1 and 8b1, but had no major effect on Ntcp and Bsep. Therefore BAs have a major effect in regulating the BA synthetic enzymes (Cyp7a1 and 8b1) but not the hepatic uptake and efflux transporters (Ntcp and Bsep).

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Inhibitive effect of bile acid on LXR ligand-induced triglyceride accumulation in cultured liver cells

Teruo Miyazaki[1,2], Akira Honda[1,2], Tadashi Ikegami[3], Yasushi Matsuzaki[1,3]
[1]Department of Development for Community Medicine, Tokyo Medical University, Japan
[2]Center for Collaborative Research, and
[3]Department of Gastroenterology, Tokyo Medical University Ibaraki Medical Center, Japan

Introduction: Hepatic fatty change is accompanied with many liver diseases and would be a cause of hepatitis and fibrosis. The synthesis of fatty acid is regulated by the LXR-SREBP-1c pathway. CDCA (chenodeoxycholic acid) and cholic acid, but not UDCA (ursodeoxycholic acid), are ligands for FXR, and consequently, the activated FXR inhibits the LXR-SREBP-1c pathway through SHP activation. UDCA has been used for treatment of fatty liver disease, but the therapeutic benefits are limited. The present study investigated the effects of bile acids and a synthetic FXR ligand on the triglyceride (TG) accumulation induced by the LXR-ligands in non-tumor hepatic cell line.

Methods: Confluent mice AML-12 cells were exposed to either endogenous (oxysterols; 4βOH, 22ROH, 25OH, 24S,25-Epoxy, 7αOH) or synthetic (To-901317) LXR-ligands for a week to establish a fatty liver model in cultured cells. Furthermore, the cells exposed to To-901317 were treated with various concentrations of CDCA, UDCA, or synthetic FXR-ligand (GW4064). The TG accumulation was histologically and biochemically evaluated by Oil-red assay and TG assay as well as mRNA expressions of enzymes involved in fatty acid synthesis.

Results: Exposure to 10 μM oxysterols (22ROH, 24S,25-Epoxy) and 1 μM To-901317 significantly induced cellular TG accumulation with significantly increased mRNA expressions of SREBP-1c, acetyl-CoA carboxylase, fatty acid synthase, and steatory-CoA desasturase-1. The TG accumulation induced by To-901317 was significantly inhibited by treatment with 1–50 μM CDCA or 1 μM GW4064, while there was no effect in UDCA. However, cell damage was observed when over 50 μM CDCA was added.

Discussion/Conclusion: The present study confirmed the inhibitive effect of CDCA and GW4064 on hepatic TG accumulation induced by LXR-ligand. Therefore, there is a possibility that FXR-ligands might be therapeutic agents for fatty liver, although higher dose of CDCA would induce hepatic damages.

AKR1B7 metabolizes bile acids and is regulated by FXR

Daniel R. Schmidt, Samuel Schmidt*, Steven A. Kliewer, David J. Mangelsdorf
Howard Hughes Medical Institute and the Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX, USA
*University of Hamburg Medical School, Hamburg, Germany

To gain insight into the function of FXR in the large intestine, transcriptional profiling experiments were performed in mouse colon treated with natural (deoxycholic acid) and synthetic (GW4064) FXR agonists. Here we show that FXR regulates expression of aldo-keto reductase 1b7 *(Akr1b7)* in both large and small intestine. AKR1B7 has previously been shown to detoxify lipid peroxides. We have now found that 3-keto bile acids are also substrates for AKR1B7. Furthermore, we show that the products of bile acid metabolism by AKR1B7, namely iso-bile acids, are less toxic to cultured cells than regular bile acids. At high concentrations bile acids promote tumorigenesis and loss-of-FXR increases susceptibility to colorectal cancer in mice. These results suggest that FXR may play a role in protecting intestinal mucosa by inducing mechanisms of bile acid detoxification.

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Synthesis and biological evaluation of ursodeoxycholic acid derivatives as glucocorticoid receptor modulators

R. Sharma[1,2], F. Majer[1], D. Prichard[2], D. Kelleher[2], A. Long[1], J.F Gilmer[1]
[1]School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Ireland
[2]Institute of Molecular Medicine, St. James's Hospital, Dublin, Ireland

Introduction: Developing novel anti-inflammatory agents lacking the side effect profile of conventional glucocorticoids has been the focus of much research over the past few decades. Ursodeoxycholic acid (UDCA), a tertiary bile acid is clinically used for the treatment of hepatic inflammatory diseases including primary sclerosing cholangitis (PSC). UDCA also exhibits cytoprotective, anti-oxidant and chemopreventative effects. UDCAs excellent safety profile during its clinical use coupled with recent studies showing that its biological effects are glucocorticoid receptor (GR) mediated make it an interesting candidate for the development of a novel GR modulator. Such a GR modulator would have potential use in the treatment of esophageal inflammatory disorders. Chronic esophageal inflammation has been linked to the development of esophageal adenocarcinoma.

Methods: A series of UDCA derivatives were synthesised and screened for ability to induce GR translocation in a high content screening assay using the esophageal SKGT-4 cell line. The most potent derivative was tested for transactivation and transrepression potential using reporter based assays. GR coactivator recruitment ability was assessed using a time-resolved fluorescence energy transfer (TR-FRET) assay.

Results: UDCA derivatives induced GR translocation in a time dependent manner with equal efficacy to that of dexamethasone 100 nM. Several of the derivatives had low micro molar potency for GR translocation. The most potent derivative could suppress TNF-α induced NF-κB transcriptional activity and also induced GRE transactivation. Interestingly the derivative was unable to displace dexamethasone from the GR ligand binding domain (LBD) in a competition binding experiment but was capable of co-activator recruitment in a TR-FRET assay. This represents a novel mechanism of action for a GR modulator.

Discussion/Conclusion: Using UDCA as a lead we have produced a series of derivatives which demonstrate a novel mechanism for GR activation. These derivatives could result in a new class of anti-inflammatory compounds.

Impaired negative feedback of hepatic bile acid synthesis by fibroblast growth factor 19 in patients with primary bile acid diarrhoea

Julian R.F. Walters[1], Sanjeev S. Pattni[1], W. Gordon Brydon[3], Tracy Dew[4], Carel Le Roux[2,4]
[1]Sections of Hepatology & Gastroenterology, [2]Metabolic Medicine, Imperial College London, [3]Western General Hospital, Edinburgh, [4]King's College Hospital, London, UK

Introduction: Chronic diarrhoea is due to primary (idiopathic) bile acid diarrhoea is under-recognised but surprisingly common, and is often diagnosed as diarrhoea-predominant IBS. Part of the problem in the recognition of the disorder has been that there has been no convincing mechanism to account for bile acid malabsorption in the majority of these patients. Several groups have produced data indicating the absence of malabsorption in primary bile acid diarrhoea. We have suggested (Clin Gastroenterol Hepatol 2009; 7: 1189) that this condition may result from excessive bile acid synthesis due to impaired negative feedback by the ileal hormone fibroblast growth factor 19 (FGF19). We aimed to confirm these findings in a prospective series of patients.

Methods: Patients with chronic watery diarrhoea and no obvious cause for their symptoms were investigated prospectively with SeHCAT tests. Fasting blood was taken for FGF19 and assayed by a commercial ELISA assay.

Results: 49 of 104 patients (47%) had SeHCAT 7d retention < 15%. After other causes were excluded, primary bile acid diarrhoea was the final diagnosis in 34 (33%), similar to previous studies (Aliment Pharmacol Ther 2009; 30: 707). Patients had a median of 7 stools/day of Bristol scale type 7. Urgency, incontinence, bloating and a raised BMI were significantly more common than in those with a normal SeHCAT test. Median FGF19 levels were lower (160 vs 276 pg/ml; $p < 0.001$).

Discussion/Conclusion: Patients with chronic diarrhoea and low SeHCAT retention have reduced FGF19 levels compared to those with normal SeHCAT. Impaired production of FGF19 is likely to be the major factor contributing to the cause of this common condition of primary bile acid diarrhoea.

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Increased hepatic bile acid synthesis prevents cholesterol gallstones in small heterodimer partner (SHP) knockout mice

David Q.-H. Wang
Gastroenterology Division and Liver Center, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA

Introduction: Lithogenic bile is mainly caused by persistent hepatic hypersecretion of biliary cholesterol, which has both hepatic and small intestinal components. Bile acid is synthesized from cholesterol by either a classical pathway resulting in the formation of cholic acid or by an alterative pathway leading to the synthesis of chenodeoxycholic acid. SHP functions as part of the negative feedback controlling bile acid synthesis by inhibiting transcription of cholesterol 7α-hydroxylase (CYP7A1) and sterol 12α-hydroxylase (CYP8B1).

Aims: We explored whether increased hepatic bile acid synthesis reduces biliary cholesterol secretion, and in turn, prevents diet-induced gallstone formation. Methods: Both gallstone and biliary lipid secretion studies were performed in male SHP (-/-) and (+/+) mice (n = 8 per group) before and during feeding a lithogenic diet (1% cholesterol, 0.5% cholic acid and 15% butterfat) for 56 days. Intestinal cholesterol absorption efficiency was determined by fecal dual-isotope ratio methods. Bile lipids and hydrophobicity indexes were measured by biochemical methods. Gene expression levels were determined by real-time PCR techniques.

Results: Deletion of the SHP gene resulted in increased expression levels of CYP7A1 and CYP8B1 in the liver, and fecal bile acid excretion was greater in (-/-) mice than (+/+) mice, suggesting that hepatic bile acid synthesis was enhanced. Gallstones were found in 60% and 100% of (+/+) mice at day 28 and 56, respectively. In contrast, no cholesterol crystals or gallstones were detected in (-/-) mice during the study. Percentages of hydrophobic taurodeoxycholic acid and hydrophobicity indexes were greater ($P < 0.05$) in (-/-) mice ($40.3 \pm 5.1\%$ and $+0.10 \pm 0.04$) than (+/+) mice ($9.3 \pm 3.0\%$ and -0.18 ± 0.05). Although intestinal cholesterol absorption efficiency was higher in (-/-) mice than (+/+) mice ($57 \pm 8\%$ vs. $32 \pm 6\%$, $P < 0.05$) due to increased biliary secretion and hydrophobicity index of bile acid pool, cholesterol saturation indexes of gallbladder bile were still lower in (-/-) mice than (+/+) mice (0.6 ± 0.2 vs. 1.5 ± 0.3, $P < 0.05$). This suggests that in (-/-) mice increased cholesterol of intestinal origin was efficiently converted to BILE ACID.

Conclusions: The loss of SHP repression of CYP7A1 expression significantly increases hepatic bile acid synthesis, and in turn, it reduces hepatic secretion of biliary cholesterol and bile cholesterol content by greatly converting the cholesterol molecules of hepatic and intestinal sources into bile acid in mice, even in challenge to the lithogenic diet. All of these changes prevent the formation of cholesterol gallstones. Thus, selective inhibition of hepatic SHP expression may provide protection against cholesterol gallstone formation.

Decreasing bile acid synthesis *with* an FXR agonist induces obesity and diabetes through the decrease of energy expenditure

Mitsuhiro Watanabe[1], Yasushi Horai[1], Kokichi Morimoto[1], Chikage Mataki[2], Hiroyuki Sato[3], Taichi Sugizaki[1], Eri Arita[1], Nao Iwasaki[1], Sander M. Houten[4], Johan Auwerx[2], Hiroshi Itho[1] and Kristina Schoonjans[2]
[1]Keio University, Tokyo, Japan
[2]EPFL, Lausanne, Switzerland
[3]National Cardiovascular Center Research Institute, Osaka, Japan
[4]Academic Medical Center, Amsterdam, The Netherlands

Over the 5 years, the field of bile acid (BA) research has undergone a considerable evolution. Besides their well-established roles in dietary lipid absorption and cholesterol homeostasis, it has recently become clear that BAs are also biological signaling molecules. BAs were shown to be natural ligands that activate FXR, which controls both the synthesis and enterohepatic recycling of BAs. We have shown that BA decreased hepatic VLDL production via FXR pathway (Watanabe et al., JCI, 2004) and increased energy expenditure, preventing obesity and insulin resistance (Watanabe et al., Nature, 2006). These observations build a strong case that BA has effects beyond the strict control of function as metabolic integrators.

We evaluated the metabolic impact of FXR activation by administering a synthetic FXR agonist (GW4064) to mice in which obesity was induced by feeding a high fat diet. The administration of GW4064 accentuated the body weight gain induced by the high fat diet, and led to a more pronounced increase in high-fat induced changes in liver and adipose mass and morphology. Glucose tolerance also detoriated after GW4064 administration. The importance of the relative contribution of primary bile acids to the BA pool and the maintenance of metabolic homeostasis is underscored by the fact that treatment with an FXR agonist decreased bile acid biosyntheses and detoriated metabolic control. Our data hence suggest that the activation of FXR is not be useful for the management of the metabolic syndrome. Manipulating the BA pool size in general, and increasing primary bile acid levels in particular, however, could be an interesting strategy to manage the metabolic syndrome.

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Loss of bile acid responsive gene retinoic acid receptor-related orphan receptor A (RORA) in Barrett's metaplasia

Shane P. Duggan[1], Murat Kirca[1,2], Fiona Behan[1], Dermot Kelleher[1,2]
[1]Department of Clinical Medicine, Institute of Molecular Medicine, Trinity College Dublin, Ireland
[2]Department of Gastroenterology, St. James' Hospital, Dublin 8, Ireland

Introduction: The involvement of gastro-esophageal-reflux-disease in the development of Barrett's metaplasia (BM) is well established. Recent evidence has begun to implicate bile acids such as the secondary bile acid deoxycholic acid (DCA) in BM and subsequent carcinogenesis. Recent work from our laboratory has identified bile acid responsive genes with altered expression in BM through a novel integrative transcriptomic profiling approach. RORA is one such postulated bile acid responsive gene with roles in lipid homeostasis, inflammation, differentiation, circadian rhythm and cancer. We now aim to validate RORA expression, bile acid-mediated induction and implications to esophageal cell signaling.

Methods: Esophageal cell lines HET-1A (normal-squamous), QhTRT (metaplastic), GohTRT (dysplastic) and SKGT4 (adenocarcinoma) were utilized. Expression of RORA was determined by semi-quantitative real time RT-PCR (ABI) and western blotting in a clinical cohort and the above cell lines.

Results: A significant reduction in RORA levels, as determined by real time RT-PCR, was observed in a cohort of BM patients ($p < 0.001$). This pattern of expression was mirrored in esophageal cell lines. HET-1A cells displayed the highest expression levels of RORA with subsequent decreases observed in QhTRT, GohTRT and SKGT4 cells. DCA-mediated (300 μm) induction of RORA expression was confirmed in all cell types by real time RT-PCR and western blotting. However, induction of RORA was lower in dysplastic cell types by comparison to HET-1A and primary squamous cells. Isoform specific RT-PCR defined RORA4 as the predominant isoform expressed in esophageal cells. At the protein level 2 distinct bands matching both RORA4 and RORA1 were induced in esophageal cells. Potential transcriptional targets of RORA were determined utilizing informatic approaches generating a regulatory network of gene expression for RORA in response to DCA.

Discussion/Conclusion: This study for the first time demonstrates the ability of secondary bile acids such as DCA to induce RORA expression in oesophageal cells and additionally validated a significant loss of this vital factor in oesophageal metaplastic tissue.

Invalidation of the vitamin D nuclear receptor promotes biliary-type liver injury

D. Firrincieli[1], S. Zuñiga[1], C. Rey[1], D. Wendum[2], E. Lasnier[3], D. Rainteau[3], A. Cadoret[1], M. Boissan[1], C. Housset[1], N. Chignard[1]
[1]UPMC Univ. Paris 06, UMR_S 938, CdR Saint-Antoine, Paris, France
[2]Service d'Anatomie Pathologique, AP-HP, Hôpital Saint-Antoine, Paris, France
[3]Service de Biochimie, AP-HP, Hôpital Saint-Antoine, Paris, France

Introduction: Vitamin D nuclear receptor (VDR) polymorphisms has been associated with primary biliary cirrhosis, a prototypical biliary disease. Because in the liver VDR is predominantly expressed in biliary epithelial cells, we hypothesized that VDR is involved in biliary-type liver diseases.

Methods: VDR knockout mice and wild type littermates were used to determine the role of VDR in biliary pathophysiology. Biliary-type disease was induced by common bile duct ligation (BDL). Liver injury was ascertained 3 days after surgery by histological observation, serum biochemical analysis and molecular analysis of liver samples. Bile acid level in the liver was evaluated by mass spectrometry.

Results: VDR knockout BDL mice displayed altered hepatic histology as compared to wild type BDL littermates. Serum biochemical analysis of alanine and aspartate aminotransferases confirmed higher hepatocellular damage. Furthermore, bile acid serum level was higher in VDR knockout BDL mice compared to control BDL mice. Hepatic bile acid concentration was not different between VDR knockout and wild type BDL mice. However, the expression of Cyp3a11 was decreased in VDR knockout mice, suggesting that VDR knockout mice are less efficient in bile acid detoxification. The expression of the bile acids transporters Bsep and Mrp3 was also reduced in VDR knockout BDL mice. Ductular reaction was lower in VDR BDL knockout mice compared to control BDL mice as assessed both by histological examination and expression of cytokeratin 19 transcripts. Finally, the expression of liver fibrosis markers was reduced in VDR knockout BDL mice compared to control BDL mice as ascertained by the analysis of TGFβ, αSMA and type I collagen mRNA expression.

Discussion/Conclusion: Our results suggest that VDR knockout BDL mice display less bile acid detoxification, ductular reaction and scarring. Taken together, these results may explain increased liver injury in these mice and suggest that VDR may have a protective role in biliary-type liver diseases.

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Identifying intermediates of extrahepatic bile acid biosynthesis and their role as ligands to nuclear receptors

W.J. Griffiths, M. Ogundare, S. Theofilopoulos, J. Sjövall, E. Arenas, Y. Wang
School of Medicine, Swansea University, UK and Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Sweden

Introduction: As a group of chemical compounds steroids, including sterols, oxysterols and bile acids, behave as ligands to numerous nuclear receptors. For example 24-, 25- and 27-hydroxycholesterols are ligands to the liver X receptors (LXRs), while chenodeoxycholic acid is a ligand to the farnesoid X receptor (FXR). The above cholesterol metabolites represent initial and ultimate metabolites in bile acid biosynthetic pathways. Although the major site of bile acid biosynthesis is the liver, bile acids can also be synthesised extrahepatically, and the goal of the current study is to identify intermediates in the bile acid biosynthetic pathways formed extrahepatically, and to asses their role as ligands to nuclear receptors in extra-hepatic tissues.

Methods: The profile of sterols, oxysterols and bile acids in human cerebrospinal fluid (CSF) and plasma was determined by liquid chromatography (LC) – tandem mass spectrometry (MS/MS) following derivatisation by charge-tagging with the Girard P (GP) reagent. Charge tagging greatly enhances the specificity and sensitivity of the assay allowing oxysterol detection at the pg/mL level. The potential of identified metabolites to act as ligands for LXR and FXR was assessed in reported assays.

Results: Analysis of CSF reveals members of the 24S-hydroxycholesterol, 25-hydroxycholesterol and the acidic pathways of bile acid biosynthesis including 24S-hydroxycholesterol, 7α,24S-dihydroxy-3-oxocholest-4-en-26-oic, 7α-hydroxy-3,24-bisoxocholest-4-en-26-oic acids; 25-hydroxycholesterol, 7α,25-dihydroxycholest-4-en-3-one; 27-hydroxycholesterol, 3β-hydroxycholest-5-en-26-oic, 7α-hydroxy-3-oxocholest-4-en-26-oic acids; and 7α-hydroxy-3-oxochol-4-en-24-oic acid. Intriguingly while 3-oxo-4-ene metabolites do not act as ligands to LXR in a neuronal cell line reporter assay some of their 3β-hydroxy-5-ene precursors do.

Discussion/Conclusion: The presence of numerous intermediates of the 24S-hydroxycholesterol pathway of bile acid biosynthesis in CSF strongly supports the hypothesis that this bile acid biosynthetic pathway is active in brain. Data from reporter assays suggest that conversion of the 3β-hydroxy-5-ene structure to the 3-oxo-4-ene equivalent may offer a route to deactivation of LXR ligands.

Invalidation of the vitamin D nuclear receptor promotes liver steatosis

Silvia Zuñiga[1,2], Delphine Firrincieli[1,2], Elisabeth Lasnier[3], Juan F. Miquel[4], Chantal Housset[1,2], Nicolas Chignard[1,2]
[1]UPMC Univ. Paris 06, UMR_S 938, CdR Saint Antoine, Paris, France
[2]Inserm, UMR_S 938, CdR Saint Antoine, Paris, France
[3]Fédération de Biochimie, AP-HP, Hôpital Saint Antoine, Paris, France
[4]Gastroenterology, Pontificia Universidad Católica de Chile, Santiago, Chile

Introduction: The vitamin D nuclear receptor (VDR) is a regulator of cholesterol and bile acid metabolism. Furthermore, hypovitaminosis D has been associated with liver steatosis. Here we examined the impact of VDR on lipid homeostasis and hepatic steatosis using VDR KO mice.

Methods: 8–12 weeks old male $VDR^{-/-}$ mice on a C57BL6J background were compared to $VDR^{+/+}$ littermates under basal conditions. A second set of experiments compared mice fed the Paigen diet (high fat 15%, cholesterol 1.5%, cholic acid 0.5%) with mice under a chow diet for 10 days. Transcript of genes involved in transport and metabolism of hepatic lipids were measured by RT-QPCR. Metabolic parameters and hepatic enzymes were assessed in serum. Histological samples were analyzed after staining with hematoxylin phloxine saffron stain (HPS) and Oil Red O.

Results: Under basal conditions, $VDR^{-/-}$ mice showed an altered hepatic expression of genes involved in biliary secretion when compared to $VDR^{+/+}$ control littermates. The bile salt transporters Ntcp and Mrp3, the phospholipid floppase Abcb4 and the cholesterol transporter Abcg8 were all significantly decreased in the $VDR^{-/-}$ mice as shown by RT-QPCR. The alteration of cholesterol metabolism was further documented in the liver of $VDR^{-/-}$ mice by the observation of a three-fold decrease in the expression levels of Cyp8b1, an enzyme involved in cholesterol catabolism ($p < 0.05$). Under basal conditions liver steatosis was observed in 57% of $VDR^{-/-}$ mice, while no steatosis was evidenced in $VDR^{+/+}$ mice. Liver steatosis appeared massive after 10 days of Paigen diet, which correlated with an altered hepatic enzyme profile.

Discussion/Conclusion: Our results suggest that lipid metabolism is altered in $VDR^{-/-}$ mice. These modifications may impact hepatic pathophysiology by inducing steatosis.

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Frizzled Homolog 3 & 10 are potential modulators of resistance to deoxycholic acid mediated cell death in an oesophageal cell line

F.M. Behan[1], S.P. Duggan[1], M.M.M. Abdel Latif[1] and D. Kelleher[1]
[1]Department of Clinical Medicine, Institute of Molecular Medicine, Trinity College Dublin, St. James' Hospital, Dublin 8, Ireland

Introduction: This study aims to identify modulators of resistance to deoxycholic acid (DCA) induced cell death in dysplastic oesophageal cell lines. A semi-automated siRNA library screen was carried out targeting G-protein coupled receptors (GPCRs) and alterations in cellular response to DCA were assessed. GPCRs were targeted as these molecules represent potential therapeutic targets for the treatment of oesophageal malignancies.

Methods: Oesophageal cell lines used: HET-1A (normal-squamous), QhTRT (metaplastic), GOhTRT (dysplastic) and SKGT4 (adenocarcinoma). Cell viability was assessed using the MTT proliferation assay. Preliminary experiments demonstrated that inhibition using siRNA targeting BCL-XL sensitized GOhTRTs to DCA (10hours@500μM) induced cell death. An automated siRNA transfection protocol was developed using the Matrix-Hydra II and Matrix-WellMate liquid handling robots with siBCL-XL incorporated as a positive control.

Results: A significant level of resistance to DCA-induced cell death was observed in the GOhTRTs compared to Het-1As. An siRNA screen targeting GPCRs identified a number of genes whose specific inhibition resulted in a sensitization of the GOhTRTs to DCA induced cell death (10hours@500μM). The top 25 targets when ranked by z-score (z) or cell viability (percentage of control) (%via) include FZD3 (z = -1.53185; %via = 68.9%), FZD10 (z = -1.39935; %via = 81.4%), CCL25 (z = -2.3465; %via = 73.70435%) and IL8Rα (z = -1.31359; %via = 81.9%). Further investigations will assess how these molecules mediate resistance to DCA induced cell death.

Discussion/Conclusion: Frizzled proteins are an integral element of the Wnt signalling pathway as they act as co-receptors for secreted Wnt proteins. This study suggests a role for frizzled homolog family members 3 & 10 (FZD3&10) as mediators of DCA induced signalling in the cell line GOhTRTs as specific inhibition of FZD3&10 results in sensitization of the GOhTRTs to DCA-induced cell death. These findings have potential implications for pathogenesis and therapeutics of oesophageal cancer. Future investigations will involve the implementation of this screening strategy with more comprehensive siRNA libraries to target a larger number of genes to potentially identify new therapeutic targets.

Deoxycholic acid impairs protein secretion and glycosylation via induction of Golgi fragmentation in oesophageal cells

Anne-Marie Byrne, Ruchika Sharma, Dermot Kelleher, Aideen Long
Trinity College Dublin, Clinical Medicine, Dublin, Ireland

Introduction: Elevated levels of deoxycholic acid (DCA) have been implicated in gastrointestinal cancer progression. We had previously shown DCA caused complete breakdown of the Golgi structure by interfering with the Golgi membrane fission process via a PKCη/PKD dependent pathway. The Golgi is an important organelle responsible for protein processing, glycosylation and secretion. Disruption of Golgi structure leads to mislocalisation of Golgi resident enzymes and impaired glycoprotein synthesis. Altered protein glycosylation and secretion are hallmarks of cancer and contribute to metastasis, inflammation and cell to cell communication. Altered glycoprotein expression has been demonstrated in Barrett's oesophagus and oesopheal cancer tissue but the mechanisms are unknown. In this study we investigated the downstream consequences of DCA-induced Golgi fragmentation with respect to protein secretion and glycosylation in normal oesophageal (HET1A) and Barrett's (QH) cell lines as a potential mechanism of neoplastic progression.

Methods: The two cell lines used in this study were the HET1A squamous oesophageal epithelial cells and QH (CP-A) Barrett's metaplastic cells. The effects of DCA (0–300 μM) on Golgi structure was assessed using immunofluorescence and quantified by high content analysis using the GE-Incell analyser 1000. DCA effects on protein secretion involved transfection of a gaussia luciferase construct and monitoring secretion of luciferase into the supernatant after DCA treatment. A panel of FITC-conjugated plant lectins (WGA, CONA, UEA-1 and PNA) was used to determine the effect of DCA on protein glycosylation which were detected by flow cytometry and fluorescence microscopy. Monoclonal antibodies to the Lewis antigens were used to assess alterations in expression in response to DCA by flow cytometry.

Results: DCA induced Golgi fragmentation and decreased protein secretion in both HET1A and QH cell lines. Alterations in localisation of FITC-labelelled WGA and CONA and a decrease in cell surface expression of WGA CONA UEA-1 was observed in HET1A and QH cells. Tn antigen expression, indicated by binding of PNA, was observed only in QH metaplastic cells. DCA did not alter Lewis antigen expression in either cell line.

Discussion/Conclusion: In this study we demonstrated DCA disrupts Golgi structure and consequently had a profound effect on protein secretion and processing functions. Decreased cell surface expression of complex glycoproteins indicates impaired fucosylation, N-linked and O-linked glycosylation processes in response to DCA which could potentially contribute to neoplastic progression.

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Bile acid signalling in diabetes and obesity: Study of Cyp7a1 transgenic mice

John Y.L. Chiang, Tiangang Li
Northeastern Ohio University Colleges of Medicine and Pharmacy Rootstown, OH, USA

Introduction: Cholesterol 7α-hydroxylase (CYP7A1) is the rate-limiting enzyme in the bile acid biosynthetic pathway that converts cholesterol to bile acids in the liver. Recent studies suggest that bile acids may play a role in regulation of lipid, glucose, and energy metabolism; however, the underlying mechanism remains unclear. The objective of this study is to investigate the role of CYP7A1 in the prevention of fatty liver, obesity and type II diabetes.

Methods: Transgenic mice carrying an ApoE3-CYP7A1 coding sequence (Cyp7a1-tg) were generated for studying diet-induced obesity, fatty liver, and insulin resistance.

Results: Cyp7a1-tg mice showed similar hepatic bile acids, cholesterol and triglycerides levels, but had a ~2-fold higher hydrophobic bile acid pool than wild type mice. When fed a Western high fat diet (HFD) for two months, Cyp7a1-tg mice were resistant to weight-gain and fatty liver, and had decreased fat mass and hepatic triglycerides and cholesterol content than wild type mice. Glucose and insulin tolerance tests showed improved glucose and insulin sensitivity in Cyp7a1-tg mice. CYP7A1-tg mice had increased hepatic VLDL secretion but maintained plasma triglyceride and cholesterol homeostasis. Indirect calorimetry revealed that HFD-fed Cyp7a1-tg mice had increased energy expenditure than wild type mice.

Discussion/Conclusion: Induction of Cyp7a1 expression with expansion of a hydrophobic bile acid pool prevents diet-induced obesity, hepatic steatosis and insulin resistance, and may be a promising therapeutic strategy for treating metabolic liver diseases, diabetes and obesity.

Role of maternal bile acid accumulation in the pathogenesis of neonatal respiratory distress syndrome

E. Herraez[1], E. Lozano[1], E. Poli[1], D. De Luca[2], P. Martinez-Becerra[1], L. Sanchez-Vicente[1], A.G. Blazquez[1], J. Vaquero[1], O. Briz[3], M.J. Monte[1], J.J.G. Marin[1], R.I.R. Macias[1]
[1]Laboratory of Experimental Hepatology and Drug Targeting, CIBERehd, University of Salamanca, Spain
[2]Division of Neonatology, Institute of Mother and Child Health, G. Salesi Women's and Children's Hospital, Polytechnical University of Marche, Rome, Italy
[3]University Hospital of Salamanca, Spain

Introduction/Aims: Respiratory distress in infants born from women with cholestasis during pregnancy has been associated to intrauterine exposure to high bile acid (BA) levels. The aim of this study was to evaluate in rats the effect of maternal hyper-cholanemia on adult and foetal lungs after complete obstructive cholestasis from days 14 to 21 of gestation.

Methods/Results: Gene expression was investigated by real-time qRT-PCR, Western blot and immunofluorescence. BA concentrations were determined enzymatically and by HPLC-MS/MS. Maternal BA pool was labelled using [^{3}H]-taurocholate to determine BA transfer to foetal tissues. Maternal cholestasis was accompanied by an increase in maternal cholanemia (x20, $p < 0.001$) together with a lower effect in foetal cholanemia (x3, $p < 0.001$). Lung BA content was markedly increased in the pup of cholestatic rats. This was accompanied by peribronchyal oedema, collapse of alveolar spaces, presence of deposits of hyaline material in the alveolar lumen, infiltration by inflammatory cells and lower abundance of IL-6 but increased myeloperoxydase activity. In maternal and foetal lungs, the expression of OATPs was not affected by cholestasis. In contrast, several ABC export pumps, such as lipid transporters (Abca1 and Mdr2), and organic anion exporters (Mrp3 and Bcrp) were up-regulated.

Discussion/Conclusion: Accumulation of BAs in foetal lung during maternal cholestasis may account for structural and functional alterations of neonatal alveoli due to direct (detergent insult and activation of phospholipase activity) and indirect (inflammatory response) effects. Moreover, changes in the expression of transport systems could affect the local handling of BAs and lipid composition of the surfactant.

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A biliary HCO_3^- umbrella protects human biliary epithelia against bile acid-induced injury

S. Hohenester, L. Maillette de Buy Wenniger, R.P. Oude-Elferink, U. Beuers
Tytgat Institute for Liver and Intestinal Research, University of Amsterdam, Amsterdam, The Netherlands

Introduction: Cholangiocytes expose a striking resistance against bile acids, which are toxic to other cell types already in the micromolar range but which are present in bile in millimolar concentrations. Protonated, apolar bile acids but not deprotonated, more polar bile salts may passively enter cholangiocytes. Protonation state of bile acids depends on their pKa and present pH. We hypothesize that human cholangiocytes alkalize bile by secreting HCO_3^- to foster deprotonation of apolar bile acids to polar bile salts. The resulting 'HCO_3^- umbrella' might be a key protective mechanism of human cholangiocytes against glycine-conjugated bile acids. Our **aim** was to test if bile acid-induced toxicity is pH-dependent and if anion exchanger 2 (AE2) protects against bile acid-induced damage.

Methods: A human cholangiocyte cell line was exposed to chenodeoxycholate (CDC), or its glycine-conjugate from 0.5 mM to 2.0 mM at pH 7.4, 7.1, 6.7 or 6.4, or after knockdown of AE2. Cell viability and apoptosis were determined by WST and caspase-3/-7 assays, respectively.

Results: CDC and GCDC (pKa 4-5) induce cholangiocyte toxicity in a pH dependent manner (fig.1). 0.5 mM CDC and 1 mM GCDC at pH 7.4 had no effect on cell viability, but at pH 6.4 decreased viability by 80.8 ± 7.0 and 81.6 ± 13.1% ($p < 0.01$ vs. pH 7.4, $n = 3$) and increased caspase activity 10- and 26-fold, respectively ($p < 0.01$ vs. pH 7.4, $n = 3$). Acidification alone had no effect. AE2 knockdown led to 4- and 2-fold enhanced apoptosis induced by 0.75 mM CDC or 2 mM GCDC at pH 7.4 ($p < 0.05$ vs. control cells, $n = 4$).

Discussion: These data support our hypothesis of a biliary HCO_3^- umbrella serving to protect human cholangiocytes against bile acid-induced injury. AE2 is a key contributor to this protective mechanism. The development and progression of cholangiopathies such as primary biliary cirrhosis may be a consequence of genetic and acquired functional defects of genes involved in maintaining the biliary HCO_3^- umbrella.

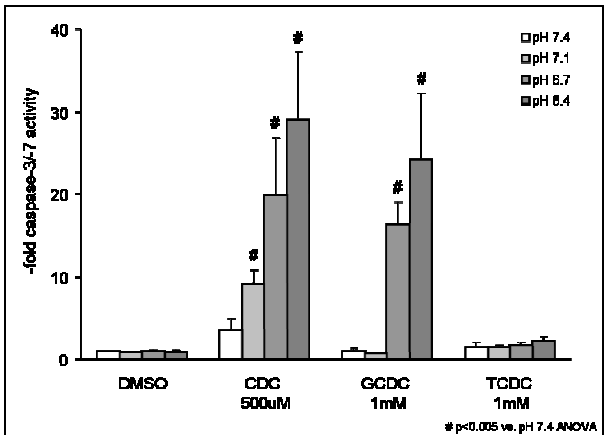

Fig. 1: Bile acid-induced BEC toxicity is pH dependent.

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Localization and function of the membrane-bound bile acid receptor TGR5 in brain

Verena Keitel, Boris Görg, Dieter Häussinger
Klinik für Gastroenterologie, Hepatologie und Infektiologie, Heinrich-Heine-Universität, Moorenstr. 5, 40225 Düsseldorf, Germany

Introduction: TGR5 (Gpbar-1) is a membrane-bound bile acid receptor, which is expressed in many tissues. TGR5 mRNA has been detected in human and mouse brain, however, it is unknown which cell types in the brain express the receptor. Aim of the present study was to identify which brain cells express TGR5. Furthermore, since bile acid concentrations are low in brain tissue, we searched for potential TGR5 ligands other than bile acids.

Methods: Expression and localization of TGR5 was determined by realtime PCR and immunofluorescence staining. Potential TGR5 ligands were analyzed in HEK293 cells, which were cotransfected with TGR5 and a cAMP sensitive luciferase gene. TGR5 expression, localization and activation were analyzed in isolated astrocytes and neurons.

Results: TGR5 was localized in astrocytes and neurons. Neurosteroids, such as 5β-pregnan-3α-ol-20-one activated TGR5 already at nanomolar concentrations. Stimulation of TGR5 in astrocytes and neurons increased intracellular cAMP and calcium levels. Furthermore, incubation of astrocytes with the neurosteroid 5β-pregnan-3α-ol-20-one induced the generation of reactive oxygen species. Treatment of cultured astrocytes with ammonia or 5β-pregnan-3α-ol-20-one led to a significant downregulation of TGR5 mRNA expression. TGR5 mRNA levels were also significantly reduced in brain tissue from patients with hepatic encephalopathy as compared to tissue from control subjects.
Furthermore, cultivation of rat astrocytes in the presence of ammonia significantly reduced TGR5 protein levels. This downregulation of TGR5 was associated with a reduced calcium response of the ammonia-treated astrocytes towards 5β-pregnan-3α-ol-20-one.

Discussion/Conclusion: The present study demonstrates that the membrane-bound bile acid receptor TGR5 is localized in both, astrocytes and neurons. TGR5, up to now regarded as a bile acid receptor, can also be activated by endogenous neurosteroids thereby increasing intracellular cAMP and calcium levels. Thus, in brain TGR5 may act as a novel neurosteroid receptor with implications for the pathogenesis of hepatic encephalopathy.

The biliary glycocalyx protects biliary epithelial cells from bile acid induced toxicity

L. Maillette de Buy Wenniger, S. Hohenester, R.P. Oude-Elferink, U. Beuers
Academic Medical Center of the University of Amsterdam, Department of Gastroenterology and Hepatology, Tytgat Institute of Liver and Intestinal Research, Amsterdam, The Netherlands

Introduction: Bile acids have detrimental effects in many cell types including hepatocytes already in the micromolar range. In the healthy liver, biliary epithelial cells (BEC) display an extraordinary resistance against millimolar concentrations of bile acids present in bile. Mechanisms by which BEC protect themselves against bile acid toxicity are not completely understood. We hypothesize that BEC form a biliary HCO_3^- umbrella stabilized by a layer of glycosylated proteins analogous to the glycocalyx on the apical side of the intestinal epithelium. The glycocalyx may contribute to protection against bile acid induced toxicity.

Methods: Expression of a glycocalyx on BEC in human and mouse liver and on a human BEC line was evaluated by electron microscopy using potassium ferricyanide and ruthenium red staining. BEC were exposed to 500 µM chenodeoxycholate (CDC, pKa > 4) at pH 6.7 after enzymatic digestion of the glycocalyx by neuraminidase (1 U/ml), respectively, or control treatment. After four hours, metabolic activity as a measure of cell viability was determined by WST assays.

Results: A glycocalyx layer of 20–40 nm was consistently detected on the apical membrane of human and mouse BEC in liver tissue and on cultured BEC. Stimulation with 500 µM CDC decreased metabolic activity of cultured BEC by 36.5 ± 8.7% in comparison to controls. Digestion of the glycocalyx with neuraminidase prior to CDC treatment exacerbated bile salt toxicity and decreased metabolic activity by 59.1%± 9.3% ($p = 0.02$ vs. control, $n = 4$).

Conclusion: Biliary epithelial cells maintain a glycocalyx layer on the luminal surface of their apical membrane *in vivo* and *in vitro*. Enzymatic digestion of this layer renders BEC more susceptible to bile acid induced toxicity. The biliary glycocalyx thus adds to the barrier function of the apical membrane of BEC against bile acid induced toxicity.

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Analysis of chenodeoxycholate-binding proteins in rat brain and pituitary by mass spectrometry coupled with cleavable affinity extraction

Nariyasu Mano[1,2], Kohei Abe[1,2], Masaru Mori[1,2], Koichi Sato[2], Hiroaki Yamaguchi[1,2], Miki Shimada[1,2], Takaaki Goto[2], Junichi Goto[1]
[1]Department of Pharmaceutical Sciences, Tohoku University Hospital, Sendai, Japan
[2]Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan

Introduction: We have reported the presence of bile acids in rat brain. Chenodeoxycholic acid (CDCA) could only be detected upon extraction with high concentration of guanidine hydrochloride, indicating that it noncovalently binds to some proteins in the brain. To identify such binding proteins, we have developed a new affinity gel in which the small molecule is immobilized via the cleavable disulfide linker. In this study, we extracted several potential CDCA-binding proteins from the cerebrum, midbrain, cerebellum, brain-steam, hippocampus, and pituitary of rat by using the cleavable affinity gel.

Methods: All tissues from Wistar rats were homogenized in phosphate buffer (pH 7.4), and they were added to CDCA-immobilized cleavable gel. After incubation for capturing binding proteins and centrifugation for removing the supernatant, the gel was mildly washed with phosphate buffer (pH 7.4). Then, we added phosphate buffer (pH 7.4) containing 0.14% DTT to the gel for collection of CDCA-binding proteins.
After SDS-PAGE, gel pieces containing the target proteins were excised, and they were in-gel digested and analyzed by mass spectrometry.

Results: We found mutual three bands on SDS-PAGE of all affinity extracted samples, and identified proteins in those bands by the mass spectrometric analysis and the subsequent database search. The potential CDCA-binding proteins were tubulin beta-2A chain/alpha-1B chain, actin cytoplasmic 1/2, and 14-3-3 protein zeta/delta. In addition, growth hormone was also extracted as a potential CDCA-binding protein from the pituitary. We demonstrated the affinity labeling of the rat recombinant growth hormone by use of chenodeoxycholyl acyladenylate as an affinity labeling reagent. We clarified that CDCA bonds to the approximate epsilon-amino groups of Lys55, Lys196, Lys205, and probably Lys192.

Discussion/Conclusion: We extracted several potential CDCA-binding proteins in rat brain and pituitary by using a cleavable affinity gel.

Transient appearance of myofibroblasts in fetal hearts promotes arrhythmias which can be prevented by ursodeoxycholic acid

Michele Miragoli[1], Siti H. Sheikh Abdul Kadir[1], Nicoló Salvarani[2], Matilda Virta[1], Sarah Wells[1], Max J. Lab[1], Viacheslav O. Nikolaev[1], Alexey Moshkov[1], Stephan Rohr[2], Catherine Williamson[3], Julia Gorelik[1]
[1]National Heart and Lung Institute, Imperial College London, London SW3 6LY, UK
[2]Department of Physiology, University of Bern, Bühlplatz 5, 3012 Bern, Switzerland
[3]Institute of Reproductive and Developmental Biology, Imperial College London, Du Cane Road, London, W12 0NN, UK

Introduction: Myofibroblasts appear during structural remodelling of the adult diseased heart. They contribute to arrhythmogenesis and mechanical and electrical dysfunction by excessive production of extracellular matrix. *In-vitro*, they depolarize rat cardiomyocytes following heterocellular gap junctional coupling. Recently it has been hypothesized that myofibroblasts might appear in the developing human heart, triggered by physiological fetal hypoxia. However, their presence and functional effects in fetal hearts (FH) have never been studied. We sought to investigate whether the presence of myofibroblasts in FH exposed to maternal cholestasis may underlie fetal arrhythmias encountered under these conditions and whether myofibroblast may serve as a direct target for antiarrhythmic therapy.

Methods: Immunohistochemistry demonstrated the transient appearance of myofibroblasts in human FH between 9 and 22 weeks of gestation. We have investigated the susceptibility to bile acid-induced arrhythmia in *in-vitro* models of the cholestatic maternal heart (MH) and FH. The MH model consisted of strands of cardiomyocytes, while for the FH model, we added a layer of rat cardiac myofibroblasts on top of cardiomyocytes.

Results: Addition of bile acids to FH but not MH models, slowed impulse conduction velocity from 19 cm/s to 9 cm/s, induced early afterdepolarizations and resulted in sustained re-entrant arrhythmias. These arrhythmic events were prevented by ursodeoxycholic acid which hyperpolarized the membrane potential of myofibroblast by modulating their potassium conductance.

Discussion/Conclusion: These data suggest that fetal myofibroblasts transiently appear in the FH where they might contribute to fetal arrhythmias during maternal cholestasis and, accordingly, may represent a novel therapeutic target for the treatment of this type of arrhythmias.

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Cholic acid feeding during gestation affects the fetal cholesterol and lipid profile. A role for the placenta in cholesterol and lipid transport?

Georgia Papacleovoulou[1], Alexandra Milona[1], Bryn Owen[1], Eugene Jansen[2], A.S. Knisely[3], Catherine Williamson[1]
[1]Maternal and Fetal Disease Group, Institute of Reproductive and Developmental Biology, Imperial College London, Du Cane Road, London W12 0NN, UK
[2]The Laboratory for Health Protection Research, National Institute for Public Health and the Environment, Bilthoven, The Netherlands
[3]Institute of Liver Studies/Histopathology, King's College Hospital, Denmark Hill, London, SE5 9RS, UK

Introduction: Developmental programming describes the ability of an identical genotype to generate more than one phenotype in response to environmental conditions. Epidemiological studies have shown that maternal malnutrition during gestation increases offspring susceptibility to the development of metabolic disease. Intrahepatic cholestasis of pregnancy (ICP) is a liver-specific disease that is characterised by increased serum bile acid (BA) levels in the second half of pregnancy. We aimed to establish the effects of cholestatic pregnancy on the metabolic profile of the fetus.

Methods: C57BL/6 mice were used (6 mice/group). Cholestatic pregnancy was induced with supplementation of 0.5% cholic acid (CA) in the standard diet (ERD) and animals were sacrificed on gestational day 18. Maternal and fetal sera were collected for biochemical analyses and maternal liver, fetal liver, and placenta were collected for lipid measurements as well as histology, gene and protein expression analysis.

Results: BA levels were increased in the fetal serum of CA-fed mothers and the hepatic BA receptor, Fxr, was activated as indicated by alterations in its target genes (reduced Cyp7a1, increased Shp and Bsep). Hepatic cholesterol and fatty acid synthesis was induced in the fetuses of CA-fed mothers as shown by increase of Srebp2, Srebp1c, Hmgcr and Fas ($p < 0.05$). Fetal hepatic cholesterol and triglycerides were also increased ($p < 0.05$). In the placenta of the CA-fed mothers, expression levels of lipogenic-related genes such as Adrp, Ldlr and Acat-2 were increased ($p < 0.05$), accompanied by raised placental cholesterol and decreased ApoB ($p < 0.05$). CA-fed maternal livers were steatotic.

Discussion/Conclusion: Cholestatic pregnancy leads to fetal cholestasis and to increased fetal hepatic cholesterol and fatty acid biosynthesis. The placental gene expression profile implies that cholesterol and lipids are accumulated in placenta instead of crossing into the fetal environment. Increased hepatic cholesterol and fatty acid biosynthesis in the fetus could present an adaptation to fulfil fetal nutritional demands.

Altered lipid homeostasis in normal and cholestatic pregnancy in mice

Georgia Papacleovoulou[1], Alexandra Milona[1], Bryn Owen[1], Eugene Jansen[2], A.S. Knisely[3] and Catherine Williamson[1]
[1]Maternal and Fetal Disease Group, Institute of Reproductive and Developmental Biology, Imperial College London, Du Cane Road, London W12 0NN, UK
[2]The Laboratory for Health Protection Research, National Institute for Public Health and the Environment, Bilthoven, The Netherlands
[3]Institute of Liver Studies/Histopathology, King's College Hospital, Denmark Hill, London, SE5 9RS, UK

Introduction: Intrahepatic cholestasis of pregnancy (ICP) is a liver-specific disease characterised by increased serum bile acid (BA) levels in the second half of pregnancy. It is multifactorial with a complex aetiology. We previously showed that normal mouse pregnancy is associated with a pre-cholestatic phenotype, as serum BA are increased, although less than in pregnant mice with cholestasis induced by a cholic acid (CA) supplemented diet, *i.e.* exogenously induced cholestasis (EIC) pregnant mice. We screened lipid profiles in normal pregnant mice and compared them with lipid profiles of EIC pregnant mice.

Methods: C57BL/6 mice were used (6 mice/group). Cholestatic pregnancy was induced with 0.5% CA in the standard diet (ERD) and animals sacrificed on gestational day 18. Day 1 of pregnant mice were used as controls. Maternal and control sera were collected for biochemical analyses. In parallel, histology, gene and protein expression assays were performed in livers.

Results: Serum total cholesterol as well as LDL- and HDL-cholesterol were decreased in normal pregnancy ($p < 0.05$), whilst in cholestatic pregnancy HDL-cholesterol was reduced, with LDL-cholesterol unaltered. Free fatty acids and triglycerides were consistently high in EIC pregnant mice. Like serum cholesterol, hepatic cholesterol was reduced in normal pregnancy (8-fold change; $p < 0.05$). Cholestatic pregnancy was also characterised by lower cholesterol levels albeit at a lower degree (3-fold change; $p < 0.05$). These data were consistent with the hepatic gene expression profile where cholesterol biosynthesis (Srebp2, Hmgcr) and transport (Abca1, Abcg1 and Ldlr) pathways were reduced in normal pregnancy ($p < 0.05$) but not in cholestatic pregnancy. Furthermore, EIC pregnant mouse livers were steatotic.

Discussion/Conclusion: Differential gestational effects take place during normal and cholestatic pregnancy. Bile acid accumulation in the maternal serum of CA-fed mice results in liver steatosis, high levels of LDL-cholesterol, accumulation of free fatty acids and triglycerides. On the other hand, cholesterol homeostatic pathways are reduced in normal mouse pregnancy.

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Bile acid toxicity SAR: Correlations between cell viability and chromatographic lipophilicity in a panel of new and known bile acids

R. Sharma[1,2], F. Majer[1], Vijaya Kumar Peta[1], Jun Wang[1], Ray Keaveney[1], D. Kelleher[2], A. Long[1], J.F Gilmer[1]
[1]School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Ireland
[2]Institute of Molecular Medicine, St. James's Hospital, Dublin, Ireland

Introduction: The molecular mechanisms and interactions underlying bile acid cytotoxicity are important to understand for intestinal and hepatic disease treatment and prevention and for the design of bile acid based therapeutics. Bile acid hydrophobicity is believed to be an important cytotoxicity determinant but this relationship is not well characterized.

Methods: In this study we synthesised new azido and other hydrophobic bile acids. We assembled a panel of 37 bile acids with a good dispersion in hydrophobicity as determined by reverse phase thin layer chromatography. R_{Mw} which is extrapolated retention in 100% aqueous phase was used as a measure of hydrophobicity. The MTT cell viability assay was used to assess cytotoxicity over 24 h in the normal esophageal cell line, the HET-1As.

Results: R_{Mw} values inversely correlated with cell viability for the entire panel of compounds ($r^2 = 0.6$) but this became more significant when non-acid compounds were excluded ($r^2 = 0.82$, $n = 29$). The association in more homologous subgroups was stronger still ($r^2 > 0.96$). None of the polar compounds were cytotoxic at 500 μM, however, not all lipophilic BAs were cytotoxic. Notably, apart from the UDCA primary amide, lipophilic neutral derivatives of UDCA were not cytotoxic. Finally, CDCA, DCA and LagoDCA were prominent outliers being more toxic than predicted by R_{Mw}.

Discussion/Conclusion: The study shows that azido substitution in bile acids imparts lipophilicity, and toxicity provided the BA is ionizable; (ii) there is an inverse correlation between R_{Mw} and toxicity that has good predictive value in homologous sets; (iii) Lipophilicity is a necessary but not sufficient characteristic for BA cytocidal activity.

Relationship between serum paraoxonase activity and bile acids level in patients with chronic liver diseases

L. Turecký[1], V. Kupčová[2], M. Szántová[2], E. Uhlíková[1]
Institut für Medizinische Chemie und Biochemie[1] and III. Medizinische Klinik[2], Medizinische Fakultät, Komensky Universität, Bratislava, Slovakia

Introduction: Chronic liver diseases are characterized by the concomitant presence of oxidative stress and a severe inflammatory response. The ubiquitous presence of antioxidant enzymes may represent an important defence mechanism in diminishing the burden of the pro-oxidant stimuli. Paraoxonase (PON) is hydrolytic enzyme synthesized in humans mainly by the liver and plays an important role in the organism's antioxidant and anti-inflammatory systems. Recently, some reports showed inhibitory effect of bile acids on the synthesis of PON.
The aim of our study was to investigate the correlation between PON activity and the levels of bile acids and some other liver parameters.

Methods: We studied 48 patients with chronic liver diseases (NASH, liver cirrhosis). The activity of PON was analyzed with paraoxon as substrate. The levels of procollagen III peptide, hyaluronic acid and TNF-alpha were determined with ELISA methods. The levels of bile acids were determined enzymatically.

Results: We found significant negative correlation between the activity of PON and serum bile acids levels. There was also correlation between the activity of PON and the levels of both fibromarkers (procollagen III peptide, hyaluronic acid) in our group of patients with chronic liver diseases.

Discussion/Conclusion: The results of our study support the hypothesis of inhibitory effect of increased level of bile acids on the synthesis of paraoxonase. Decreased PON activity enhances oxidative stress, which is an important factor in the activation of fibrogenesis. Negative correlation between PON activity and the levels of fibromarkers supports this role of oxidative stress in fibrogenesis.

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Chemical synthesis of the (25R)- and (25S)-epimers of 3α,7α,12α-trihydroxy-5α-cholestan-27-oic acid as well as their corresponding glycine and taurine conjugates

Takashi Iida[1], Shoujiro Ogawa[1], Matthew D. Krasowski[2], Lee R Hagey[3], Alan F. Hofmann[3]
[1]Department of Chemistry, College of Humanities and Sciences, Nihon University, Tokyo, Japan
[2]Department of Pathology, University of Iowa Hospital and Clinics, Iowa City, IA, USA
[3]Department of Medicine, University of California, San Diego, CA, USA

Introduction: Naturally occurring bile acids and bile alcohols differ markedly in their chemical structures; such chemical diversity results from the evolution of differing biochemical pathways that serve to convert cholesterol into conjugated bile salts. To our knowledge, a synthetic route from C_{24} 5β-bile acids to epimeric (25*R*)- and (25*S*)-3α,7α,12α-trihydroxy-5α-cholestan-27-oic acids (5α-THCAs) has not hitherto been reported, although synthesis of the 5β-epimers was reported some years ago. We herein report chemical synthesis of (25*R*)- and (25*S*)-epimers of 5α-THCA as well as that of their corresponding glycine and taurine conjugates.

Methods: As shown in the figure, our synthetic pathway from cholic acid (CA) involves *allo*-cholic acid as a key intermediate. Then, the target (25*R*)- and (25*S*)-5α-THCAs (**1a** and **1a'**) were prepared from *allo*-CA in six steps. The principal reactions employed were (1) iodination of 3,7,12-triformyl-5α-cholan-24-ol with I_2/triphenyl-phosphine, (2) nucleophilc substitution of the iodo derivative with diethylmethyl malonate/NaH, and (3) complete hydrolysis of the resulting 3,7,12-triformyl-25-methyl-26,27-diethyl ester with KOH followed by decarboxylation of the resulting geminal dicarboxylic acid with LiCl to give a mixture of (25*R*)- and (25*S*)-5α-THCAs (**1a** and **1a'**). After isolation of the individual epimers by HPLC, each epimer was *N*-acylamidated with glycine or taurine to yield the desired compounds **1b, 1b', 1c** and **1c'**.

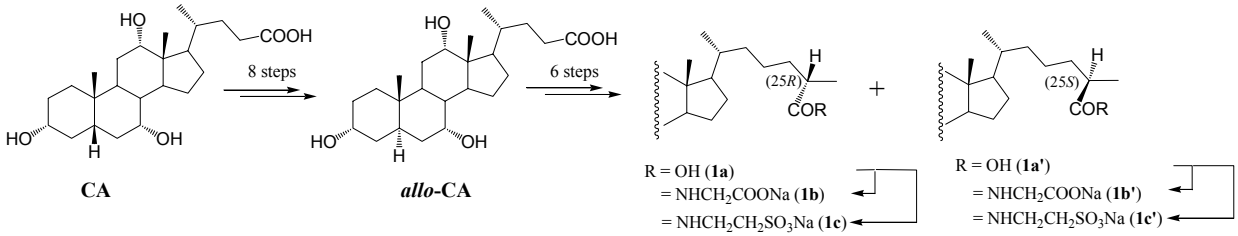

Fig.

Results: All the reactions proceeded cleanly to give the desired compounds in good isolated yields. Overall yield of (25*R*)- and (25*S*)-5α-THCAs (**1a** and **1a'**) from CA in 14 steps was *ca.* 14%. The structures of the isolated compounds epimeric at C-25 were established definitively by 2D-NMR techniques. The corresponding conjugates were then prepared by *N*-acylamidation of **1a** and **1a'** with glycine or taurine using diethyl phosphorocyanidate and triethylamine as coupling agents.

Discussion/Conclusion: The unconjugate and *N*-acylamidated conjugates with glycine or taurine of epimeric C_{27} 5α-THCAs were prepared from CA. These authentic reference compounds should be useful for their identification in biological fluids as well as studies on the biosynthesis of bile acids from cholesterol.

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Itch intensity in cholestasis correlates with autotaxin but not bile salts levels

Andreas E. Kremer[1], Job J. Martens[1], Ulrich Beuers[1], Ronald P.J. Oude Elferink[1]
[1]Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, University of Amsterdam, The Netherlands

Introduction: Pruritus is a common symptom of patients with cholestatic disorders. Bile salts and opioids have been held responsible for the induction of pruritus, but a relation with itch intensity has never been established. Screening sera of cholestatic patients for neuronal activation we have recently identified lysophosphatidic acid (LPA) as main activator. LPA, which is formed from lysophosphatidylcholine by the enzyme autotaxin (ATX), was increased only in those cholestatic patients suffering from pruritus.

Methods: Autotaxin activity was measured enzymatically in diluted serum samples of patients with chronic cholestatic disorders (mainly primary biliary cirrhosis [PBC] and primary sclerosing cholangitis; $n = 72$) and healthy controls (HC; $n = 202$). Total serum bile salts (TBS) were quantified by enzymatic assays, μ-opioid activity by receptor binding assay. In mice, scratch activity after intradermal pruritogen injection was quantified using a magnetic device.

Results: TBS and μ-opioids did not show any correlation with itch intensity. In contrast, ATX activity highly correlated with intensity of pruritus ($n = 52$, $r = 0.63$, $p < 0.0001$). Furthermore, ATX was markedly increased in sera of cholestatic patients with vs. without pruritus (2.6-fold, $p < 0.0001$). In PBC patients who underwent nasobiliary drainage both, itch intensity (-80%, $p < 0.01$) and autotaxin activity (-45%, $p = 0.01$), decreased during drainage and returned to pre-treatment levels when pruritus had returned, whereas TBS showed no correlation with itch intensity. In end-stage liver disease when cholestasis is most pronounced and bile salt concentrations reach their highest values pruritus often subsides. Interestingly, in-vitro ATX activity was suppressed by the 3,7-dihydroxy-bile salts chenodeoxy-cholate, ursodeoxycholate and their glyco- and tauro-conjugates. LPA, but not bile salts, injected intradermally into mice, induced scratch responses.

Conclusion: Our data suggest that autotaxin and its product, LPA, play a key role in cholestatic pruritus. The inhibitory effect of dihydroxy-bile salts on ATX activity could explain the enigmatic amelioration of pruritus in terminal liver failure.

Direct involvement of the muscarinic M_2 receptor in bile acid-induced fetal arrhythmia in intrahepatic cholestasis of pregnancy

Siti H. Sheikh Abdul Kadir[1,2], Michele Miragoli[1], Shadi Abu-Hayyeh[2], Alexey V. Moshkov[1], Verena Keitel[3], Viacheslav O. Nikolaev[1], Catherine Williamson[2], Julia Gorelik[1*]
[1]National Heart and Lung Institute, Imperial College London, London SW3 6LY, UK
[2]Institute of Reproductive and Developmental Biology, Imperial College London, Hammersmith Campus, Du Cane Road, London, W12 0NN, UK
[3]Clinic of Gastroenterology, Hepatology and Infectiology, Heinrich-Heine-University, Moorenstr. 5, 40225 Düsseldorf, Germany

Introduction: Intrahepatic cholestasis of pregnancy (ICP) is a common disease affecting up to 5% of pregnancies which can cause fetal arrhythmia and sudden intrauterine death. We previously demonstrated that bile acid taurocholate (TC) which is raised in the bloodstream of ICP can acutely alter the rate and rhythm of contraction and induce abnormal calcium destabilization in cultured neonatal rat cardiomyocytes (NRCM). Apart from their hepatic functions bile acids are ubiquitous signalling molecules with diverse systemic effects mediated by either the nuclear receptor FXR or by a recently discovered G-protein coupled receptor TGR5. We aim to investigate the mechanism of bile-acid induced arrhythmogenic effects in an *in-vitro* model of the fetal heart.

Methods: Levels of bile acid transporters and nuclear receptor FXR were studied by quantitative real time PCR, western blot and immunostaining which showed low levels of expression.

Results: We did not observe functional involvement of the canonical receptors FXR and TGR5. Instead, we found that TC binds to the muscarinic M_2 receptor in NRCM and serves as a partial agonist of this receptor in terms of inhibitory effect on intracellular cAMP and negative chronotropic response. Pharmacological inhibition and siRNA-knockdown of the M_2 receptor completely abolished the negative effect of TC on contraction, calcium transient amplitude and synchronisation in NRCM clusters.

Discussion/Conclusion: We conclude that in NRCM the TC-induced arrhythmia is mediated by the partial agonism at the M_2 receptor. This mechanism might serve as a promising new therapeutic target for fetal arrhythmia.

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The effect of bile acids and bile acid analogues on store-operated calcium entry (SOCE) in RBL-2H3 cells, HepG2 cells, clone9 cells, and rat hepatocytes

Michiko Yoshii, Ai Tarao, Fukumi Kodama, Koichiro Ozawa
Division of Clinical Pharmacotherapeutics, Programs for Applied Biomedicine, Graduate School of Biomedical Sciences Hiroshima University, Kasumi, 1-2-3, Minami-ku, Hiroshima, Japan, E-Mail: ymichik@hiroshima-u.ac.jp

An increase in cytosolic Ca^{2+} levels in mast cells that are stimulated via the IgE receptor or thapsigargin provides an essential signal for exocytotic release of granules, histamine release etc. This increase of cytosolic Ca^{2+} level is associated with release with Ca^{2+} stores located in the endoplasmic reticulum and influx of external Ca^{2+} by a mechanism, referred to as SOCE. Meanwhile in primary rat hepatocytes, SOCE plays a role in the maintenance of agonist- induced calcium oscillations. In the present study we investigated inhibitory effect of bile acids and bile acid analogues on histamine release from RBL-2H3 cells and on SOCE in RBL-2H3 cells and cultured hepatocytes.
We compared with the inhibitory effect of histamine release with UDC-OH and other CRAC channel inhibitors (SKF-96365 or YM-58483). YM-58483 (10 μM) and UDC-OH (75 μM) reduce to 10% of control histamine release and SKF-96365 (100 μM) poorly reduces by 30% control histamine release.
Figure 1 shows the change in $[Ca^{2+}]_{in}$ induced with DNP-BSA with UDC-OH and following with SKF-96365 or YM-58483. SKF-9635 and YM-58483 reduced further the $[Ca^{2+}]_{in}$ reduce with UDC-OH. Possibly histamine release inhibitory mechanism of UDC-OH is different from SKF-96365 and YM-58483. These reduce was caused with inhibition of external Ca^{2+} influx that concerns with at leaset two proteins, STIM1 a Ca^{2+} sensor and Orai1 a channel protein.

Results/Discussion: In 29th Symposium on Biomembrane-drug Interaction, we demonstrated that bile acids or bile alcohols had inhibitory effect of histamine release from mast cells and one of the C_{24} bile alcohols, UDC-OH was most effective inhibitor.
In this study we investigated these proteins and TRPC5 that interacts with STIM1, with Blue Native page-western blotting or Blue Native page-SDS page-western blotting. SKF-96365 or YM-58483 treatment induced STIM1 aggregation even without Tg stimulation. UDC-OH treatment didn't induce STIM1 aggregation without Tg stimulation, and induced STIM1 aggregation with Tg stimulation. Orai1 protein interacted with STIM1 was observed in control and UDC-OH treatment samples from immunoblotting results. From immunoblotting results of TRPC5, no defference was observed between SKF-96365, YM-58483, and UDC-OH treated samples from control samples.
It remains possible that UDC-OH interacts with Orai1 directly and stops effluxing Ca^{2+}, or UDC-OH interacts with $iPLA_2\beta$ and stops effluxing Ca^{2+} by $iPLA_2\beta$-dependent pathway.

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7-Alpha-hydroxy-4-cholesten-3-one can be a clinically useful circulating marker for liver and intestinal disease

M. Axelson
Department of Clinical Chemistry, Karolinska Institutet and Hospital, Stockholm, Sweden

Introduction: Several biochemical tests for liver and intestinal dysfunction exist, yet there is a need for additional tests. We have previously demonstrated that the levels of 7-alpha-hydroxy-4-cholesten-3-one (C4) in plasma reflect rates of bile acid synthesis in man. Analysis of C4 has since then been widely used to study short- and long-term effects of various factors, such as drugs, individual bile acids, diets, alcohol etc. on bile acid production in humans. However, analysis of C4 has clinically mainly been limited to diagnose patients with chronic diarrhoea caused by bile salt malabsorption. Since the rate of bile acid synthesis depend on the condition of both the liver and the intestine, we have now studied the possibility to clinically use C4 as a more general marker for liver and intestinal diseases in patients.

Methods: Plasma levels of C4 from patients with various liver and intestinal diseases have been analysed.

Results: The results show that most patient groups with liver disease had subnormal levels of C4 and, as expected, patient groups with ileal dysfunction or ileal resection had pathologically elevated levels. Many of the patients with fat malabsorption had, however, normal levels of C4. In order to permit a large number of samples to be processed which is required for a clinical method in routine use, a rapid and sensitive isotope-dilution liquid-chromatography-tandem mass spectrometry (LC-MS/MS) method for C4 in plasma was developed (A. Lövgren Sandblom, I. Björkhem and M. Axelson).

Discussion/Conclusion: The results suggest that analysis of 7-alpha-hydroxy-4-cholesten-3-one (C4) in plasma can provide valuable information about the condition of the liver and intestines when investigating patients with suspected liver or intestinal dysfunction.

Metabolic profile of IBS patients with bile acid malabsorption compared to those with normal bile acid turnover rate

Antal Bajor[1], Mats Rudling[2], Kjell-Arne Ung[3], Jenny Wallin[1], Magnus Simrén[1]
[1]Department of Internal Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[2]Center for Biosciences and Nutrition, Department of Medicine, Karolinska Institute at Karolinska University Hospital, 141 86 Stockholm, Sweden
[3]Department of Internal Medicine, Kärnsjukhuset, Skövde, Sweden

Introduction: The removal of cholesterol from the human body is either by direct excretion to the bile or by catabolism through bile acids (BAs). However, it is not known whether the BA metabolism mirrored by the ^{75}SeHCAT test is correlated to plasma lipid profiles and other cardiovascular risk factors. BA malabsorption is commonly diagnosed using the ^{75}SeHCAT test or with the plasma marker of BA synthesis (C4). After absorption into the enterocyte bile acids trigger the secretion of the gut hormone FGF-19 which in turn exerts a strong feedback inhibition of BA synthesis in the liver.

Aims: to assess whether the turnover rate of BAs measured by the ^{75}SeHCAT test have an impact on plasma lipid levels, BA synthesis, FGF-19, or body mass index (BMI).

Methods: Irritable bowel syndrome patients (IBS) fulfilling the ROME II criteria referred to our centre has been consecutively included in the study. Exclusion criteria were previous intestinal or gallbladder surgery, celiac disease, diabetes mellitus or any inflammatory bowel disease. All patients performed a ^{75}SeHCAT test. The plasma C4, FGF-19, triglyceride (TG), total cholesterol (TC), and HDL cholesterol concentrations were analyzed.

Results: Patients with BA malabsorption n = 25, (7 day ^{75}SeHCAT retention < 10%) compared to patients with ^{75}SeHCAT > 10%, n = 95, had higher plasma TC (1.5 mmol/L vs. 1.1 mmol/L, $p < 0.05$), C4/chol (11.6 vs. 3.6, $p < 0.01$), BMI (25.8 vs. 23.2, $p < 0.01$) but lower HDL (1.56 mmol/L vs. 1.77 mmol/L, $p < 0.05$) and FGF-19 (99 pg/mL vs. 150 pg/mL, $p < 0.05$) levels.
The cholesterol concentration did not differ.

Discussion/Conclusion: In IBS patients, idiopathic BA malabsorption is linked to higher BMI and plasma triglycerides and lowered HDL cholesterol concentrations whereas total cholesterol is unaltered.

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Is the bile acid pool enlarged in IBS patients with bile acid malabsorption?

Antal Bajor[1], Mats Rudling[2], Kjell-Arne Ung[3], Jenny Wallin[1], Magnus Simrén[1]
[1]Department of Internal Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[2]Department of Endocrinology, Metabolism & Diabetes and Center for Biosciences and Nutrition, Department of Medicine, Karolinska Institute at Karolinska University Hospital, 141 86 Stockholm, Sweden
[3]Department of Internal Medicine, Kärnsjukhuset, Skövde, Sweden

Introduction: Idiopathic bile acid malabsorption (IBAM) is commonly diagnosed using the ^{75}SeHCAT test or from estimation of bile acid (BA) synthesis by measuring the plasma marker C4. The patophysiology of BAM is unknown, but a previous study has shown an enlarged BA pool which in turn overrides the BA absorption capacity of the ileum.

Aim: To attempt to estimate the BA pool from ^{75}SeHCAT and BA synthesis measures and to compare such data from IBS patients with BAM and other IBS patients with normal BA metabolism.

Methods: IBS patients fulfilling the Rome II criteria referred to our centre were consecutively included in the study, irrespective of the bowel habit. They performed a seven day retention ^{75}SeHCAT test. Blood samples were taken after overnight fasting for analysis of C4. We assumed steady state conditions so where hepatic synthesis of BAs should equal fecal BA losses. The ^{75}SeHCAT test measures the fraction in % of the BA pool left in the enterohepatic circulation after 7 days and the fecal BA losses are in turn [100% – (^{75}SeHCAT test)].
We estimated the magnitude of the BA pool by using the formula [100 x C4 / (100% – ^{75}SeHCAT%).

Results: Altogether 116 patients performed the ^{75}SeHCAT test and C4 analyses and 22 of them had ^{75}SeHCAT retention < 10 on day 7 (IBAM). These patients had significantly larger estimated BA pool compared to patients with ^{75}SeHCAT > 10% (12.1 vs. 6.5 arbitrary units) $p < 0.01$.

Discussion/Conclusion: Patients with IBAM might have an enlarged BA pool compared to patients with normal ^{75}SeHCAT test. Our data are in line with the thinking that IBAM may be caused by excessive hepatic BA synthesis resulting in an enlarged BA pool which in turn overrides the ileal uptake capacity. However our current estimates needs to be validated by other tests such as isotope dilution methods.

Genetic susceptibility to intrahepatic cholestasis of pregnancy

Peter H. Dixon[1], Christopher A. Wadsworth[2], Jennifer Chambers[1], Jennifer Donnelly[3], Sharon Cooley[3], Sheba Jarvis[1], Ralph Kubitz[4], Frank Lammert[5], Hans-Ulrich Marschall[6], Anna Glantz[7], Shahid Khan[2], John Whittaker[8], Michael Geary[3], Catherine Williamson[1]
[1]Maternal and Fetal Disease Group, Institute of Reproductive and Developmental Biology, Imperial College London, Du Cane Road, London W12 0NN, UK
[2]Department of Hepatology and Gastroenterology, Imperial College London, UK
[3]The Rotunda Hospital, Parnell Square, Dublin 1, Ireland
[4]Department of Gastroenterology, Hepatology and Infectious Diseases, University of Düsseldorf, Moorenstr. 5, 40225 Düsseldorf, Germany
[5]Department of Medicine I, Saarland University Hospital, Kirrberger Str. 1, 66421 Homburg, Germany
[6]Karolinska Institutet, Department of Medicine at Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden
[7]Mödrahälsovårdsöverläkare i Göteborg, Primärvårdskansliet, Lillhagsparken 6, 422 50 Hisings Backa, Sweden
[8]London School of Hygiene and Tropical Medicine, University of London, 105a Keppel Street Building, London WC1E 7HT, UK

Introduction: Intrahepatic cholestasis of pregnancy (ICP) has a complex aetiology. Genes mutated in progressive familial intrahepatic cholestasis (PFIC) have been implicated in disease pathogenesis in several populations. Heterozygous mutations of these canalicular transporters occur in a subset of ICP cases. Genetic variation around the bile acid sensor FXR *(NR1H4)* has also been described, and recently a population susceptibility allele (the 444A variant of *ABCB11*) identified. We sought to considerably expand population based genetic analysis of ICP by investigation of common variation around six candidate loci with biological plausibility for a role in ICP: *ABCB4, ABCB11, ABCC2, ATP8B1, NR1H4* and *FGF19*.

Methods: 563 ICP patients of white western European origin together with 642 controls from the Rotunda Thrombophilia study were analysed. In total 83 markers were selected from the HapMap dataset using Tagger (Haploview 4.1, build 22; MAF > 0.05, pair-wise comparisons only) capturing the majority of common genetic variation around the six loci. Genotyping was performed by a proprietary allelic discrimination assay (KBioscience, Cambridge, UK). Following QC including conformation of Hardy-Weinberg equilibrium, SNP data were analysed by Armitage's trend test and haplotypes were analysed with Haplostats.

Results: 78 markers passed quality control and Hardy-Weinberg tests. Single SNP analysis identified 6 SNPs clustered in *ABCB11* and 6 SNPs in *ABCB4* that showed significant evidence of association. The minimum Bonferroni corrected p-value for *ABCB11* was 3.7×10^{-4} *(rs3815676)* and for *ABCB4* 3.4×10^{-7} *(rs2109505)*. The dataset was investigated further by haplotype analysis across all six loci, which identified significant differences in frequencies between cases and controls for *ABCB4* (global p-value 9.6×10^{-6}) and *ABCB11* (global p-value 0.0036).

Discussion/Conclusion: Our analysis of a large cohort of ICP cases has identified a key role for common variation around the *ABCB4* and *ABCB11* loci, expanding on the genetic factors known to play a role in susceptibility to this disease.

Determination of multi-conjugated bile acids in the urine of patients with Niemann-Pick disease, type C by liquid chromatography/electrospray ionization tandem mass spectrometry

Masamitsu Maekawa[1], Yasushi Misawa[2], Ayako Sotoura[2], Osamu Sakamoto[3], Toshihiro Ohura[3], Daiki Abukawa[4], Takehiko Inoue[5], Kousaku Ohno[5], Akina Muto[6], Hajime Takei[6], Hiroshi Nittono[6], Shoujiro Ogawa[7], Genta Kakiyama[7], Takashi Iida[7], Alan F. Hofmann[8], Junichi Goto[9], Miki Shimada[2,9], Nariyasu Mano[2,9]
[1]Innovation of New Biomedical Engineering Center, Tohoku University, Sendai, Japan
[2]Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan
[3]Department of Pediatrics, Tohoku University Hospital, Sendai, Japan
[4]Department of General Pediatrics, Miyagi Children's Hospital, Sendai, Japan
[5]Faculty of Medicine, Tottori University, Yonago, Japan
[6]Junshin Clinic Bile Acid Institute, Tokyo, Japan
[7]College of Humanities and Sciences, Nihon University, Tokyo, Japan
[8]Department of Medicine, University of California, CA, USA
[9]Department of Pharmaceutical Sciences, Tohoku University Hospital, Sendai, Japan

Introduction: Niemann-Pick disease, type C (NPC) is a neurovisceral lipid storage disorder caused by NPC gene defects, characterized clinically by hepatosplenomegaly, vertical gaze palsy, dystonia, and progressive neurodegeneration. It has been reported that abnormal amounts of unusual multi-conjugated bile acids were excreted in the urine of an NPC patient. To reveal a definite diagnosis of NPC relied on the detection of those bile acids, we developed a highly sensitive quantitative method with liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) for 3β,7β-dihydroxy-5-cholen-24-oic acid, sulfated at C-3, N-acetylglucosaminidated at C-7, and glycine-, taurine-, or non-conjugated at C-24.

Methods: LC/ESI-MS/MS was performed using an API-5000 system equipped with an ESI probe under negative ion detection mode. LC separation was achieved with isocratic elution from YMC Pack Pro C18 using 20 mM ammonium acetate buffer (pH 5.5)/methanol (1:1, v/v), at a flow rate of 0.2 mL/min. The SRM the transitions of *m/z* 672 to 97, *m/z* 364 to 433, and *m/z* 389 to 460 were used for quantitation of nonamidated, glycine-amidated, and taurine-amidated form with collision energy of -70 eV, -30 eV, and -40 eV, respectively.

Results: We analyzed and compared concentration of urinary multi-conjugated bile acids in patients with NPC, other metabolic abnormalities, and healthy volunteers. Those unusual bile acids were detected in large amounts from the urine of NPC patients. And small quality of them were found in the urine of other metabolic abnormalities and slightly healthy volunteers, although the urine of a patient with 3β-dehydroxysteroid dehydrogenase deficiency includes those bile acids with the almost the same quality as an NPC patient.

Discussion/Conclusion: We developed a determination method for unusual multi-conjugated bile acids in the urine of an NPC patient by LC/ESI-MS/MS. And we found that large amounts of them were detected in the urine of patients with NPC and 3β-hydroxysteroid dehydrogenase deficiency.

Antibiotic treatment improves absorption of saturated fatty acids in a mouse model for cystic fibrosis

M. Wouthuyzen-Bakker[1], M.J.C. Bijvelds[2], H.R. de Jonge[2], H.J. Verkade[1]
[1]Pediatric Gastro-Enterology and Hepatology, Beatrix Children's Hospital, University Medical Center Groningen, The Netherlands
[2]Department of Biochemistry, Erasmus University Medical Center Rotterdam, The Netherlands

Introduction: In cystic fibrosis (CF) patients, pancreatic enzyme replacement therapy does not completely correct fat malabsorption. Non-pancreatic mechanisms are suggested to play a role in the remaining fat malabsorption, like small intestinal bacterial overgrowth (SIBO). We aimed to determine the contribution of SIBO to fat malabsorption under CF conditions.

Methods: Homozygous ΔF508 mice and wild type littermates received antibiotic treatment (ciprofloxacin and metronidazol) or placebo for three weeks. Before and after treatment, fat malabsorption was quantified by a 72 hours fat balance test. The competence of fat digestion (triglyceride hydrolysis) and of fatty acid uptake were assessed separately by determining plasma appearance of stable isotope-labelled fats, originating from intragastrically administered tri-1-^{13}C-tripalmitin and 1-^{13}C-stearate. After termination, bacterial load in the small intestine was quantified via qPCR and faecal bile salts by gas chromatography.

Results: Bacterial load did not differ between ΔF508 and wild type mice and was not reduced in either genotype after antibiotic treatment. In ΔF508 mice, antibiotic treatment improved the absorption of saturated fatty acids. Plasma concentrations of ^{13}C-fats from tri-1-^{13}C-palmitin and 1-^{13}C-stearate were non-significantly higher in the antibiotic treated ΔF508 mice. Antibiotic treatment reduced faecal excretion of primary and secondary bile salts in ΔF508 mice compared to placebo treatment (primary, 11 ± 5 vs. 19 ± 3 μmol/100 gram BW/day, $p < 0.05$; secondary, 0.4 ± 0.2 vs. 7.0 ± 0.9 μmol/100 gram BW/day, $p < 0.01$, respectively).

Discussion/Conclusion: We conclude that antibiotics improve fat absorption and the enterohepatic circulation of bile salts in homozygous ΔF508 mice, but the mechanism does not seem to involve treatment of SIBO.

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Pleiotropic quantitative trait loci (QTL) influence phenotypes in the ATP8B1-deficient mouse

Bull, Laura N.[1]; Sanford, Ukina[1]; Shah, Sohela[1]; Robinson, Jacquelynn[1]; Paulusma, Coen[2]; Grenert, J.P.[1,3]; Knisely, A.S.[4]; Oude-Elferink, Ronald P.[2]; Sen, Saunak[1,5]
[1]Liver Center, University of California San Francisco, San Francisco, CA, USA
[2]Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, The Netherlands
[3]Department of Pathology, University of California San Francisco, San Francisco, CA, USA
[4]Institute of Liver Studies, King's College Hospital, London, UK
[5]Department of Epidemiology and Biostatistics, University of California San Francisco, San Francisco, CA, USA

Introduction: ATP8B1-deficient *(Atp8b1$^{-/-}$)* mice serve as a model of FIC1/ATP8B1 deficiency in humans. Phenotypes in the *Atp8b1$^{-/-}$* mouse are influenced by strain background. To map QTL modifying phenotypes in *Atp8b1$^{-/-}$* mice, we performed genetic mapping studies using mice of C57Bl/6J (B6) and 129S4 backgrounds, and backcross and intercross strategies.

Methods: *Atp8b1$^{-/-}$* mice (121 backcross, 245 intercross) were phenotyped: At age ≥ 3 months, mice were fed a diet supplemented with 0.5% cholate for 6 days, then sacrificed. DNA samples were genotyped for 242 informative markers; serum collected before and after diet administration was assayed for cholesterol, ALP, AST, ALT, and bilirubin; and concentrations of cholesterol, phospholipid and bile salts in bile were measured. We performed QTL analysis for 14 traits, using J/qtl. Pleiotropic QTL were defined as regions linked to ≥ 4 traits, including at least 1 trait that reached genomewide significance (established using 10,000 permutations), with remaining traits attaining pointwise significance.

Results: Four genomic regions contain pleiotropic QTL. The phenotypes influenced vary between QTL, but in total encompass: initial and final serum cholesterol, ALP, AST, and ALT; final total serum bilirubin; rate of weight loss on cholate diet; liver: body weight ratio; and biliary phospholipid concentration. For 3 of the 4 pleiotropic QTL, serum cholesterol (initial or final) yielded the most significant evidence of linkage. B6 alleles were usually associated with greater phenotypic abnormalities than were 129 alleles.

Discussion/Conclusion: We have genetically mapped 4 pleiotropic modifier QTL of ATP8B1 deficiency in the mouse. The sequence variants underlying these loci likely influence processes central to manifestation of ATP81 deficiency. Their identification will enhance our understanding of the role of ATP8B1, and the human orthologs of genes at these loci may be modifier genes in human ATP8B1 deficiency.

The connection between gut dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases

H. Duboc[1], D. Rainteau[1,2], M. Maubert[1], G. Thomas[1,2], V. Barbu[2], L. Humbert[1,2], G. Despras[5], C. Bridonneau[6], F. Dumetz[6], J.P. Grill[1], J. Masliah[2], L. Beaugerie[3], J. Cosnes[3], O. Chazouillères[4], R. Poupon[4], C. Wolf[1], J.M. Mallet[5], P. Langella[6], G. Trugnan[1], H. Sokol[1,3], P. Seksik[1,3]
[1]ER7, Université Pierre et Marie Curie, 27 rue de Chaligny, Paris, France
[2]Département de Biochimie B et LCBGM, Hôpital Saint Antoine AP-HP, Paris, France
[3]Département de Gastro-entérologie et Nutrition, Hôpital Saint Antoine, AP-HP, Paris, France
[4]Département d'Hépatologie – Centre National de Référence des Maladies Inflammatoires des Voies Biliaires, Hôpital Saint Antoine, AP-HP, Paris, France
[5]UMR 7203 Laboratoire des Biomolécules. Ecole Normale Supérieure, Paris, France
[6]Institut MICALIS, INRA, Jouy en Josas, France

Introduction: Gut microbiota is involved in bile acids (BA) metabolism, and a decrease in *Faecalibacterium prausnitzii* has been repeatedly reported in the dysbiosis associated with inflammatory bowel disease (IBD). The aim of this study was to investigate the impact of IBD-associated dysbiosis on BA metabolism.

Methods: Thirty patients with colonic IBD were compared with 18 healthy subjects (HS). Fecal and serum BA profiles comprising 28 different molecular species were assessed by high-performance liquid chromatography coupled with tandem mass spectrometry. Fecal microbiota composition was assessed by quantitative real-time PCR. The effects of *F. prausnitzii* on BA (deconjugation and dehydroxylation activities) were studied *in vitro*, and was the involvement of BA molecular species in inflammatory pathways, using Caco-2 cells stimulated by IL-1β. Fecal BA profiles in germ-free and conventionalized mice were also compared to correlate dysbiosis and BA dysmetabolism.

Results: Gut microbiota dysbiosis in active IBD patients was characterized by a significant decrease in the *F. prausnitzii-to-E. coli* ratio. In feces, conjugated BA proportions were higher in active IBD patients compared with controls (8.1 ± 2.1% *vs* 2.8 ± 0.6%, $P = 0.05$, respectively), while secondary BA proportions were lower (45.8 ± 8.3% in IBD *vs* 93.2 ± 1.5% in controls, $P = 0.002$). Interestingly, active IBD patients exhibited higher levels of fecal 3-OH-sulfated BA compared with controls (14.2 ± 3.5% *vs* 0.7 ± 0.2%, $P = 0.002$, respectively). Secondary BA *in vitro* exerted anti-inflammatory effects, but sulfation of secondary BA abolished their anti-inflammatory properties.

Discussion/Conclusion: BA dysmetabolism is linked to IBD-associated dysbiosis. The present results suggest loss of microbiota function in BA metabolism in IBD patients. Also, altered BA transformation in the gut lumen can erase the anti-inflammatory effects of some BA species on gut epithelial cells, a process that may be involved in the chronic inflammation seen in IBD.

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Fast methods to measure amidated bile acids in liver transplant patients: Fluorescent side-chain analog derivatives can capture Na^+

FERREIRA Humberto E.C.S.[1,2,*], BARBOSA Jorge[3], LOPES António[5], VILCA-MELENDEZ Hector[4], HEATON Nigel[4], BORDADO João C.M.[2]
[1]Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal. E-mail: hecsf@ist.utl.pt
[2]Departamento de Engenharia Química, Instituto Superior Técnico, Av. Rovisco Pais, 1096 Lisboa codex, Portugal
[3]Laboratório Nacional de Investigação Veterinária, Estrada de Benfica, nº701, 1549-011 Lisboa, Portugal
[4]Liver Transplant Surgical Service, King's College Hospital, Denmark Hill, London, England SE5 9RS, UK
[5]Colloids, Polymers and Surfaces Lab, ITQB-UNL, Av. da República, Estação Agronómica Nacional, 2780-157 Oeiras, Portugal

Introduction: The need for fast methods to obtain complete bile acid profiles, in organ evaluation (1) and follow-up of human liver transplants (2) has been defined.

Methods: By using HPLC-ESI-MS^n we have fully characterized solvents, reagents, products and side-products of reactions needed for fast and efficient fluorescent tagging (for HPLC analysis) of side-chain analogs of amidated bile acids. Light Scattering Spectroscopy (LSS) and Fluorescence/UV-Absortion Spectroscopy were used to follow reactions and cluster formation of the identified dimers, under controlled dissolution conditions. 3D molecular modelling originated a detailed picture of the intermolecular interactions that stabilize the dimers around the Na^+ cation.

Results: The combined use of LSS and MS revealed that the products of the derivatization reactions form dimer clusters with sodium cations included, that are insoluble in the reaction solvent, thus triggering a dissolution study that identified ethanol as an efficient solvent for the monomeric pyrene derivatives. Dissolution studies in water and acetonitrile with a novel surfactant were carried out and showed that the detection signal is dependent on the 3D configuration, and aggregation state, of the dimers formed during the reaction.
The MS/MS transitions identified can now be used to detect and quantify bile acids derivatives in the fentomole range, by HPLC-MS/MS, provided the injection occurs at 15 min/RT, before the aggregation state produces massive precipitation of the reaction product. At this point in time the fluorescence signal is maximized and 4 times higher than the reference redissolution in ethanol. This hyperchromic effect is abolished by ethanol redissolution originating a signal that was expected.

Discussion/Conclusion: The use of these optimized reactions in clinical liver transplantation may have clear advantages over other standard techniques (3). Namely, the proposed reactions are faster in obtaining results (under 4 h per sample), do not use carcinogenic compounds, and are less costly per sample since no initial group separation is needed.

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Bile acid retention and a common polymorphism in the ABCB11 gene affect the antiviral treatment response in chronic hepatitis C independently of the IL28B genotype

Johannes Schmitt[1*], Katharina Baur[1*], Rika Iwata[1], Bruno Stieger[2,3], Joachim C. Mertens[1], Tobias Müller[4], Julia Braun[5], Athanasios Vergopoulos[6], Ina V. Martin[1,7], Stephanie Bibert[8], Pierre-Yves Bochud[8], Beat Müllhaupt[1,9], Thomas Berg[4,10], Andreas Geier[1,3,9]; Swiss Hepatitis C Cohort Study Group
[1]Clinic for Gastroenterology & Hepatology and [2]Division of Clinical Pharmacology, University Hospital Zurich (USZ), 8091 Zurich, Switzerland; [3]Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, 8001 Zurich, Switzerland; [4]Medical Department Hepatology & Gastroenterology, Charité, Campus Virchow-Klinikum, Universitätsmedizin Berlin, Berlin, Germany; [5]Biostatistics Unit, Institute of Social und Preventive Medicine, University of Zurich, 8001 Zurich, Switzerland; [6]Institute for Clinical Chemistry, University Hospital Zurich (USZ), 8091 Zurich, Switzerland; [7]Department of Internal Medicine II, Aachen University (RWTH), University Hospital (UKA), 52074 Aachen, Germany; [8]Infectious Diseases Service, Department of Medicine, University Hospital and University of Lausanne (CHUV), 1011 Lausanne, Switzerland; [9]Swiss Hepatopancreatobiliary (HPB)-Center, University Hospital Zurich (USZ), 8091 Zurich, Switzerland; [10]Clinic for Gastroenterology and Rheumatology, Department of Hepatology, Universitiy Clinic Leipzig, Leipzig, Germany. Correspondence: andreas.geier@usz.ch

Introduction: The outcome of HCV infection and the likelihood of a sustained virologic response (SVR) to antiviral therapy depend on both viral and host characteristics. In vitro studies demonstrated that bile acids (BA) interfere with antiviral Interferon-effects. We investigate the influence of BA concentrations and an *ABCB11* polymorphism associated with lower transporter expression on viral load and SVR with respect to the *IL28B* genotype.

Methods: 451 Caucasian HCV-patients treated with PEG-Interferon and ribavirin were included. *ABCB11* 1331T>C and *IL28B* rs12979860C>T was genotyped and plasma BA levels were determined.

Results: The 1331C allele was slightly overrepresented in HCV-patients compared to controls. In HCV-patients, a significant difference between patients achieving SVR versus non-SVR was observed for HCV-2/3 (5 vs. 9 µmol/L; p = 0.0001), while median BA levels in HCV-1 were marginally elevated. Elevated BA levels > 8 µmol/L were significantly associated with SVR (58.3% vs. 36.3%; OR 2.46; p = 0.0001). This difference was significant for HCV-2/3 (90.7% vs. 67.6%; p = 0.002) but marginal in HCV-1 (38.7% vs. 27.8%; p = 0.058). SVR was equal between *ABCB11* genotypes for HCV-1, but was increased for HCV-2/3 (TT 100 vs. CC 78%; OR 1.42; p = 0.043). *IL28B* genotype had no influence on these associations (multivariate analysis SVR in all HCV pts: OR bile acids 1.66; p = 0.009; OR *IL28B* rs12979860 Tvs.C 1.62; p = 0.016; MVA SVR in HCV-2/3: OR bile acids 2.54; p = 0.041; OR *IL28B* rs12979860 Tvs.C 0.73; p = 0.449). No correlation between BA levels and HCV RNA was detected for any HCV genotype.

Discussion/Conclusion: The higher allelic frequency of *ABCB11* 1331C in HCV-patients compared to controls may be a hint towards a causal role of increased BA for HCV chronicity. Our data support a role for BA as host factor affecting therapy response in HCV-2/3 patients, whereas a weaker association was found for HCV-1. Bile acid-mediated effects are independent of the *IL28B* genotype.

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Supplementation of retinoic acid to ursodeoxycholic acid reduces bile acid pool size and liver fibrosis in bile duct ligated rats by mechanisms verified in human hepatic cell cultures

Hongwei He, Albert Mennone, James L. Boyer, and Shi-Ying Cai
Department of Internal Medicine and Yale Liver Center, Yale University School of Medicine, New Haven, CT 06520, USA

Introduction: Cholestasis leads to liver fibrosis, cirrhosis, and eventually liver failure. Despite limited benefits, ursodeoxycholic acid (UDCA) is the only FDA approved treatment for certain cholestatic disorders. Alternative therapies have been tried but have not been successful based on limited clinical trails. Retinoic acid (RA) is a ligand for the nuclear receptors RAR and RXR, but also possesses immunomodulatory effects. It is used to treat acute promyelocytic leukemia and inflammatory disorders such as psoriasis, acne, and rheumatoid arthritis. Further, RA represses the expression of CYP7A1 in human hepatocytes, the rate-limiting enzyme in converting cholesterol into bile acid.

Aim: To determine whether supplementation of RA with UDCA and RA might be superior to UDCA alone for cholestasis.

Methods: Bile duct ligation (BDL) rats were treated with PBS, UDCA, all-trans RA (atRA), or UDCA + atRA daily by gavage for 14 days. Liver histology was blindly assessed. Bile salt concentrations, liver hydroxyproline, and gene expression were analyzed.

Results: UDCA + atRA treatment substantially improved animal growth rate, and significantly reduced liver fibrosis and bile duct proliferation, and nearly eliminated liver necrosis after BDL. Reductions in bile salt pool size and liver hydroxyproline content were also seen with atRA and atRA + UDCA treatments when compared to UDCA treatment alone. AtRA + UDCA also significantly reduced liver mRNA and/or protein expression of Tgf-β1, Col1A1, Mmp2, Ck19, α-Sma, Cyp7a1, Tnf-α and IL-β1 consistent with the anti-fibrotic effects. Finally, atRA (1 or 5 μM) alone or in combination with 50 μM UDCA greatly repressed CYP7A1 expression in human hepatocytes, and significantly inhibited COL1A1, MMP2, and α-SMA expression and/or activity in primary human hepatic stellate cells and LX-2 cells.

Discussion/Conclusion: Our findings suggest that atRA reduces bile salt pool size and liver fibrosis and may be an effective supplemental therapy with UDCA for cholestatic liver diseases.

The antifibrotic effect of nor-ursodeoxycholic acid in thio-acetamide-induced liver fibrosis reversal

O. Lukivskaya[1], E. Naruta[1], E. Belanovskaya[1], H.-D. Tauschel[2], V. Buko[1]
[1]Division of Biochemical Pharmacology, Institute of Pharmacology and Biochemistry, Grodno, Belarus
[2]Dr. Falk Pharma GmbH, Freiburg, Germany

Introduction: We studied whether nor-ursodeoxycholic acid (nor-UDCA), the C23 homologue of ursodeoxycholic acid (UDCA) is able to reverse advanced TAA-fibrosis in rat. The aim of this study was to compare antifibrotic effects of nor-UDCA and UDCA in a rat model of thioacetamide (TAA)-induced liver fibrosis reversal.

Methods: Advanced liver fibrosis was induced by TAA treatment (200 mg/kg, i.p.) twice a week for 12 weeks. Resolution of fibrosis was assessed after 2 months of TAA withdrawal. During this period, fibrotic rats were daily administered with UDCA (40 and 80 mg/kg) and nor-UDCA (corresponds to 40 and 80 mg/kg of UDCA) by oral gavage.
The severity of liver fibrosis was assessed by morphometric evaluation of liver slides stained with Azan-Mallory and hydroxyproline (Hyp) determination. Serum markers of fibrosis including tissue-inhibitor of metalloproteinases, collagens-I, -III, -IV types, procollagen III-NT, hyaluronate, laminin and TGFβ1 contents, were evaluated by ELISA techniques.

Results: The TAA treatment resulted in advanced fibrosis/cirrhosis with complete fibrous septa formation and dramatic increase in liver Hyp content. These signs of fibrosis were less pronounced in rats with TAA withdrawal. All the studied serum fibrosis markers were significantly elevated in rats treated with TAA for 3 m and decreased after 2 m of TAA withdrawal. Only treatment with the high dose of nor-UDCA significantly decreased the total and relative liver Hyp contents whereas UDCA and the lowest dose of nor-UDCA did not change these parameters which are a “gold standard” for liver fibrosis. Both the tested compounds decreased serum TGFβ and collagen-IV type contents, whereas other serum markers did not differ from the placebo-treated group with fibrosis resolution.

Discussion/Conclusion: Our study suggests that the high dose of nor-UDCA (corresponds to 80 mg/kg of UDCA), but not UDCA, demonstrated a pronounced antifibrotic effect in the model of TAA-induced liver fibrosis reversal.

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The effect of ursodeoxycholic acid (UDCA) therapy after surgical correction for biliary atresia

W. de Vries[1], J.B.F. Hulscher[2], D.C. Aronson[3], H.J. Verkade[1], also on behalf of NeSBAR
Departments of [1]Pediatric Gastro-Enterology and Hepatology, [2]Pediatric Surgery, Beatrix Children's Hospital, University Medical Center Groningen, The Netherlands; [3]Surgery/Pediatric Surgery, Radboud University Medical Center, Nijmegen, The Netherlands

Introduction: Biliary atresia (BA) is a cholestatic disease of infancy, caused by obliteration of bile ducts. The effect of therapy with ursodeoxycholic acid (UDCA) on the transplant-free survival of BA after surgical correction is not well established. We retrospectively evaluated the effect of UDCA therapy in a national cohort of BA patients.

Methods: Patient data were retrieved from the Netherlands Study group on Biliary Atresia Registry (NeSBAR) database (1987–2008). Patients were categorized in UDCA-therapy and non-UDCA groups. Patients receiving prednisolon were excluded from the analysis. Dosage of UDCA was 20 mg/kg/day (median duration 738 days). Outcome measures were clearance of jaundice (bilirubin < 20 µmol/l within six months post-surgery) and 2-year transplant-free survival.

Results: Of 214 BA patients, 28 (13%) received UDCA therapy whereas147 (69%) did not receive UDCA. Treatment groups were similar with respect to prevalence of BA subtypes, age at surgery and preoperative laboratory values. Post-operative antibiotic prophylaxis use was similar in both groups, except ciprofloxacin which was given more often in the UDCA group (25% vs 6%, $p < 0.01$). Clearance of jaundice was 57% in the UDCA-group and 31% in the non-UDCA group ($p < 0.05$).Two-year transplant-free survival was 75% in the UDCA-group and 48% in the non-UDCA group ($p < 0.01$). Transplant-free survival of patients receiving ciprofloxacin was similar to patients receiving other or no antibiotics.

Discussion/Conclusion: Post-surgical adjuvant therapy on UDCA alone was associated with a beneficial effect on clearance of jaundice and short-term (2-year) transplant-free survival in children with BA. We speculate that UDCA adjuvant therapy might postpone the need for liver transplantation.

Management of ursodeoxycholic acid with vitamin E in non-alcoholic steatohepatitis

Krasimira Kalinova, Katya Todorova, Mariana Penkova, Maya Gulubova
University Hospital Stara Zagora, Bulgaria

Background/Aims: Despite a proposed role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis (NASH), antioxidant approaches have not been investigated sufficiently in the therapy of nonalcoholic steatohepatitis. Nonalcoholic steatohepatitis can progress to cirrhosis and for which there is no recognized therapy. UDCA and vitamin E have been considered separately as therapeutic options and have not been shown to be effective.

Methods: This was an open-labeled, prospective, randomized study enrolling patients with histologically proven fatty liver disease who had chronically elevated alanine aminotransferase, despite a three-month reducing diet. Patients consuming alcohol (more than 20 g/day) were excluded. Patients with elevated aminotransferase levels and drinking less than 40 g alcohol/week with biopsy-proven NASH were randomly assigned to receive UDCA 12–15 mg x kg^{-1} x day^{-1} with vitamin E 400 IU twice a day (UDCA/Vit E), UDCA with placebo (UDCA/P), or placebo/placebo (P/P). After 2 years, they underwent a second liver biopsy. Biopsy specimens were collected, blinded, and scored by a single liver pathologist.

Results: There was no significant change in body mass index before and after the treatment in both groups. At the end of six months of therapy, serum aspartate aminotransferase and aminotransferase levels significantly decreased in both treatment options. Forty eight patients were included, 15 in the UDCA/Vit E group, 18 in the UDCA/P group, and 15 in the P/P group; 8 patients dropped out, none because of side effects. Baseline parameters were not significantly different between the 3 groups. Body mass index remained unchanged during the study. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels diminished significantly in the UDCA/Vit E group. Neither the AST nor the ALT levels improved in the P/P group and only the ALT levels in the UDCA/P group. Histologically, the activity index was unchanged at the end of the study in the P/P and UDCA/P groups, but it was significantly better in the UDCA/Vit E group, mostly as a result of regression of steatosis. Alanine aminotransferase decreased to normal levels in 17 of 27 (63%) and in 16 of 29 patients (55%), respectively, in the two groups.

Conclusions: However, treatment of NASH with UDCA for 12 months resulted in significant improvement in alkaline phosphatase, ALT, GGT, and hepatic steatosis. The possible benefit of UDCA therapy should be further investigated in the context of a randomized, controlled trial. Two years of treatment with UDCA in combination with vitamin E improved laboratory values and hepatic steatosis of patients with NASH. Larger trials are warranted.

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Long-term ursodeoxycholic acid treatment of cholestatic liver diseases in childhood – Clinical and biochemical effects

Krasimira Kalinova, Marlena Panaiotova, C. Georgiev, K. Georgiev
University Hospital Stara Zagora, Bulgaria

Background: In adults with chronic cholestatic liver disorders, controlled studies have shown a reduction of clinical, biochemical and possibly histological parameters during long-term medication with ursodeoxycholic acid (UDCA). It is not yet clear, however, whether similar effects can be achieved in children. Therefore, we retrospectively evaluated the use of UDCA in typical liver diseases of childhood.

Methods: 18 children were treated for at least 18 months (age at start of therapy 5–7, median 36 months; diagnosis: biliary atresia n = 8, Alagille's syndrome n = 4, intrahepatic biliary hypoplasia n = 5, Byler disease n = 1). Pruritus, liver cell injury, cholestasis, synthetic liver function and weight and height for age before medication with UDCA (7–26, mean 12 mg/kg BW/d) was compared to values after 3, 6, 12, 18 and 24 months of therapy, with special attention towards possible adverse effects.

Results: No adverse effects of UDCA necessitating modification of therapy were encountered. During the first year of medication, weight for age improved in 15 patients, but pruritus in only four. During UDCA treatment, GIDH and gamma GT decreased significantly. GOT and GPT declined in the majority of patients. No significant changes of bilirubin and parameters of liver synthesis were seen.

Conclusion: Long-term medication with UDCA appears to be safe in children. Thus, controlled studies of UDCA medication in children are justified, and are urgently needed to further investigate the prognostic significance of the positive effects of UDCA identified in this retrospective analysis.

Farnesoid X receptor: A new target for treatment of diarrhoeal disease?

Niamh Keating, Fiona Dunlevy, Joseph B. Ward, Magdalena S. Mroz,
Orlaith B. Kelly, Stephen J. Keely
Department of Molecular Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland

Introduction: The farnesoid X receptor (FXR) is currently under investigation as a therapeutic target for liver and metabolic disorders. However, although endogenous bile acids are known to be important regulators of colonic fluid and electrolyte transport, whether FXR is important in this regard is unknown. Here we investigated the role of FXR in regulating colonic secretory function and the potential for targeting FXR in treatment of diarrhoeal diseases.

Methods: Cl^- secretion, the primary driving force for colonic fluid secretion, was measured as changes in short-circuit current across voltage-clamped T_{84} cells or muscle-stripped mouse colon in Ussing chambers.

Results: Pretreatment of T_{84} cells with the FXR agonist, GW4064 (2 μM), induced nuclear translocation of FXR and inhibited Ca^{2+}- and cAMP-evoked secretory responses to 55.6 ± 8.4 and 72.0 ± 4.0% of controls, respectively ($n = 7$; $p < 0.01$). Effects of GW4064 were concentration-dependent (0.1–10 μM) and maximal after 24 hrs. An FXR antagonist, guggulesterone (5 μM), inhibited the antisecretory effects of GW4064. Although GW4064 potently stimulated FGF-19 production from T_{84} cells, SU5042, an FGF receptor antagonist, did not alter its antisecretory actions. To determine the molecular target of GW4064 we analyzed the activity of transport proteins comprising the Cl^- secretory pathway. GW4064 inhibited Na^+/K^+ ATPase pump activity to 62.1 ± 4.1% of control values ($n = 5$; $p < 0.01$). This effect was not due to altered pump expression or trafficking, or to depletion of cellular ATP. Finally, intraperitoneal injection of GW4064 (100 mg/kg) to mice attenuated Ca^{2+}- and cAMP-stimulated secretory responses across colonic tissues by 31.2 ± 6.9% and 34.6 ± 7.6%, respectively ($n = 6$, $p < 0.05$).

Discussion/Conclusion: FXR agonists exert antisecretory actions *in vitro* and *in vivo* and may represent a new class of drug that directly targets epithelial secretory function for treating diarrhoeal diseases.

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The therapeutic potential of metabolically stable derivatives of ursodeoxycholic acid in diarrhoeal disease: An in vitro and in vivo study

O. Kelly[1], E. Pastorini[2], R. Pellicciari[2], F.E. Murray[1], A. Roda[2], A.F. Hofmann[3], S.J. Keely[1]
[1]Departments of Molecular Medicine/Gastroenterology, Royal College of Surgeons in Ireland, Dublin, Ireland
[2]Department of Pharmaceutical Sciences, University of Bologna, Italy
[3]Department of Medicine, University of California, San Diego, USA

Introduction: Despite the global prevalence of diarrhoeal diseases, there are no anti-diarrhoeal drugs available that directly target epithelial transport processes. While ursodeoxycholic acid (UDCA) is widely and safely used in clinical practice, little is known of its effects on intestinal fluid and electrolyte transport. Here, we examined effects of UDCA and its metabolite, lithocholic acid (LCA), on colonic epithelial secretion.

Methods: Chloride (Cl^-) secretion, the driving force for intestinal fluid secretion, was measured as changes in short circuit current across voltage-clamped T_{84} cells and muscle-stripped mouse colon. Confocal microscopy and surface biotinylation were used to assess abundance/surface expression of transport proteins.

Results: Unlike their parent bile acid, chenodeoxycholic acid, UDCA and LCA were devoid of prosecretory actions in T_{84} cells. However, acute UDCA (500 μM) pretreatment rapidly attenuated secretory responses to the Ca^{2+} and cAMP-dependent agonists, carbachol (CCh) and forskolin (FSK) to 14.5 ± 4.2% and 40.1 ± 7.4% of controls, respectively ($n = 12$, $p < 0.001$). Antisecretory effects of UDCA were apparent at 50 μM and maximal at 1 mM. In contrast, in an *in vivo* mouse model, intraperitoneal UDCA (100 mg/kg) enhanced responses to CCh and FSK to 285 ± 42% and 215 ± 54% of controls, respectively ($n = 5$, $p < 0.05$). We hypothesised this prosecretory effect to be due to bacterial metabolism of UDCA to LCA. Accordingly, we found that LCA (50–200 μM) enhanced secretion *in vitro* and the stable UDCA analogue, 6-methyl UDCA (6MUDCA), which is not metabolised to LCA was antisecretory both *in vitro* and *in vivo*. Further investigation of molecular targets of UDCA in T_{84} cells revealed that the bile acid significantly inhibited Na^+/K^+-ATPase pump activity and basolateral K^+ currents without altering cell surface expression of these proteins.

Conclusion: Metabolically stable UDCA analogues may represent a novel class of anti-diarrhoeal drug that acts directly at the level of transport protein function.

The farnesoid X receptor (FXR) agonist obeticholic acid (INT-747, 6α-ethyl chenodeoxycholic acid) in combination with ursodeoxycholic acid (UDCA) increases plasma FGF-19 concentrations but not bile acid concentration or profile in primary biliary cirrhosis (PBC)

Hanns-Ulrich Marschall[1], Velimir Luketic[2], Andrew Mason[3], Keith Lindor[4], Gideon Hirschfield[5], Stuart Gordon[6], Marlyn Mayo[7], Kris Kowdley[8], Albert Parés[9], Michael Trauner[10], Lise Eliot[11], Alan F. Hofmann[12], Cathi Sciacca[11], David Shapiro[11]
[1]Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Sweden
[2]Virginia Commonwealth University, Richmond, VA, USA
[3]University of Alberta, Edmonton, AB, Canada
[4]Mayo Clinic, Rochester, MN, USA
[5]University of Toronto Western Hospital, Toronto, ON, Canada
[6]Henry Ford Health System, Detroit, MI, USA
[7]UT South Western Medical Center, Dallas, TX, USA
[8]Virginia Mason Medical Center, Seattle, WA, USA
[9]Digestive Diseases Institute, Barcelona, Spain
[10]Medical University of Vienna, Austria
[11]Intercept Pharmaceuticals, Inc, San Diego, CA, USA
[12]University of California San Diego, San Diego, CA, USA

Introduction: Obeticholic acid (OCA), [INT-747, 6α-ethylchenodeoxycholic acid (6α-ECDCA)], is a semi-synthetic derivative of CDCA that is a potent FXR agonist. It has shown anticholestatic and antifibrotic efficacy in nonclinical studies. Fibroblast growth factor 19 (FGF-19) is an ileal protein hormone, directly regulated by FXR, that is required for physiological downregulation of bile acid synthesis. An international double-blind, placebo-controlled, parallel-group study of OCA in combination with UDCA was conducted in PBC patients. Changes in serum FGF-19 levels and bile acid (BA) profile and concentration were assessed as a secondary objective.

Methods: 165 PBC patients with alkaline phosphatase values 1.5-10x the upper limit of normal were randomized to receive placebo or 10, 25 or 50 mg of OCA for 12 weeks, once daily in addition to their continuing dose of UDCA (mean: 16 mg/kg/day). Blood samples were taken on Day 0 and Day 85 to determine FGF-19 by ELISA and bile acids including OCA by HPLC-MSMS.

Results: FGF-19 increased significantly vs. placebo in a dose-related manner across OCA dose levels. Plasma OCA concentrations increased with OCA dose but comprised less than 5% of total BA. Pretreatment BA concentrations [total BAs: 16.7; 1.1–315.1 µmol/L; UDCA: 11.3; 0.1–24.1 µmol/L (median; range)] were not changed by OCA treatment; nor were there changes in other major bile acids. Two subjects at the highest OCA dose (50 mg/day) had extremely high UDCA and FGF-19 concentrations and were discontinued from the study due to worsening bilirubin levels.

END OF TREATMENT	Placebo	OCA 10 mg	OCA 25 mg	OCA 50 mg
FGF-19[1], change from baseline [ng/mL]	15.6 (-131 – +356)	115.4*** (-74 – +714)	145.9*** (-71 – +2268)	213.4*** (-292 – +57005)
Total bile acids[1] [µmol/L]	13.1 (2.8 – 96.5)	13.8 (1.9 – 174.9)	11.7 (0.9 – 187.5)	15.9 (1.6 – 548.4)
Total UDCA[1] [µmol/L]	9.1 (1.2 – 59.1)	11.0 (0.6 – 145.8)	9.7 (0.3 – 93.9)	10.1 (1.1 – 315.0)
Total OCA[1] [µmol/L]	–	0.1 (0 – 1.9)	0.2 (0 – 2.4)	0.4 (0 – 30.1)
OCA in total BA[2] [%]	–	2.5 (5.3)	3.0 (4.5)	3.4 (4.5)

[1][Median (range)]; [2]Mean (± SD); *** $p < 0.001$ vs. placebo.

Discussion/Conclusion: In patients with PBC, OCA statistically significantly increased FGF-19 plasma concentrations in a dose-related manner, consistent with its FXR agonist properties. There were no clinically or statistically relevant effects of OCA treatment on UDCA or total BA concentrations.

Ursodeoxycholic acid has antiinflammatory effects in a model of colitis in the rat

P. Martínez-Moya*; I. Romero-Calvo[#], P. Requena*, A. Zarzuelo[#], M.D. Suárez*, O. Martínez-Augustin*, J.J.G. Marín[+], F. Sánchez de Medina[#]
*Departments of Biochemistry and Molecular Biology II, CIBERehd, [#]Department of Pharmacology, CIBERehd, School of Pharmacy, University of Granada, and [+]Department of Physiology and Pharmacology, CIBERehd, University of Salamanca, Spain

Introduction: The denomination inflammatory bowel disease (IBD) comprises a group of chronic inflammatory diseases of the digestive tract, ulcerative colitis and Crohn's disease being the most important conditions. These diseases of unknown etiology are characterized by periods of activity and remission. Although there are effective treatments they have frequent side effects and therefore there is active research going on to improve IBD management. Bile acids (BAs) are secreted to the intestinal lumen to facilitate fat digestion, as well as lipid and liposoluble vitamin absorption. BA, like ursodeoxycholic acid (UDCA), are currenly being used to treat cholestasis or primary biliary cirrhosis, because of their choleretic, cytoprotective and immunomodulatory effects. In fact, UDCA has been reported to exert an anti-inflammatory effect, whereas deoxycholic acid is regarded as predominantly proiinflammatory.

Methods: In this study we study the hypothesis that BA may have an intestinal anti-inflammatory effect. To do so we used the model of experimental colitis in rats induced by the administration of trinitrobenzenesulfonic acid (TNBS).

Results: Our results indicate that UDCA ameliorates experimental colonic inflammation but only at a relatively high dose (50 mg/kg/day), while it was inactive at 10 and 25 mg/kg/day. Deoxycholic acid was essentially neutral. The therapeutic effect was evidenced, among others, by a higher body weight recovery, a diminished affected to total mucosal area and lower alkaline phosphatase activity in treated vs. control (TNBS treated) animals.

Discussion/Conclusion: Our results indicate that UDCA is anti-inflammatory in an animal model of intestinal inflammation and could potentially be useful in the treatment of IBD.

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Voluntary wheel running beneficially affects cholesterol turnover and atherosclerosis in hypercholesterolemic mice

M. Meissner[1], E. Lombardo[2], R. Havinga[1], U.J.F. Tietge[1], F. Kuipers[1,3], A.K. Groen[1,3]
[1]Department of Pediatrics, [3]Department of Laboratory Medicine, Center for Liver, Digestive, and Metabolic Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[2]Department of Biochemistry, Academic Medical Center, Amsterdam, The Netherlands

Introduction: Regular physical activity beneficially affects risk for atherosclerosis but underlying mechanisms are not fully understood. We questioned whether voluntary wheel running provokes specific modulations in cholesterol turnover that translate into a decreased atherosclerotic burden in hypercholesterolemic mice.

Methods: Male Ldlr-deficient mice (8 weeks old) had either access to a voluntary running wheel for 12 weeks (RUN) or remained sedentary (CONTROL). Both groups were fed a high cholesterol diet. Running activity and food intake were recorded. At 12 weeks of intervention, feces, bile and plasma were collected to determine fecal, biliary and plasma parameters of cholesterol metabolism and plasma cytokines. Atherosclerotic lesion size was determined in the aortic arch.

Results: RUN weighed less ($p = 0.002$) while food consumption was increased by 17% ($p = 0.004$). Plasma cholesterol levels were decreased by 12% ($p = 0.035$) and plasma lipoprotein profiles were improved in RUN compared to CONTROL. Running modulated cholesterol catabolism by enhancing cholesterol turnover: RUN displayed an increased biliary bile acid secretion (by 42%, $p = 0.007$) and increased fecal neutral sterol (by 34%, $p = 0.002$) and bile acid (by 93%, $p = 0.009$) outputs compared to CONTROL indicating that reverse cholesterol transport was increased in RUN. Importantly, aortic lesion size was decreased by ~45% in RUN ($p = 0.0327$).

Discussion/Conclusion: Voluntary wheel running reduces atherosclerotic burden in hypercholesterolemic mice. An increased cholesterol turnover, specifically its catabolism into bile acids, may underlie the beneficial effect of voluntary exercise in mice.

The effects of bile acid sequestration and voluntary wheel running on cholesterol turnover and atherosclerosis in hypercholesterolemic mice

M. Meissner[1], H. Wolters[1], R. Havinga[1], R. Boverhof[1], V. Bloks[1], F. Kuipers[1,2], A.K. Groen[1,2]
[1]Department of Pediatrics, [2]Department of Laboratory Medicine, Center for Liver, Digestive, and Metabolic Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Introduction: Although bile acid sequestrants (BAS) are widely utilized as cholesterol lowering agents, nothing is known about the effects their on atherosclerotic lesion size. We questioned whether BAS, by modulating cholesterol metabolism, results in a decreased atherosclerotic lesion size in hypercholesterolemic mice. Because pharmaceutical interventions are often co-prescribed to lifestyle interventions and we recently found decreased atherosclerotic lesion size in hypercholesterolemic mice exposed to a voluntary running wheel (RUN), we further questioned the effect of a combination treatment of BAS and RUN on cholesterol metabolism and atherosclerotic lesion size in hypercholesterolemic mice.

Methods: Male Ldlr-deficient mice (8 weeks old) remained sedentary (WD), were treated with 2% (wt/wt in diet) Colesevelam HCl (BAS), had access to a voluntary running wheel (WD RUN), or were exposed to BAS RUN. All groups were fed a high cholesterol diet for 12 weeks. Running activity and food intake were recorded. At 12 weeks of intervention, feces, bile and plasma were collected to determine fecal, biliary and plasma parameters of cholesterol metabolism. Atherosclerotic lesion size was determined in the aortic arch.

Results: BAS RUN ran more than RUN ($p < 0.05$). BAS and BAS RUN displayed ~3-fold decreases in plasma cholesterol levels ($p < 0.001$), improved plasma lipoproteins profiles, ~2-fold increases in fecal neutral sterol ($p < 0.001$) and bile acid ($p = 0.01$) outputs, decreases in biliary secretions of cholesterol (~6-fold, $p < 0.0001$), bile acids and phospholipids (both ~2-fold, $p < 0.001$) and decreased hepatic total, free and esterified cholesterol contents (all $p < 0.01$) vs. WD while no effect was observed in RUN. Compared to WD, atherosclerotic lesion size decreased by ~80% in both BAS and BAS RUN, ($p<0.0001$) while a non-significant 20% decrease was found in RUN.

Discussion/Conclusion: BAS benefits parameters of cholesterol metabolism and reduces atherosclerotic burden in hypercholesterolemic mice the combination treatment BAS RUN had no additive effects.

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Acute one day resin treatment rapidly and strongly reduces plasma FGF19 levels and stimulates bile acid and cholesterol synthesis – Three responses lasting at least 36 hours

B. Sjöberg, S. Straniero, B. Angelin, M. Rudling
Department of Endocrinology, Metabolism & Diabetes, Karolinska Institute M63, Karolinska Univ Hospital, Huddinge, 141 86 Stockholm, Sweden

Introduction: Synthesis and turnover of bile acids (BAs) is crucial in cholesterol homeostasis. We previously showed that BA synthesis has a diurnal rhythm in man partly regulated from the intestine via FGF19 (JIM-06). We here investigated the temporal changes in cholesterol and BA metabolism following interruption of BA circulation with a resin.

Methods: 10 normal subjects were bled regularly from 9 day 1 until 17 hrs day 2. On day 1, 4 g of cholestyramine was taken with each meal (4 x 4 g). Plasma markers for BA and cholesterol synthesis and FGF19 were determined with plasma lipids.

Results: BA synthesis was induced within 6 hrs after first resin dose in parallel with diminished levels of FGF19. BA synthesis increased continuously during night reaching a 4-fold induction in the morning day 2. Interestingly plasma TG increased all night reaching doubled levels in all individuals in the morning day 2. The diurnal rhythm of BA synthesis remained day 2 although at a 4-fold higher level.

Discussion/Conclusion: The acute interruption of BA circulation in man increases BA synthesis within hours and this is preceded by pronounced reductions in plasma FGF19 that persist at least 36 hrs. The diurnal changes in BA synthesis remain upon acute treatment with cholestyramine whereas BA and cholesterol synthesis are induced for at least 3 days. Our data show that in studies on FGF15 or BA synthesis it is crucial to seponate cholestyramine many days before blood sampling.

Primary bile acid therapy (glycocholic acid) ameliorates fat-soluble vitamin malabsorption in patients with genetic defects in bile acid conjugation

Kenneth D.R. Setchell and James E. Heubi
Department of Pathology and Laboratory Medicine and Department of Gastroenterology and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA

Inborn errors in bile acid synthesis are now a well-characterized category of metabolic liver disease with 7 known defects having been described to date. Primary bile acid therapy with cholic acid has proven highly effective for the treatment of these disorders, resulting in suppression in the atypical hepatotoxic bile acids synthesized and a concomitant normalization in liver function tests and improvements in liver histology. Some years ago we first reported the first case of a patient with defective bile acid conjugation resulting from a deficiency in the bile acid CoA:amino acid N-acyl transferase (BAAT) and since then, 11 patients have been identified by mass spectrometric analysis of urine, serum and bile which sre characterized by high concentrations of unconjugated cholic acid accompanied by glucuronides and sulfates, and a complete lack of glycine and taurine conjugated bile acids. Mutations in the gene encoding BAAT confirmed the defect to reside at the point of transfer of glycine and taurine to the unconjugated bile acid intermediate, cholic acid. The phenotype of this disorder is variable with patients having mild/severe liver disease, there may or may not be poor growth, but in all cases there is a severe fat-soluble vitamin malabsorption that long-term leads to rickets. Since thes epatient synthesize unconjugated cholic acid, treatment with requires a conjugated bile acid. After oral administration of glycocholic acid (GCA) at a dose of 15 mg/kg bw/d, it was observed that GCA is absorbed and secreted in bile, and as a result of increased intraluminal bile acid levels, this leads to a correction of the fat-soluble vitamin malabsorption as evidenced from the plasma vitamin D and E pharmacokinetic concentration curves following single oral administration of vitamin D_2, and vitamin E. GCA has been well tolerated in all patients treated for up to 2 years and there have been no reported side effects. In conclusion, defective bile acid amidation should be considered as a possible cause in cases of unexplained fat-soluble vitamin malabsorption and glycocholic acid therapy is effective in improving the fat-soluble vitamin status. These findings attest to the crucial role conjugated bile acid play in fat-soluble vitamin absorption.

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Acute but not chronic resin treatment persistently increases serum triglycerides concomitantly with reduced serum bile acid levels

B. Sjöberg, S. Straniero, B. Angelin, M. Rudling
Department of Endocrinology, Metabolism & Diabetes, Karolinska Institute M63, Karolinska Univ Hospital, Huddinge, 141 86 Stockholm, Sweden

Introduction: In man, BA synthesis has a diurnal rhythm independent of cholesterol synthesis. Cholestyramine reduces LDL cholesterol levels through increased LDL receptor numbers. Of unclear reason, serum triglycerides (TGs) frequently increase. The aim was here to collect information regarding the acute and chronic responses following this treatment.

Methods: Ten subjects received one day or long-term treatment with cholestyramine. Plasma lipids bile acids and serum markers for bile acid and cholesterol synthesis were assayed.

Results: Ten hours after last intake of cholestyramine acutely all subjects were hypertriglyceridemic (+80%) while BA synthesis was significantly induced 4-fold. Serum BAs were significantly reduced. Following 3 wks of chronic resin treatment subjects were normotriglyceridemic, had 5-fold increased BA-synthesis but had no reductions in their serum levels of BAs.

Discussion/Conclusion: Hypertriglyceridemia was a consistent finding in all subjects immediately following the initiation of resin treatment. Interestingly, plasma BAs were low at this moment. This response was absent during chronic resin treatment when plasma BAs were normal. The data point toward a possibly important role of BA deficiency with reduced FXR signalling as a possible cause for why acute but not chronic resin-treatment increases serum triglycerides.

Ursodeoxycholic acid and fibrate make an excellent combination for the treatment of cholestasis in patients with primary biliary cirrhosis

Akira Honda[1], Tadashi Ikegami[1], Teruo Miyazaki[1] Makoto Nakamuta[2], Hajime Takikawa[3], Yasushi Matsuzaki[1]
[1]Tokyo Medical University Ibaraki Medical Center, Ibaraki, Japan
[2]Kyushu Medical Center National Hospital Organization, Fukuoka, Japan
[3]Teikyo University School of Medicine, Tokyo, Japan

Introduction: Ursodeoxycholic acid (UDCA) improves cholestasis in patients with the early stage of primary biliary cirrhosis (PBC). However, a complete normalization of liver biochemistry is not always achieved by UDCA alone. Recent reports have suggested that fibrates have additional effects on PBC patients refractory to UDCA. To explore the mechanism of the additional improvement by fibrates, we studied cholesterol and bile acid metabolism in PBC patients treated with UDCA and bezafibrate.

Methods: Study 1 compared untreated PBC patients (Scheuer's stage I or II; n = 30) with controls (n = 30). Study 2 compared before and after treatment with UDCA (600 mg/day for 12 weeks) in untreated PBC patients (n = 20). Study 3 compared before and after treatment with additional bezafibrate (400 mg/day for 4 weeks) in PBC patients using UDCA (n = 10). Serum biomarker sterols for cholesterol and bile acid metabolism were analyzed by HPLC-MS/MS, and serum FGF-19 levels were measured by ELISA.

Results: In untreated PBC patients, serum ALP, γGTP, LDL-cholesterol (LDL-C) and triglycerides (TG) were significantly elevated compared with those in controls. Serum FGF-19 (a marker of endogenous bile acid secretion into duodenum), 7α-hydroxy-4-cholesten-3-one (C4; a marker of bile acid synthesis), and plant sterols (markers of cholesterol absorption) were not significantly different from controls, while serum lathosterol (a marker of cholesterol synthesis) was decreased by 35%. After treatment with UDCA, serum ALP and γGTP reduced 40%, but LDL-C and TG did not change significantly. Both FGF-19 and plant sterols increased about 35% and lathosterol and C4 did not change by UDCA treatment. Additional treatment with bezafibrate significantly decreased lathosterol and C4 by 35% and 72%, respectively. FGF-19 and plant sterols also tended to be decreased. Serum ALP and γGTP further reduced 45%, and LDL-C and TG also decreased.

Discussion/Conclusion: UDCA improved cholestasis by replacing hydrophobic bile acids with hydrophilic UDCA and by increasing bile flow. However, it did not down-regulate cholesterol and bile acid synthesis and stimulated cholesterol absorption. In contrast, bezafibrate down-regulated bile acid and cholesterol synthesis and reduced cholesterol absorption, which makes up for weak points of UDCA and leads to further improvement of cholestasis.

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The ^{75}SeHCAT test is essential in the diagnostic work-up of diarrhoea though dispensable in ileal Crohn's or small bowel resection

Nima Mottacki[1], Kjell-Arne Ung[1], Anders Kilander[2], Antal Bajor[2]
[1]Department of Internal Medicine, Kärnsjukhuset, Skövde, Sweden
[2]Department of Internal Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden

Introduction: The ^{75}SeHCAT test mirrors the turnover rate of bile acids and is used to diagnose bile acid malabsorption in diarrhoea.
We aimed to study the distribution of ^{75}SeHCAT test values in different diagnostic groups in patients with chronic diarrhoea. The prevalence of idiopathic bile acid malabsorption (IBAM) was also evaluated.

Methods: The 2112 ^{75}SeHCAT tests performed between 1989–2005 in patients with chronic diarrhoea at Sahlgrenska University Hospital were reviewed. Medical records, clinical work-up and final diagnoses were evaluated. Patients were excluded if a diagnosis could not be determined. ^{75}SeHCAT values in each diagnostic group were compared with data from healthy controls. Retention < 10% on day 7 is considered abnormal.

Results: Diagnoses were established in 1602 of the 2112 tests. Median 7 day retention values were: in controls (n = 31) 38.0% (range 8.0–91.0), IBAM 4.3% (n = 308) (0–10.0), IBS (n = 392) 21.0% (range 0.01–75.0), post-cholecystectomy diarrhoea (n = 231) 6.5% (range 0.01–85.0), collagenous colitis (n = 171) 16.0% (range 0.01–69.0), Crohn's disease with diarrhoea (n = 58) 1.4% (range 0–27.0%), post-ileocecal resection (n = 58) 2.4% (range 0.01–54.00) (range 0.01–54.0), lymphocytic colitis (n = 53) 15.0 (range 0.60–67.0), coeliac disease (n = 53) 27.0% (range 0.1–97.0) and ulcerative colitis (n = 38) 20.0% (range 1.0–64.6). 49.1% of all tested patients had ^{75}SeHCAT retention < 10%. ^{75}SeHCAT retention values differed significantly from controls in all diagnostic groups ($p < 0.001$), excepting those with coeliac disease ($p = 0.084$).

Discussion/Conclusion: Considering the high prevalence of bile acid malabsorption the ^{75}SeHCAT test is recommendable in investigating chronic diarrhoea. Diarrhoea per se seems to lower ^{75}SeHCAT values. For patients with ileal Crohn's disease and after small bowel resection the ^{75}SeHCAT test may be omitted, as these diseases lead overwhelmingly to low retention.

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