Falk Symposium 184

XXII International Bile Acid Meeting

Hepatic and Extrahepatic Targets of Bile Acid Signalling

September 14 – 15, 2012
Hilton Vienna Hotel
Vienna, Austria

Abstracts

Poster Abstracts

FALK FOUNDATION e.V.

Leinenweberstr. 5
79108 Freiburg
Germany

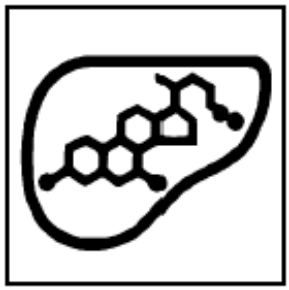
www.falkfoundation.org

Abstracts of Invited Lectures
Poster Abstracts

Falk Symposium 184

XXII International Bile Acid Meeting

HEPATIC AND EXTRAHEPATIC TARGETS OF BILE ACID SIGNALING



Vienna (Austria)
September 14 – 15, 2012

Scientific Organization:
U. Beuers, Amsterdam (The Netherlands)
D. Häussinger, Düsseldorf (Germany)
M. Trauner, Vienna (Austria)

CONTENTS

Session IV

Bile acids and disease

Chair:
U. Beuers, Amsterdam
P.A. Dawson, Winston-Salem

Session V

Therapeutic potential of bile acids I

Chair:
A. Stiehl, Heidelberg
M. Trauner, Vienna

Session VI

Therapeutic potential of bile acids II

Poster Abstracts

1. Intrahepatic cholestasis of pregnancy levels of sulfated progesterone metabolites inhibit FXR resulting in a pro-cholestatic phenotype
S. Abu-Hayyeh, G. Papacleovoulou, A. Lövgren-Sandblom, S. Ravat, V. Nikolova, J. Chambers, H.-U. Marschall, M. Parker, C. Williamson (London, GB; Stockholm, Gothenburg, SE)

2. Bile acid modulation of brain microRNAs in the double transgenic APP/PS1 mouse model of Alzheimer's disease
J.D. Amaral, P.M. Borralho, A.F. Nunes, Y. Zeng, A.L. Sarver, C.J. Steer, C.M.P. Rodrigues (Lisbon, PT; Minneapolis, US)

3. The expression of bile salt export pump (BSEP, ABCB11) and FIC1 ATPase (FIC1, ATP8B1) are regulated by microRNA-33 (mIR-33) which is downregulated in experimental cholestasis
M. Ananthanarayanan, R.M. Allen, T.J. Marquart, C.J. Albert, F.J. Suchy, D.A. Ford, A. Baldan (New York, St. Louis, Aurora, US)

4. Cholesterol metabolism in gallstone disease: Analysis of circulating markers of sterol homeostasis
M. Bertolotti, P. Loria, E. Pellegrini, M. Del Puppo, C. Galbusera, S. Ognibene, E. Baldelli, C. Anzivino, L. Carulli, N. Carulli (Modena, Monza, IT)

5. Reduction of bile acid synthesis *in vivo* in patients receiving total parenteral nutrition
M. Bertolotti, L. Carulli, C. Anzivino, E. Baldelli, M. Del Puppo, M.R. Odoardi, P. Loria, N. Carulli (Modena, Monza, IT)

6. Novel treatment options to improve protein folding and enhance pre-mRNA splicing in ATP8B1 deficiency
I.T.G.W. Bijsmans, W.L. van der Woerd, R.H.J. Houwen, S.F.J. van de Graaf (Utrecht, NL)

7. Targeting specific FXR isoforms in the bile acid biosynthetic pathway
M. Boesjes, J. Hageman, V.W. Bloks, H. Havinga, F. Kuipers, A.K. Groen (Groningen, NL)

8. Taurine pretreatment ameliorates liver injury and periportal Bsep expression following warm experimental ischemia and reperfusion
S. Braun, P. Cebula, T. Kolb, C. Strucks, P. van Caster, D. Herebian, E. Mayatepek, I. Bauer, B. Pannen, D. Häussinger, M.G. Donner (Düsseldorf, DE)

9. ABCG2 plays an important role in the placental barrier for bile acids
O. Briz, A.G. Blazquez, M.R. Romero, R. Rosales, M.J. Monte, J. Vaquero, R.I.R. Macias, D. Cassio, J.J.G. Marin (Salamanca, ES; Orsay, FR)

10. Bile acid sequestration increases transintestinal cholesterol excretion in mice
G. Brufau, A. Bleeker, M. Schonewille, F. Kuipers, A.K. Groen (Groningen, NL)

11. The Src-family kinase Fyn mediates hyperosmolarity-induced Mrp2- and Bsep-retrieval from the canalicular membrane
M. Cantore, R. Reinehr, A. Sommerfeld, M. Becker, D. Häussinger
(Düsseldorf, DE)

12. Hyperosmolarity-induced retrieval of bile salt transport systems is mediated by oxidative stress and sensitive to cyclic AMP
M. Cantore, A. Sommerfeld, R. Reinehr, D. Häussinger (Düsseldorf, DE)

13. Slowed small intestinal transit: The second motility defect in cholesterol cholelithogenesis is caused by sterol enrichment of intestinal sarcolemmal membranes
M.C. Carey, M. Xie, V.R. Kotecha, J.D. Andrade, J.G. Fox
(Boston, Cambridge, US)

14. Hyperhomocysteinemia and -homocysteinbilia: Unrecognized signals and perhaps new perils of cholesterol cholelithogenesis
M.C. Carey, J. Zhang, D.E. Handy, Y. Wang, G. Bouchard, J. Selhub,
J. Loscalzo (Boston, Charlestown, US)

15. The miRNA-21/PDCD4 pathway in the response of rat hepatocytes to bile acids: Deciding between cell death and survival
R.E. Castro, M.B. Afonso, D.M.S. Ferreira, P.M. Rodrigues, P.M. Borralho,
C.M.P. Rodrigues (Lisbon, PT)

16. Bile salt toxicity in sandwich-cultured rat hepatocytes (SCRH): Intracellular exposure to glycine conjugates appears to be the main determinant of bile salt toxicity
S. Chatterjee, P. Augustijns, P. Annaert (Leuven, BE)

17. Analysis of liver inflammation in mice invalidated for the vitamin D nuclear receptor
N. Chignard, T. Denaes, D. Firrincieli, C. Housset (Paris, FR)

18. A randomized controlled trial of budesonide with ursodeoxycholic acid versus methotrexate plus ursodiol in primary biliary cirrhosis
I. Copaci, L. Micu, E. Mindrut, M. Voiculescu (Bucharest, RO)

19. Modulation of intestinal microflora affects hepatic bile acid synthesis in mice
C. Degirolamo, S. Rainaldi, S. Murzilli, A. Moschetta
(Sta. Maria Imbaro, Bari, IT)

20. In vitro analysis of splicing mutations of *ABCB4* using minigene constructs
P.H. Dixon, M. Willsden, C. Williamson (London, GB)

21. Efficacy of ursodeoxycholic acid in the treatment of nonalcoholic fatty liver disease
O.P. Dudanova, I.A. Belavina (Petrozavodsk, RU)

22. Cellular effects of disease-causing variations of the hepatobiliary transporter ABCB4
T. Falguières, J. Gautherot, J.-L. Delaunay, D. Delautier, A.-M. Durand-Schneider, C. Housset, M. Maurice, T. Ait-Slimane (Paris, FR)

23. Ursodeoxycholic acid inhibits activation of miR-34a/SIRT1/p53, a pro-apoptotic pathway associated with disease severity in non-alcoholic fatty liver
D.M.S. Ferreira, P.M. Borralho, M.B. Afonso, P.M. Rodrigues, M.V. Machado, H. Cortez-Pinto, C.M.P. Rodrigues, R.E. Castro (Lisbon, PT)

24. Vitamin D nuclear receptor deficiency promotes E-cadherin cleavage and bile duct rupture in bile duct-ligated mice
D. Firrincieli, S. Zuniga, C. Rey, D. Wendum, E. Lasnier, D. Rainteau, A. Cadoret, M. Boissan, C. Housset, N. Chignard (Paris, FR)

25. Carbon-11 labelled cholylsarcosine: A PET-tracer for conjugated bile acids
K. Frisch, S. Jakobsen, M. Sorensen, O.L. Munk, A.K.O. Alstrup, P. Ott, A.F. Hofmann, S. Keiding (Aarhus, DK; San Diego, US)

26. Combined therapy with low-dose ursodeoxycholic acid in the treatment of nonalcoholic steatohepatitis in obese patients
A. Genunche-Dumitrescu, D. Badea, M. Badea, P. Mitrut, A. Badea (Craiova, RO)

27. A helical structure of the N-terminal part of the TGR5 C-terminus is required for receptor membrane localization
C.G.W. Gertzen, B. Schmitz, B. Nisius, V. Keitel, H. Gohlke (Düsseldorf, DE)

28. Fenofibrate, a peroxisome proliferator-activated receptor alpha (PPARalpha) agonist, up-regulates human MDR3/ABCB4
N.S. Ghonem, M. Ananthanarayanan, C.J. Soroka, J.L. Boyer (New Haven, US)

29. Bile acids stabilize their receptor FXR by blocking ubiquitin-proteasome-mediated degradation
G. Gil, W.M. Pandak, L. Zhang, L. Ouyang, Z.X. Cronk (Richmond, US)

30. Sulfonylurea receptor in myofibroblasts is a target for ursodeoxycholic acid: A new avenue for anti-arrhythmic therapy
J. Gorelik, F. Schultz, M. Miragoli, M.J. Lab, V.O. Nikolaev, C. Williamson (London, GB)

31. Are reduced levels of cholestenoic acids a cause of motor neuron disease?
W.J. Griffiths, Y. Wang, S. Theofilopoulos, E. Arenas, P.T. Clayton, M.T. Bassi (London, GB; Stockholm, SE; Lecco, IT)

32. Human FXR regulates SHP expression through direct binding to an LRH-1 binding site, independent of an IR-1 and LRH-1
J. Heegsma, M.O. Hoeke, M. Hoekstra, H. Moshage, K.N. Faber (Groningen, NL)

33. Protective effect of biliverdin and biliverdin reductase against bile acid-induced toxicity in liver cells
F. Herraez, E. Gonzalez Sanchez, M.J. Perez, N.S. Nytofte, O. Briz, M.A. Serrano, M.J. Monte, F. Jimenez, F. Gonzalez-San Martin, J.J.G. Marin (Salamanca, ES; Nuuk, GL)

34. Association of FXR polymorphisms with cholelithiasis
S. Hirobe-Jahn, S. Harsch, O. Renner, A. Strohmeyer, S. Schimmel, E.F. Stange (Stuttgart, DE)

35. Vitamin A deficiency (VAD) aggravates liver damage in obstructive cholestasis and is characterized by an altered bile acid profile and excessive bile duct damage
M. Hoekstra, M.O. Hoeke, J. Heegsma, H. Moshage, K.N. Faber (Groningen, NL)

36. Dissecting the molecular pathways regulating FXR transactivation and transrepression
D.A.A. Hollman, E.C.L. Willemsen, R.M. Gadaleta, A. Milona, R. van Gent, V. Massafra, A.B. Brenkman, S.W.C. van Mil (Utrecht, NL)

37. Combination therapy of primary biliary cirrhosis with ursodeoxycholic acid and a dual PPARα/PXR agonist, bezafibrate
A. Honda, T. Ikegami, T. Miyazaki, M. Nakamuta, H. Takikawa, Y. Matsuzaki (Ibaraki, Fukuoka, Tokyo, JP)

38. Deoxycholic acid-FXR signalling is crucial for the bile acid metabolic phenotypes in Cyp8b1$^{-/-}$ mice
X. Hu, Y. Bonde, G. Eggertsen, M. Rudling (Stockholm, SE)

39. Resistance to diet-induced obesity conferred by dietary bile acid supplementation in mice is strain specific
K. Hüttinger, T. Fromme, M. Klingenspor (Freising, DE)

40. Increased serum liver X receptor ligand oxysterols in patients with non-alcoholic fatty liver disease
T. Ikegami, A. Honda, T. Miyazaki, Y. Matsuzaki, H. Hyogo, S. Tazuma, K. Tokushige, E. Hashimoto, K. Inui (Ibaraki, Hiroshima, Tokyo, Nagoya, JP)

41. Identification of *S*-acyl glutathione conjugates of bile acids in human bile by means of LC/ESI-MS
S. Ikegawa, N. Hori, T. Iida, A.F. Hofmann, K. Mitamura (Higashi-Osaka, Tokyo, JP; San Diego, US)

42. Gut microbiota regulates bile acid composition throughout the enterohepatic system
S. Islam, J. Felin, S. Jäntti, T. Hyötyläinen, A. Wahlström, H.-U. Marschall, B. Angelin, M. Oresic, F. Bäckhed (Gothenburg, SE; Espoo, FI; Stockholm, SE)

43. Intestinal bile acid transporter expression in inflammatory bowel disease
J. Jahnel, P. Fickert, C. Högenauer, M. Trauner (Graz, Vienna, AT)

44. Ezetimibe increases transintestinal cholesterol secretion in humans
L. Jakulj, T. van Dijk, T. Boer, R. Boverhof, E.G. Stroes, A.K. Groen (Amsterdam, Groningen, NL)

45. A novel major epimer of varanic acid from the bile of monitor lizards: Taurine-conjugated (*24R, 25S*)-3α,7α,12α,24-tetrahydroxy-5β-cholestan-27-oic acid
N. Kato, T. Iida, S. Ogawa, M. Une, K. Mitamura, S. Ikegawa, L.R. Hagey, A.F. Hofmann (Tokyo, Noda, Kure, Higashi-Osaka, JP; San Diego, US)

46. TGR5 (Gpbar-1) has anti-apoptotic and proliferative effects in isolated cholangiocytes and is overexpressed in human cholangiocarcinomas
V. Keitel, M. Reich, R. Reinehr, A. Sommerfeld, K. Cupisti, W.T. Knoefel, D. Häussinger (Düsseldorf, DE)

47. Ursodeoxycholic acid inhibits Toll-like receptor-2 and -4 driven cytokine release from human colonic epithelium
O. Kelly, J. Ward, C. Jefferies, F.E. Murray, S.J. Keely (Dublin, IE)

48. Ursodeoxycholic acid influences the expression of p27kip1 but not FoxO1 in patients with non-cirrhotic primary biliary cirrhosis (PBC)
A. Kempinska-Podhorodecka, J. Kopycinska, E. Elias, P. Milkiewicz, M. Milkiewicz (Szczecin, PL)

49. Ursodeoxycholic acid affects expression of CAR but not PXR mRNA in patients with non-cirrhotic primary biliary cirrhosis (PBC)
M. Klak, M. Milkiewicz, E. Wunsch, E. Elias, P. Milkiewicz (Szczecin, PL; Birmingham, GB)

50. Short-term feedback regulation of bile salt uptake by bile salts in rat liver
S. Kluge, O. Domanova, T. Berlage, C. Stross, A. Helmer, V. Keitel, D. Häussinger, R. Kubitz (Düsseldorf, St. Augustin, Bonn, DE)

51. Wild-type bile and "Byler bowel": Diarrhoea and hepatic steatosis after liver transplantation in *ATP8B1* disease
A.S. Knisely, L.N. Bull, S.A. Kocoshis (London, GB; San Francisco, Cincinnati, US)

52. *nor*UDCA protects CBDL mice from bile acid-induced collecting duct tubular injury
E. Krones, M.J. Pollheimer, F. Durchschein, M. Trauner, P. Fickert (Graz, Vienna, AT)

53. Bile salt hydrophobicity causes cirrhosis and cholestasis in new mouse models for PFIC disease
C. Kunne, M. de Graaff, S. Duijst, D.R. de Waart, R.P.J. Oude Elferink, C. Paulusma (Amsterdam, NL)

54. Bile acids regulate autophagy in colonic epithelial cells
N. Lajczak, M.S. Mroz, S.J. Keely (Dublin, IE)

55. Evolution of substrate specificity for the bile salt transporter ABST (SLC10A2)
D. Lionarons, J.L. Boyer, S.-Y. Cai (New Haven, US)

56. Role of bile acids as cocarcinogenic agents in the development of cholangiocarcinoma
R.I.R. Macias, E. Lozano, M.J. Monte, L. Sanchez-Vicente, F. Herraez, O. Briz, J.J.G. Marin (Salamanca, ES)

57. Development of an analytical method for focused metabolomics of bile acid conjugates
M. Maekawa, T. Inoue, K. Ohno, H. Nittono, T. Iida, A.F. Hofmann, J. Goto, M. Shimada, N. Mano (Sendai, Yonago, Tokyo, JP; San Diego, US)

58. An intact biliary glycocalyx adds to the barrier function of the apical membrane that protects cholangiocytes against toxic bile salts
L. Maillette de Buy Wenniger, S. Hohenester, S.J. van Vliet, R.P.J. Oude Elferink, U. Beuers (Amsterdam, NL; Aachen, DE)

59. High levels of ursodeoxycholic acid act as FXR antagonist and deplete liver cholesterol due to increased bile acid synthesis in morbidly obese patients
H.-U. Marschall, M. Müller, T. Claudel, M. Wagner, P. Fickert, G. Zollner, D. Silbert, K. Lackner, A. Thorell, M. Trauner
(Gothenburg, Stockholm, SE; Graz, Vienna, AT)

60. The farnesoid X receptor (FXR) agonist obeticholic acid (OCA) increases plasma FGF-19 concentrations and decreases bile acid synthesis in primary biliary cirrhosis (PBC)
H.-U. Marschall, V.A. Luketic, A. Lövgren-Sandblom, L. Benthin, K.V. Kowdley, G. Hirschfield, A. Mason, K.D. Lindor, S. Gordon, C. Vincent, R.W. Chapman, M. Mayo, A.K. Burroughs, A. Parés, D.E.J. Jones, C. Schramm, L. Eliot, A.F. Hofmann, D. Shapiro
(Gothenburg, SE; Richmond, US; Stockholm, SE; Seattle, US; Toronto, Edmonton, CA; Rochester, Detroit, US; Montreal, CA; Oxford, GB; Dallas, US; London, GB; Barcelona, ES; Newcastle upon Tyne, GB; Hamburg, DE; San Diego, US)

61. Intrahepatic cholestasis of pregnancy is not associated with intrauterine fetal death but with gestational diabetes and preeclampsia
H.-U. Marschall, E. Wikström Shemer, J.F. Ludvigsson, O. Stephansson
(Gothenburg, Stockholm, SE)

62. Gallstone disease in Swedish twins is linked to the Gilbert variant of *UGT1A1*
H.-U. Marschall, M. Krawczyk, F. Grünhage, D. Stalberg Katsika, C.A. Einarsson, F. Lammert (Gothenburg, SE; Homburg, DE; Stockholm, SE)

63. FXR acetylation: What are the functional consequences on target gene expression?
V. Massafra, A. Milona, D.A.A. Hollman, E.C.L. Willemsen, S.W.C. van Mil (Utrecht, NL)

64. Bile acid sequestration with or without voluntary wheel running dramatically reduces atherosclerosis in hypercholesterolemic mice
M. Meissner, R. Boverhof, H. Wolters, R.A. de Boer, R. Havinga, V.W. Bloks, F. Kuipers, A.K. Groen (Groningen, NL)

65. Exploring a role for Cyp17a1 in the pathogenesis of cholestasis
A. Milona, E.C.L. Willemsen, C. Williamson, S.W.C. van Mil
(Utrecht, NL; London, GB)

66. Progressive familial intrahepatic cholestasis (PIFC) type II – 15 years treatment with UDCA
M. Miloshevski, V. Serafimoski, V. Calovska, M. Genadieva-Dimitrova, O. Bernard (Skopje, MK; Paris, FR)

67. Activation of FXR upregulates fatty acid β-oxidation and reduces triglyceride accumulation in human but not in mouse hepatocytes
T. Miyazaki, A. Honda, T. Ikegami, Y. Matsuzaki (Ibaraki, JP)

68. Farnesoid X receptor exerts antisecretory actions on colonic epithelium – A new target for development of antidiarrhoeal drugs?
M.S. Mroz, N. Keating, J. Ward, S. Amu, P. Fallon, S.J. Keely (Dublin, IE)

69. Intrahepatic cholestasis of pregnancy levels of sulfated progesterone metabolites downregulate hepatic LXRα
V. Nikolova, S. Abu-Hayyeh, G. Papacleovoulou, M.G. Parker, C. Williamson (London, GB)

70. Induction of FGF19 but not ASBT by bile acids in short-term explant culture of human ileum
J.D. Nolan, S.L. Kennie, J.H. Zhang, I.M. Johnston, T. Dew, P.H. Dixon, C. Williamson, J.R.F. Walters (London, GB)

71. Chemical synthesis of 7α,12α-dihydroxy-cholest-4-en-3-one and its 12-deoxy analogue (C4): Key intermediates in the biosynthesis of bile acids from cholesterol
K. Omura, Y. Kimoto, T. Iida, O. Ogawa, K. Mitamura, S. Ikegawa, L.R. Hagey, A.F. Hofmann (Tokyo, Noda, Higashi-Osaka, JP; San Diego, US)

72. Chronic prednisolone treatment strongly influences bile salt and cholesterol fluxes in mice
C. Out, A. Laskewitz, R. Havinga, T. van Dijk, R. Boverhof, H. Verkade, F. Kuipers, A.K. Groen (Groningen, NL)

73. Gut microbiota have profound effects on host bile acid and cholesterol metabolism
C. Out, J. Hageman, T. Bos, R. Boverhof, R. Havinga, F. Kuipers, H. Verkade, A.K. Groen (Groningen, NL)

74. The cholesterol metabolite, 5-cholesten-3beta, 25-diol 3-sulfate, promotes hepatic proliferation in mice
W.M. Pandak, X. Zhang, Q. Bai, G. Kakiyama, L. Xu, J.K. Kim, S. Ren (Richmond, US; Shanghai, RC)

75. Programming of metabolic disease in mouse female offspring from cholestatic pregnancy
G. Papacleovoulou, S. Abu-Hayyeh, E. Nikolopoulou, B. Owen, V. Nikolova, E. Jensen, A.S. Knisely, C. Williamson (London, GB; Belthoven, NL)

76. TGR5 knockout mice are highly susceptible to lipopolysaccharide treatment
M. Reich, V. Keitel, D. Häussinger (Düsseldorf, DE)

77. Involvement of the peroxisomal ABC-transporter Pmp70 in the intracellular transport of bile salts in rat hepatocytes
K.P. Rembacz, J. Woudenberg, Z. Du, F.A.J. van den Heuvel, E.Z. Jonkers, F. Stellaard, H. Moshage, K.N. Faber (Groningen, NL)

78. The acidic pathway of bile acid biosynthesis: Role in oxysterol sulfation, lipid metabolism and inflammatory responses
S. Ren, L. Xu, Q. Bai, J.K. Kim, S. Chu, P. Hylemon, D.M. Heuman, W.M. Pandak (Richmond, US)

79. Understanding human metabolic effects of resins from monitoring responses following acute or chronic treatment
M. Rudling, S. Straniero, B. Angelin, B. Sjöberg (Stockholm, SE)

80. Circulating FGF19 is strongly increased in human extrahepatic cholestasis
M. Rudling, R. Hultcrantz, A. Bergqvist, B. Angelin (Stockholm, SE)

81. Liver HMG-CoA reductase is contributing to transintestinal cholesterol excretion
M. Schonewille, J. Hageman, A.K. Groen, G. Brufau (Groningen, NL)

82. Nor-UDCA and UDCA protect against arrhythmia in a model of the fetal heart
F. Schultz, M. Miragoli, A. Bhargava, M. Trauner, C. Williamson, J. Gorelik (London, GB; Vienna, AT)

83. Successful treatment of bile acid synthetic defects with oral cholic acid – Results from a 20+ year experience
K.D.R. Setchell, J.E. Heubi (Cincinnati, US)

84. Use of novel fluorescent variants of cholic acid to study intracellular sorting of bile acids
S. Shajari, K.P. Rembacz, M. Hoekstra, E.Z. Jonkers, M.A. Miranda, F. Stellaard, H. Moshage, K.N. Faber (Groningen, NL; Valencia, ES)

85. Bile acid flux is a more powerful signal than FGF-19 in the regulation of CYP7A1 in rabbits
Q. Shang, M. Saumoy, G. Salen, G. Xu (East Orange, Newark, US)

86. The N-terminal part of the TGR5 C-terminus is essential for receptor membrane localization
L. Spomer, V. Keitel, D. Häussinger (Düsseldorf, DE)

87. Lysophosphatidylcholine induces apoptotic cell death in biliary epithelial cell: Implication to biliary oncogenesis associated with pancreaticobiliary maljunction
A. Sugiyama, K. Kanno, M. Saito, K. Fujita, N. Kishikawa, M. Okamoto, Y. Kikuchi, K. Yokobayashi, S. Kushihata, M. Mizooka, T. Saeki, M. Nakashima, S. Tazuma (Hiroshima, JP)

88. Identification of functionally relevant lysine residues that modulate human farnesoid X receptor (hFXR) function
A.-Q. Sun, Y. Luo, D.S. Backos, S. Xu, N. Balasubramaniyan, P. Reigan, F. Suchy (Aurora, US)

89. Effect of 4-phenylbutyrate on the biliary transport maximum of taurocholate reduced by lipopolysaccharide in rats
H. Takikawa, N. Sano, M. Adachi, K. Miura, M. Mikami, M. Aiso, Y. Takamori, A. Tanaka (Tokyo, JP)

90. Hypertension enhances hepatic steatosis through oxidative stress associated with down-regulated expressions of LXR and FXR: Implication to the future strategy for non-alcoholic fatty liver diseases (NAFLD) by nuclear receptor ligands
S. Tazuma, T. Ikuta, K. Kanno, M. Nakashima, A. Sugiyama, N. Kishikawa, H. Kodama, M. Okamoto, Y. Kikuchi, S. Kushihata, K. Yokobayashi, M. Mizooka, T. Saeki, K. Arihiro (Hiroshima, JP)

91. Monitoring bile acid transport in single living cells using a genetically encoded fret sensor
S.F.J. van de Graaf, M. Merkx, S.W.C. van Mil, L.W.J. Klomp, I.T.G.W. Bijsmans, M.V. Golynskiy, L.M. van der Velden (Utrecht, Eindhoven, NL)

92. Genetic inactivation of the bile salt export pump in mice leads to massive cholesterol excretion
M.Y.M. van der Wulp, T.H. van Dijk, V.W. Bloks, A.K. Groen, H.J. Verkade (Wageningen, Groningen, NL)

93. Atp8B1 deficiency in mice, mimicking PFIC type 1, reduces the synthesis and pool size of the bile salt cholate
H.J. Verkade, K.E. Gooijert, C. Kunne, R. Boverhof, F. Stellaard, C.C. Paulusma (Groningen, Amsterdam, NL)

94. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-betamuricholic acid, a naturally occurring FXR antagonist
A. Wahlström, S. Islam, J. Felin, H.-U. Marschall, K. Bamberg, F. Bäckhed (Gothenburg, Mölndal, SE)

95. Reduced fibroblast growth factor 19 production in patients with primary bile acid diarrhoea
J.R.F. Walters, I.M. Johnston, J.D. Nolan, S.S. Pattni, K.D. Bardhan, P.H. Dixon, C. Williamson (London, GB)

96. Bile acid precursors and oxysterol 7alpha-hydroxylase deficiency
Y. Wang, W.J. Griffiths, P. Crick, P.T. Clayton, A.A. Morris (Swansea, London, Manchester, GB)

97. The G protein-coupled bile acid receptor, TGR5, is expressed on colonic epithelial cells and regulates ion transport
J. Ward, M. Mroz, S.J. Keely (Dublin, IE)

98. Bile acid binding resins affect diverse molecules to improve metabolic syndrome
M. Watanabe, K. Morimoto, N. Kaneko-Iwasaki, T. Sugizaki, Y. Horai, E. Arita, K. Murahashi, K. Schoonjans, J. Auwerx, H. Itho (Tokyo, Kanagawa, JP; Lausanne, CH)

99. Microscopy based method for the quantification of subcellular localization of hepatobiliary tranporters
K. Weissenberger, S. Kluge, J. Stindt, D. Häussinger, R. Kubitz (Düsseldorf, DE)

100. A prospective national study of severe intrahepatic cholestasis of pregnancy
C. Williamson, V. Geenes, B. North, M. Knight (London, Oxford, GB)

101. FXR- and BDL-mediated transcriptional regulation of hepatic and extrahepatic glucuronidation in a humanized *tgUGT1A* mouse model
A. Winkler, S. Kalthoff, N. Freiberg, M.P. Manns, C.P. Strassburg (Hannover, DE)

102. A novel di-leucine motif at the N-terminus of human Organic Solute Transporter-beta (hOST-beta) is essential for protein interaction and membrane localization
S. Xu, A.-Q. Sun, D.S. Backos, Y. Luo, P. Reigan, F.J. Suchy (Aurora, US)

103. Bile acids and sphingosine-1 phosphate receptor signaling in cholangiocarcinoma progression
H. Zhou, R. Zhao, X. Zhou, D.J.W. Campbell, A.E. Sirica, P.B. Hylemon (Richmond, US)

104. Reduced FGF19 level in bile of patients with primary sclerosing cholangitis
S.J.L.B. Zweers, A. Shiryaev, T.H. Karlsen, P.L.M. Jansen, F.G. Schaap (Amsterdam, NL; Oslo, NO)

Session I

Metabolism and transport of bile acids

Regulation of canalicular ABC transporters by their membrane microenvironment

Bruno Stieger
Department of Clinical Pharmacology and Toxicology, University Hospital, 8091 Zurich, Switzerland

Canalicular bile formation in essence leads to the production of a detergent by hepatocytes. The first biological barrier to be exposed to this detergent solution is the canalicular membrane, which consequently needs special biophysical properties to resist the detergent action of canalicular bile salts. The canalicular membrane of rat liver contains microdomains, which can be induced by detergents or bile salts. These microdomains are enriched in cholesterol and contain ATP-binding cassette (ABC) transporters necessary for canalicular bile formation. Microdomains may constitute a protective element against the detergent action of bile salts. Cholesterol is known to regulate the activity of various membrane-bound enzymes and transporters. We use the insect cell system to investigate the influence of membrane cholesterol content on the activity of the bile salt export pump (BSEP) and the multidrug resistance-associated protein 2 (MRP2), two key ABC-transporters needed for canalicular bile formation. Cholesterol loading of insect cells stimulates markedly the transport activity of BSEP and MRP2. Kinetic analysis of BSEP activity revealed classic Michaelis-Menten kinetics in membranes with low and with high cholesterol content. In contrast, kinetic analysis of MRP2 in membranes with low cholesterol content revealed a mild cooperativity for small molecular weight substrates and classic Michaelis-Menten kinetics for a high molecular weight substrate. In membranes with high cholesterol content, MRP2 displayed classic Michaelis-Menten kinetics for small and high molecular weight substrates. Both transporters have an increased vmax in high cholesterol membranes demonstrating a positive regulation of these transporters by cholesterol. In conclusion, membrane cholesterol content regulates transport activity of these two transporters and in the case of MRP2 also affects the substrate binding.

Bile acids are upstream regulators of the Hippo growth control pathway

Sayeepriyadarshini Anakk[1], Randy L. Johnson[3], Milton J. Finegold[2], David D. Moore[1]
[1]Department of Molecular and Cellular Biology, [2]Department of Pathology, Baylor College of Medicine, [3]Department of Biochemistry and Molecular Biology, UTMDACC, Houston, TX 77030, USA

The Hippo pathway regulates organ size and cell proliferation. Loss of Hippo kinase or over expression of its target YAP (Yes Associated Protein) results in enlarged liver and spontaneous liver tumorigenesis. We found that the phenotype of mice with highly elevated bile acid levels is very similar to that of mice with targeted deletion of Hippo pathway components, including increased YAP activation, hepatomegaly, progenitor cell proliferation and spontaneous tumorigenesis. Bile acid treatment is sufficient to induce YAP activation in mice as well as primary mouse hepatocytes. YAP is over expressed in bile ducts as well as hepatocytes in human patients with biliary dysfunction and hepatic bile acid overload. Thus, bile acids can regulate liver growth and tumorigenesis via the Hippo pathway.

Molecular basis and mechanism of Rotor syndrome

M. Jirsa
Center of Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Bilirubin, a breakdown product of heme, is normally glucuronidated and excreted by the liver into bile. Failure of this system can lead to a buildup of conjugated bilirubin in the blood, resulting in jaundice. The mechanistic basis of bilirubin excretion and hyperbilirubinemia syndromes is largely understood, but that of Rotor syndrome, an autosomal recessive disorder characterized by conjugated hyperbilirubinemia, coproporphyrinuria, and near-absent hepatic uptake of bromsulphthalein, indocyanine green and cholescintigraphy radiotracers, has remained enigmatic.

In our study we analyzed 8 Rotor syndrome families. We found that subjects affected with Rotor syndrome carry mutations in the *SLCO1B1* and *SLCO1B3* genes predicted to cause complete and simultaneous deficiencies of the organic anion transporting polypeptides OATP1B1 and OATP1B3. These liver-specific detoxification-limiting proteins mediate uptake and clearance of conjugated and unconjugated bilirubin, unconjugated bile salts, thyroid hormones, conjugated steroids, numerous xenobiotics and toxins, and countless drugs, drug metabolites and their conjugates across the sinusoidal membrane of hepatocytes. OATP1B1 polymorphisms have previously been linked to drug hypersensitivities.

Using mice deficient in Oatp1a/1b and in the multispecific sinusoidal export pump Abcc3, we found that Abcc3 secretes bilirubin conjugates into the blood, while Oatp1a/1b transporters mediate their hepatic reuptake. Transgenic expression of human OATP1B1 or OATP1B3 restored the function of this detoxification-enhancing liver-blood shuttle in Oatp1a/1b-deficient mice. Within liver lobules, this shuttle may allow flexible transfer of bilirubin conjugates (and probably also drug conjugates) formed in upstream hepatocytes to downstream hepatocytes, thereby preventing local saturation of further detoxification processes and hepatocyte toxic injury. The same proteins (OATP1B1 and OATP1B3) may also be responsible for clearance of bilirubin conjugated in extrahepatic tissues.

Disruption of hepatic uptake of bilirubin glucuronides due to coexisting OATP1B1 and OATP1B3 deficiencies explains Rotor-type hyperbilirubinemia. Moreover, OATP1B1 and OATP1B3 null mutations may confer a substantial risk of drug toxicities.

The expression of bile salt export pump (BSEP, ABCB11) and FIC1 ATPase (FIC1, ATP8B1) are regulated by microRNA-33 (mIR-33) which is downregulated in experimental cholestasis

Meena Ananthanarayanan[2], Ryan M. Allen[1], Tyler J. Marquart[1], Carolyn J. Albert[1], Frederick J. Suchy[3], David A. Ford[1] and Angel Baldan[1]
[1]Department of Molecular Biology and Biochemistry, St. Louis University School of Medicine, St. Louis, MO, [2]Section of Digestive Diseases, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT and Department of Pediatrics, University of Colorado Denver School of Medicine, Aurora, CO, USA

Introduction: MicroRNAs are small 20-25 nt noncoding RNAs shown to regulate a number of biological processes by binding to specific recognition motifs in the 3'-UTR of cognate mRNAs. We showed that the evolutionarily conserved mIR-33, downregulates the sterol transporter ABCA1 (PNAS 2010;107:12228). Whether mIR-33 binds to and regulate the expression of bile acid transporter mRNAs and their effects on bile secretion are unknown.

Methods: Functional analysis of mIRNA binding sites were evaluated by cloning the 3'-UTR of ABCB11 and ATP8B1 mRNAs downstream of luciferase reporter genes.

Results: Human and rodent *BSEP/Bsep* mRNAs contained mIR-33 recognition sequences overlapping the stop codon and 732- 751 nt after the stop codon respectively. Evolutionarily conserved mIR-33 binding sites were also found in the 3'-UTR of *ATP8B1/atp8b1* mRNAs. Cotransfection 3'-UTR of constructs into HEK293 cells in the presence or absence of a plasmid that overexpresses miR-33 confirmed that the 3'UTRs of these genes responded to miR-33. miR-33 overexpression resulted in ~40% decrease in luciferase activity when fused to the 3'UTR or the putative responsive elements of *ATP8B1/atp8b1* or *ABCB11/abcb11.* Mutations preventing the binding of the seed sequence of the miRNA abolished the response to miR-33. To verify whether mIR-33 regulates ABCB11 and ATP8B1 in human liver, Huh7 cell line was transfected with mIR-33 plasmid. qPCR analysis of ABCB11 and ATP8B1 message levels revealed that mIR-33 overexpression resulted in 24.8 and 25.7% decrease in levels respectively compared to a GFP control. To see whether mIR-33 expression levels are altered during cholestasis, mIR-33 was quantified post-CBDL. Expression of mIR-33 decreased to 35.1, 72.8 and 61% of sham-operated controls at 1, 3 and 7 days after CBDL suggesting adaptive regulation to allow maximal expression of BSEP and FIC1.

Discussion/Conclusion: Together, these results suggest that mIR-33 may play a major role in bile secretion by controlling the expression of BSEP/FIC1.

Session II

Bile acid signaling I

TGR5 and atherosclerosis

Kristina Schoonjans
Laboratory of integrative systems and physiology, École Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland

Low-grade chronic inflammation, obesity and insulin resistance are all risk factors intimately linked with the metabolic syndrome. Interestingly, many of the signaling pathways that control inflammatory responses also intersect with those that control nutrient metabolism. Earlier work from our laboratory has demonstrated that BAs, through TGR5 activation, can exert systemic metabolic effects by promoting energy expenditure and GLP-1 secretion, thereby preventing obesity and insulin resistance during high fat diet feeding. More recently, we demonstrated that activation of TGR5 signaling suppresses pro-inflammatory cytokine production in macrophages via a mechanism that involves inhibition of NFkB nuclear translocation. TGR5 activation with the TGR5-specific agonist INT-777 also inhibits acetylated LDL uptake and loading and attenuates the development of atherosclerosis in $LDLR^{-/-}$ mice fed a high cholesterol diet. These data underscore the TGR5-NFkB signaling pathway as a novel target to prevent and combat chronic inflammatory diseases such as atherosclerosis.

TGR5 in the nervous system

Verena Keitel and Dieter Häussinger
Klinik für Gastroenterologie, Hepatologie und Infektiologie, Moorenstr. 5, 40225 Düsseldorf, Germany

TGR5 (Gpbar-1) is a plasma membrane-bound, G_s-protein coupled bile acid receptor, which is expressed in various tissues including intestine and brain (1, 2).
In the intestine TGR5 was detected in enteroendocrine L-cells, in immune cells as well as in neurons and astrocytes of the submucosal and myenteric plexus (3–5). While expression of TGR5 in L-cells has been linked to increased glucagon-like peptide-1 secretion and regulation of glucose homeostasis (5), the function of the receptor in the enteric nervous systems is mostly unknown. However, administration of TGR5 ligands to mice delayed gastric emptying and small bowel transit time (3). This is line with findings from a genetic analysis which revealed an association of the C-allel of the common TGR5 single nucleotide polymorphism rs11554825 and faster small bowel transit time (6). This exon 1 SNP is located outside the coding sequence of TGR5 and has been associated with decreased TGR5 expression levels (7, 8).
In the central nervous system TGR5 has been detected in the plasma membrane of astrocytes, neurons and microglia (9). In these cells TGR5 acts as a receptor for neuroactive steroids, which is activated by nanomolar concentrations of 5β-pregnan-3α-ol-20-one (pregnanolone) and micromolar concentrations of 5β-pregnan-3α-17α-21-triol-20-one and 5α-pregnan-3α-ol-20-one (allopregnanolone). In cultured rat astrocytes and neurons stimulation of TGR5 led to adenylate cyclase activation, elevation of intracellular calcium levels and the generation of reactive oxygen species. Furthermore, TGR5 mRNA expression was downregulated in astrocytes in the presence of neurosteroids and ammonia. A significant reduction of TGR5 mRNA was also detected in cerebral cortex from cirrhotic patients dying with hepatic encephalopathy (HE) as compared to brains from non-cirrhotic patients, suggesting a role for TGR5 in the pathogenesis of HE (9).
TGR5 is localized in astrocytes and neurons of the enteric and central nervous system, where the receptor has been linked to intestinal motility and the pathogenesis of hepatic encephalopathy, respectively.

Reference List:

1. Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, et al. A G protein-coupled receptor responsive to bile acids. J Biol Chem 2003;278:9435–9440.
2. Maruyama T, Miyamoto Y, Nakamura T, Tamai Y, Okada H, Sugiyama E, et al. Identification of membrane-type receptor for bile acids (M-BAR). Biochem Biophys Res Commun 2002;298:714–719.
3. Poole DP, Godfrey C, Cattaruzza F, Cottrell GS, Kirkland JG, Pelayo JC, et al. Expression and function of the bile acid receptor GpBAR1 (TGR5) in the murine enteric nervous system. Neurogastroenterol Motil 2010;22:814–825.

4. Reimann F, Habib AM, Tolhurst G, Parker HE, Rogers GJ, Gribble FM. Glucose sensing in L cells: a primary cell study. Cell Metab 2008;8:532–539.
5. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, et al. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab 2009;10: 167–177.
6. Camilleri M, Vazquez-Roque MI, Carlson P, Burton D, Wong BS, Zinsmeister AR. Association of bile acid receptor TGR5 variation and transit in health and lower functional gastrointestinal disorders. Neurogastroenterol Motil 2011;23:995–999, e458.
7. Hov JR, Keitel V, Laerdahl JK, Spomer L, Ellinghaus E, ElSharawy A, et al. Mutational characterization of the bile acid receptor TGR5 in primary sclerosing cholangitis. PLoS One 2010;5:e12403.
8. Keitel V. Bile acids as extrahepatic and interorgan signaling molecules. In: Häussinger D, Keitel V, Kubitz R, eds. Hepatobiliary Transport in Health and Disease.Berlin: DeGruyter Publishing, 2012. 117–129.
9. Keitel V, Görg B, Bidmon HJ, Zemtsova I, Spomer L, Zilles K, et al. The bile acid receptor TGR5 (Gpbar-1) acts as a neurosteroid receptor in brain. Glia 2010;58: 1794–1805.

Bile acids in the gut-liver axis: The FXR-FGF15/19 axis

Antonio Moschetta
Clinica Medica "A. Murri," University of Bari, Bari, Italy and Laboratory of Lipid Metabolism and Cancer, Department of Translational Pharmacology, Consorzio Mario Negri Sud, 66030 Santa Maria Imbaro, Chieti, Italy

We will present novel data connecting the FXR-FGF15 axis with the modification of bile acid metabolic pathways in health and disease. First we will discuss the relevance of the regulation of this axis in intestinal and hepatic inflammatory and cancer disease (cholestasis, cholesterol gallstone disease and colon cancer). Then, we will specifically focus on the potential relationship between microbiota, bile acids and FXR-FGF15 axis. We will show how modification of the intestinal microbiota via oral administration of specific bacteria strains can directly modulate the rate of hepatic bile acid production. Indeed, using specific transgenic animal model we will underscore the importance of the FXR-FGF 15 axis in the microbiome-induced changes in whole body bile acid homeostasis.

Bile acid and nuclear receptor signaling in liver diseases, obesity and diabetes

John Y.L. Chiang, Tiangaing Li, Jessica M. Francl, and Shannon Boehme
Department of Integrative Medical Sciences, Northeast Ohio Medical University, Rootstown, OH 44272, USA

Non-alcoholic fatty liver disease (NAFLD) is associated with over-nutrition, obesity and insulin resistance. Bile acids play a key role in lipid, glucose and energy metabolism. However, the underlying molecular mechanisms linking bile acid signaling to metabolic homeostasis are far from clear. Cholesterol 7α-hydroxylase (CYP7A1) is the rate-limiting enzyme in bile acid synthesis and plays a critical role in regulating cholesterol and bile acid metabolism. We reported recently that *Cyp7a1* transgenic mice (*Cyp7a1*-tg) were resistant to Western high fat diet-induced fatty liver, obesity and insulin resistance. Increasing bile acid synthesis stimulates cholesterol synthesis and biliary bile acid and cholesterol secretion, and maintains lipid homeostasis. Recent studies have implicated microRNA-33 (miR-33), which is co-localized in intron of the SREBP-1c and SREBP-2 genes, in inhibiting translation of several genes in cholesterol transport and fatty acid metabolism. We hypothesized that Increasing bile acid synthesis stimulates hepatic cholesterol synthesis but maintains cholesterol and bile acid homeostasis, which may be controlled by a feedback loop involving miR-33. We used microarray gene profiling to identify differentially regulated genes and pathways in *Cyp7a1*-tg mice. Adenovirus-mediated gene delivery was used to study the effects of miR-33 on cholesterol and bile acid metabolism in mice. Ingenuity Pathway Analysis of microarray results identified sterol biosynthesis as the top differentially regulated pathway in *Cyp7a1*-tg mice compared to wild type controls. Top 13 up-regulated genes identified in chow-fed *Cyp7a1*-tg mice are involved in cholesterol synthesis and are target genes of steroid response element binding protein 2 (SREBP2). This is consistent to ~11-fold increase in hepatic cholesterol synthesis rate in *Cyp7a1*-tg mice. Interestingly, both SREBP2 and miR-33 were induced in *Cyp7a1*-tg mice. Adenovirus-mediated miR-33 expression in wild type mice repressed the mRNA levels of hepatic bile acid synthesis genes CYP7A1, CYP8B1 and CYP27A1, and a hepatic bile acid uptake transporter NTCP. Expression of miR-33 in mice decreased total bile acid pool size by ~25%. Furthermore, miR-33 decreased serum cholesterol by 50% but increased hepatic cholesterol by ~20%, which is likely due to repression of ABCA1-mediated cholesterol efflux and CYP7A1-mediated conversion of cholesterol into bile acids. Interestingly, miR-33 increased the size of gallbladder and bile acids, cholesterol and phospholipid contents. Transient transfection assay in HepG2 cells showed that miR-33 strongly inhibited the reporter activity of a miRNA reporter construct containing a 3'-UTR (nt +1-200) of CYP7A1 mRNA fused downstream of the luciferase gene. This study revealed that induction of CYP7A1 stimulated conversion of cholesterol to bile acids, and subsequent induction of SREBP2 and miR-33. Increasing expression of miR-33 inhibited bile acid synthesis and bile acid uptake into hepatocytes. Our results revealed a novel role of miR-33 in regulating hepatic cholesterol and bile acid homeostasis. Antagonizing miR-33 may be a therapeutic approach to increase bile acid synthesis for treating metabolic disorders such as hyperlipidemia and NAFLD. (Supported by NIH grants R37DK58379 and R01DK44442).

Monitoring bile acid transport in single living cells using a genetically encoded FRET sensor

Stan F.J. van de Graaf[1], Maarten Merkx[2], Saskia W.C. van Mil[1], Leo W.J. Klomp[1], Ingrid T.G.W. Bijsmans[1], Misha V. Golynskiy[2*], Lieke M. van der Velden[1*]
[1]Department of Metabolic Diseases, University Medical Center Utrecht, The Netherlands
[2]Laboratory of Chemical Biology, Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands
*L.M.vdV and M.V.G contributed equally to this work

Introduction: Bile acids are pivotal for the absorption of dietary lipids and vitamins, and function as important regulators of glucose homeostasis, lipid metabolism and energy expenditure. Current methods to study cellular bile acid homeostasis and transport provide limited time and spatial resolution. Here, we describe a genetically encoded fluorescent sensor that allows for spatiotemporal monitoring of bile acid dynamics in single living cells.

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

Results: Changes in subcellular concentration of multiple bile acid species were detected as robust and reversible changes in Förster Resonance Energy Transfer (FRET) in multiple cell types. Influx of cell-impermeable bile acids was visualized in the presence of bile acid transport proteins. Uptake of cyprinol sulphate, the zebrafish bile alcohol, required expression of zebrafish ASBT. Similarly, Import of taurine- and glycine-conjugated bile acids was shown to depend on the expression of active NTCP. Combined cellular visualization of bile acid uptake with fluorescent labeling of cell surface resident NTCP showed that the low bile acid uptake activity of several NTCP variants was due to loss of transporter activity, as plasma membrane expression was unaffected. The reversible nature of the sensor also enabled measurements of bile acid efflux in living cells, and expression of the organic solute transporter αβ (OSTαβ) resulted in efflux of conjugated chenodeoxycholic acid.

Discussion/Conclusion: Genetically encoded fluorescent bile acid sensor (BAS) was developed that allows real time intracellular imaging of bile acid homeostasis in single living cells.

Session III

Bile acid signaling II

The sphingosine-1-phosphate receptor 2 ($S1P_2$) is activated by conjugated bile acids in primary rodent hepatocytes and in vivo: Implications for regulating hepatic glucose and lipid metabolism

Phillip B. Hylemon[1,4], Kazuaki Takabe[2,3], Masayuki Nagahashi[3], Sarah Spiegel[2], William M. Pandak[4], Weiren Xu[5], Pat Bohdan[1] and Huiping Zhou[1,4]
Departments of Microbiology and Immunology[1], Biochemistry and Molecular Biology[2], Surgery[3], McGuire Veterans Affairs Medical Center[4], Virginia Commonwealth University, Richmond, VA 23298, USA; and Tianjin Key Laboratory of Molecular Design and Drug Discovery, Tianjin Institute of Pharmaceutical Research[5], Tianjin, China

Abstract: Previous studies suggest that the extracellular regulated kinase (ERK1/2) and protein kinase B (AKT) play a yet to be defined role in regulating hepatic lipid metabolism. Conjugated bile acids have been reported to activate the ERK 1/2 and AKT signaling pathways in primary hepatocytes and *in vivo* in a pertussis toxin (PTX) and dominant-negative $G\alpha_1$ sensitive manner. However, the G-protein coupled receptor(s) (GPCR) responsible for activation of these signaling pathways have not been identified nor has the effect of their activation on lipid metabolism been elucidated.

Methods: GPCRs in the lipid activated phylogenetic family were screened for activation of the ERK 1/2 and AKT pathways by the addition of taurocholate (TCA) to HEK293 cell cultures expressing the genes encoding various members of this family. JTE-013, a $S1P_2$ antagonist, and a recombinant lentivirus encoding $S1P_2$ shRNA was used to inhibit and down-regulate the $S1P_2$ receptor, respectively. Bile fistula rats were used to determine if JTE-013 would block induction of SHP by TCA *in vivo*. $S1P_2^{-/-}$ mouse hepatocytes were used to measure the effects on ERK1/2 and AKT activation by TCA. The Glide docking method (Schrödinger Suite 2009 program) was used to predict TCA and S1P binding to $S1P_2$.

Results: $S1P_2$ was the only GPCR in the lipid family significantly activated by TCA. TCA was calculated to hydrogen bond to leucine 173 in the $S1P_2$ binding pocket. The activation of the ERK1/2 and AKT pathways was significantly inhibited by JTE-013 and $S1P_2$ shRNA in primary rodent hepatocytes. JTE-013 significantly inhibited SHP mRNA induction by TCA in bile fistula rats. Primary hepatocytes from $S1P_2^{-/-}$ animals were blunted in the activation of ERK1/2 and AKT by TCA. Finally, $S1P_2^{-/-}$ mouse livers were markedly enlarged and laden with large amounts of cellular lipids as compared to matched wild-type mice.

Conclusion: The activation of $S1P_2$ and FXR by conjugated bile acids aid in the regulation hepatic lipid metabolism during the feed/fast cycle.

$\alpha_5\beta_1$-Integrins are sensors for tauroursodeoxycholate in liver

Holger Gohlke[1], Birte Schmitz[1], Annika Sommerfeld[2], Roland Reinehr[2], Dieter Häussinger[2]
[1]Institut für Pharmazeutische und Medizinische Chemie, Heinrich-Heine-Universität, Universitätsstr. 1, 40225 Düsseldorf, Germany
[2]Klinik für Gastroenterologie, Hepatologie und Infektiologie, Heinrich-Heine-Universität, Moorenstr. 5, 40225 Düsseldorf, Germany

Introduction: Ursodeoxycholic acid, which is *in vivo* converted to its taurine conjugate tauroursodeoxycholate (TUDC), is a mainstay for the treatment of cholestatic liver disease. Earlier work showed that TUDC exerts its choleretic properties in the perfused rat liver in an $\alpha_5\beta_1$ integrin-mediated way. However, the molecular basis of TUDC-sensing in the liver is unknown.

Methods: Rat livers were perfused and analysed by immunofluorescence staining and Western blot technique thereafter. Cell culture experiments were performed in either primary rat hepatocytes or in a stably expressing FLAG-Ntcp-EGFP HepG2 cell line. Molecular dynamics (MD) simulations of complex structures of the ectodomain of $\alpha_5\beta_1$ integrin bound to either TUDC, taurocholate (TC), or the integrin-antagonistic peptide GRGDSP were performed and analysed with respect to conformational changes in the head region.

Results: TUDC (20 µmol/L) induces in perfused rat liver and human HepG2 cells the rapid appearance of the active conformation of the β_1 subunit of $\alpha_5\beta_1$ integrins, followed by an activating phosphorylation of extracellular signal regulated kinases. TUDC-induced kinase activation was no longer observed after β_1 integrin knockdown in isolated rat hepatocytes using siRNA or in the presence of GRGDSP in perfused rat liver. TUDC-induced β_1 integrin activation occurred predominantly inside the hepatocyte and required TUDC uptake via the Na^+/taurocholate cotransporting peptide. The MD simulations of $\alpha_5\beta_1$ integrin with TUDC bound revealed significant conformational changes within the head region that have been linked to integrin activation before.

Discussion/Conclusion: It is concluded that TUDC has the unique property to directly interact with $\alpha_5\beta_1$ integrins inside the hepatocyte. The resulting conformational change triggers β_1 integrin activation and initiates integrin-dependent signalling, which explains not only the choleretic and cytoprotective effects of this bile acid but also its hepatocyte-specificity.

The heart as a target of bile acid signaling

Moreshwar Desai, M.D.[1] and Saul J. Karpen, M.D. Ph.D.[2]
[1]Baylor College of Medicine, Houston, TX, USA
[2]Emory University School of Medicine, Atlanta, GA, USA (skarpen@emory.edu)

There are numerous extrahepatic manifestations of cirrhosis that impact upon both the duration and quality of a patient's life. These targets include the kidneys, musculature, endocrine system, immune system, brain, and heart. The etiologies of these extra-hepatic manifestations of cirrhosis are broad and include consequences of impaired hepatic function (e.g., detoxification) but recent evidence points towards responses of these target organs to elevated levels of biliary constituents in the circulation. Along this line of enquiry, we hypothesized that elevated levels of circulating bile acids, inherent to cirrhotic pathophysiology, contributes to altered cardiac structure and function in these patients. We explored conductive, functional and signaling pathways in the heart in a well-defined mouse model of biliary cirrhosis – the DDC model. Within 3 weeks of initiation of biliary cirrhosis, mice were more fatigued in a treadmill test, and their hearts were larger (both in weight and by MRI), had poorer echocardiographic function, marked activation of hypertrophic signaling pathways (e.g., AKT) and cardiac gene targets (e.g., 20-fold upregulation of β Myosin heavy chain RNA). Moreover, bile acids that stimulate TGR5 signaling activated AKT signaling in isolated myocardiocyte cultures. Intriguingly, both mouse and human cardiomyocytes express high levels of TGR5, suggesting a rational plausible linkage between circulating levels of bile acids and cardiac targeting. In sum, in the DDC as well as several other cholestatic models, bile acid signaling is present in mouse cardiac tissues in ways that recapitulates hypertrophic and functional impairments seen in patients with biliary cirrhosis (i.e., infants with biliary atresia).

References:

Desai MS, Zainuer S, Kennedy C, Kearney D, Goss J, Karpen SJ. Cardiac structural and functional alterations in infants and children with biliary atresia, listed for liver transplantation. Gastroenterology. 2011;141(4):1264–72.

Desai MS, Shabier Z, Taylor M, Lam F, Thevananther S, Kosters A, Karpen SJ. Hypertrophic cardiomyopathy and dysregulation of cardiac energetics in a mouse model of biliary fibrosis. Hepatology. 2010;51(6):2097–107.

Gaskari SA, Honar H, Lee SS. Therapy insight: Cirrhotic cardiomyopathy. Nat Clin Pract Gastroenterol Hepatol. 2006;3(6):329–37.

Bile acid signaling in non-canonical targets: Implications for treatment of portal hypertension

Vijay Shah
Professor of Medicine, Physiology and Cancer Cell Biology, Vice-Chair, Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA

Farnesoid X Receptor (FXR) and TGR5 mediate important signaling functions of bile acids in diverse cell types including those residing in the hepatic vasculature. Indeed, recent work has identified these bile acid receptors as potential regulators of vascular structure and function. For example, FXR activates endothelial cells through pathways requiring MMP-9 and FAK. TGR5 has been implicated in eNOS activation in liver endothelial cells. FXR signaling is also thought to protect from activation of the hepatic stellate cell although it remains uncertain as to whether these effects are through direct actions of bile acids on hepatic stellate cells, due to indirect actions of bile acids on epithelial cell injury that leads to hepatic stellate cell activation, or some combination of both. These studies as well as others have led to the development of pharmacological agonists of FXR that are being evaluated for treatment of liver fibrosis and portal hypertension. This seminar and chapter will focus on these non-canonical signaling actions of bile acids and their receptors and also on preclinical/clinical studies that may eventually emanate from this line of investigation.

Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-betamuricholic acid, a naturally occurring FXR antagonist

Annika Wahlström[1]*, Sama Islam[2]*, Jenny Felin[2], Hanns-Ulrich Marschall[1], Krister Bamberg[3], and Fredrik Bäckhed[2]
Sahlgrenska Academy, Institute of Medicine, Departments of [1]Internal Medicine and [2]Molecular and Clinical Medicine - Sahlgrenska Center for Cardiovascular and Metabolic Research/Wallenberg Laboratory, University of Gothenburg, S-413 45 Gothenburg, Sweden; [3]AstraZeneca R&D Mölndal, S-431 83 Mölndal, Sweden

Introduction: Farnesoid-X-receptor (FXR) is a nuclear receptor that is activated by bile acids and plays a key role in the regulation of bile acid synthesis and homeostasis. Bile acids are synthesized from cholesterol in the liver and further metabolized by the gut microbiota into secondary bile acids. In this study we aimed to investigate if the effects of the gut microbiota on bile acid homeostasis are mediated through FXR.

Methods: *Fxr*-deficient mice were rederived as germ-free (GF) in order to study the impact of the gut flora on bile acid metabolism through FXR. The expression of FXR target genes in the liver and distal ileum was analyzed. Taurine-conjugated beta-muricholic acid (TBMCA) was tested for direct FXR activity in a co-activator recruitment assay.

Results: Rederivation of *Fxr*-deficient mice as GF showed that the gut microbiota regulates expression of fibroblast growth factor-15 (FGF15) in the ileum and cholesterol-7α-hydroxylase (CYP7A1) in the liver by FXR-dependent mechanisms. Since GF mice have elevated bile acid levels, in particular TBMCA, but reduced FXR activation we hypothesized that GF bile contained a FXR antagonist. In agreement with this hypothesis we identified TBMCA as a potent endogenous FXR antagonist.

Discussion/Conclusion: We propose that the gut microbiota modulates bile acid synthesis by changing the bile acid profile and by alleviating FXR inhibition in the small intestine. In summary, we demonstrate that the microbial suppression of biosynthetic genes in the liver is consistent with increased FXR-dependent activation of FGF15 in the ileum due to reduced levels of TBMCA.

Session IV

Bile acids and disease

FXR activation inhibits inflammation

Saskia W.C. van Mil
Department of Metabolic Diseases, UMC Utrecht, The Netherlands

The nuclear bile salt sensor farnesoid X receptor (FXR) is activated by endogenous bile salts and regulates transcription of genes involved in bile salt, glucose and cholesterol homeostasis by classical transactivation mechanisms. FXR is most highly expressed in liver and intestine and upon activation, binds FXR responsive elements (FXRE) in promoters of target genes as a heterodimer with Retinoid X Receptor (RXR). Recently, it became apparent that FXR activation can also strongly inhibit inflammation in different models of intestinal inflammation. We demonstrated that FXR activation in the intestine improves clinical symptoms and histology in the DSS (dextran sulfate sodium) and TNBS (2,4,6-trinitrobenzenesulfonic acid) murine models of colitis. These beneficial effects are detected in wild type mice treated with a semi-synthetic FXR ligand (6-ECDCA), but not in FXR-/- mice and untreated wild type mice. Also, FXR activation inhibits the increase of epithelial permeability and pro-inflammatory cytokine mRNA expression in the intestinal mucosa. We show that the mechanism is most probably via tethering transrepression of NF-κB, resulting in decreased NF-kB target gene expression upon docking of ligand-bound FXR to NF-κB at its respective DNA binding sites. The multi-level protection against intestinal inflammation provides a clear rationale to further explore FXR agonists as a novel therapeutic strategy for inflammatory bowel disease.

The role of autotaxin in cholestatic itch

Ronald Oude Elferink[1], Andreas Kremer[1], Remco van Dijk[1], Dagmar Tolenaars[1], Catherine Williamson[2], Ulrich Beuers[1]
[1]Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, The Netherlands, [2]Maternal and Fetal Disease Group, Institute of Reproductive and Developmental Biology, Imperial College London, London, England

Itch (pruritus) is a prominent and distressing symptom of nearly all cholestatic liver diseases. The mechanism of itch induction is not understood. However, relief of cholestasis or interruption of the enterohepatic circulation leads to a rapid resolution of itch. We hypothesized that patients with cholestasis accumulate compounds in the systemic circulation that either directly or indirectly induce itch perception. To test this hypothesis we screened sera from cholestatic itchy and non-itchy patients for its capacity to stimulate neuroblastoma cells. This screening revealed increased calcium signalling by cholestatic itchy vs. non-itchy sera. Analysis of the active compound pointed towards lysophosphatidic acid (LPA). LPA in serum is formed from lysophosphatidylcholine by the enzyme autotaxin (which is a lysophospholipase D). We could show that serum autotaxin activity in itchy cholestatic patients was significantly increased compared to non-itchy patients suggesting that increased serum autotaxin levels play a role in the generation of cholestatic itch.
We have subsequently set up an assay for scratching behaviour in mice. Intradermal injection of LPA in mice caused a dose-dependent scratch behaviour.
We analyzed sera from various patients with other forms of itch, including patients with atopic dermatitis, lymphoma and uremia and observed that in those patients there was no correlation between the occurrence of itch and elevated autotaxin. This suggests that increased systemic autotaxin is a specific aspect of cholestatic itch.
Treatment of cholestatic patients with the bile acid binding resin colesevelam reduced serum bile acids but neither reduced itch nor serum ATX levels. In contrast, treatment with MARS (Molecular Adsorbents Recirculation System) or nasobiliary drainage improved itch significantly and also significantly lowered serum ATX. Treatment with rifampicin improved itch and reduced serum ATX activity.
Highest levels of serum ATX were observed in women with intrahepatic cholestasis of pregnancy (ICP). This syndrome, observed in about 1% of women during the third trimester of pregnancy, is defined by itch combined with mild cholestasis. The itch disappears soon after delivery as does the increased ATX levels. Interestingly, however, analysis of serum ATX activity in women previously suffering from ICP was found to be significantly higher than control women, suggesting a genetic predisposition to elevated ATX levels.
These results results strongly suggest that autotaxin plays a role in cholestatic itch. We hypothesize that ATX hydrolyzes lysophosphatidylcholine into LPA near nerve endings of itch neurons, thereby potentiating signalling through these neurons. This hypothesis is in line with the observed expression of LPA receptors on neurons.

Update on the genetics of PBC

Dr. Gideon Hirschfield
Centre for Liver Research, Institute of Biomedical Research, University of Birmingham, Birmingham, B15 2TT, UK

Background/Aims: A mixture of environmental and genetic risk underpins the development of primary biliary cirrhosis (PBC), the classic autoimmune biliary disease.

Methods: Based on an understanding of the latest genetic research insights, this review summarises current knowledge on the genetic risk factors for PBC development.

Results: Prior to the application of genome-wide level genetic surveys, the only established genetic risk locus for PBC was HLA. Repeated studies have confirmed an important class II HLA association with PBC that varies depending on the ancestry of the patient. Non-HLA risk has been speculative for some time, but only recently robustly confirmed, by the application of genome-wide association studies. A number of studies now performed have highlighted a growing list of variants encompassing many shared immunoregulatory loci. Most striking of the observations has been the apparent importance of IL-12 (and closely related cytokines), with genetic risk seen across a biological pathway encompassing the cytokine, receptor subunits, as well as upstream and downstream mediators.

Conclusion: Genetic risk studies in PBC have confirmed the classic autoimmune nature of the disease and highlighted a strong role for immune regulatory pathways that may be amenable to targeting by novel medications. Complete appreciation of genetic risk in disease will however require increased cohort sizes and application of whole genome sequencing, as well as recognition that epigenetic and environmental (including microbiome) influences remain additional very important factors to be resolved if better understanding of disease pathogenesis is to lead to rational therapy.

Update on ICP genetics

Frank Lammert
Department of Medicine II, Saarland University Medical Center, Homburg, Germany

Pregnancy represents a physiological challenge for transport systems in liver and placenta, since high concentrations of hormone metabolites (e.g., estradiol glucuronides and progesterone sulfates) and nutrients have to be taken up and secreted at fluid barriers. Several subtypes of intrahepatic cholestasis of pregnancy (ICP) might be differentiated: (i) primary ICP of unknown origin; (ii) ICP due to deficiency of the ATP-binding cassette transporter B4 (often coupled with low phospholipid-associated cholelithiasis), and (iii) secondary ICP due to other liver diseases (e.g. hepatitis C virus infection, primary biliary cirrhosis, non-alcoholic steatohepatitis). Early family studies indicated an increased familial risk for ICP, since the risk of a sister to develop the disease is 12 times as high as the risk of an unrelated pregnant woman. This difference might be due in part to shared environmental factors, but an increased frequency of ICP (around 6%) is maintained in Amerindian patients after migration to the United States, indicating a genetic background (1).

The first real clues to the genetic factors underlying ICP were published in 1999 and came from mothers of children that were born with progressive familial intrahepatic cholestasis (PFIC) type 3 (2): A functionally detrimental genetic variant (c.1721delT) in the *ABCB4* gene encoding the hepatocanalicular phosphatidylcholine floppase was found to co-segregate with ICP in the index patient and her female relatives suffering from ICP. Gauging the contribution of hepatocanalicular transporters towards ICP has proven less straightforward than finding the genes affected in congenital cholestasis, as most ICP cases are likely to be caused by an unfavourable combination of predisposing gene variants rather than a single variant of high penetrance. Genetic heterogeneity was confirmed when Savander et al. (3) analysed Finnish pedigrees of ICP patients for haplotype transmission: Both segregation of haplotypes and multipoint linkage analysis excluded the *ABCB4, ABCB11* and *ATP8B1* loci in the studied pedigrees. Family studies, singular cases with high-penetrance variants and the studies of *ABCB4* haplotypes in ICP cohorts were subsequently able to confirm the contribution of this gene towards ICP (Table 1), particularly in the subgroup of women with raised γ-GT and very high bile acid levels (4).

The central and functionally unique bile salt export pump ABCB11, which causes either progressive familial intrahepatic cholestasis (PFIC) or benign recurrent intrahepatic cholestasis (BRIC) type 2 by virtue of functional variants, has been identified as a common risk factor for ICP in cohort studies (Table 1): Homozygous presence of the common variant of c.1331C>T, corresponding to the amino acid exchange p.A444V, conveys a 3-fold risk of developing ICP (5, 6). Screening the complete coding sequence of *ABCB11* and its regulator *FXR* in ICP patients with low γ-GT levels revealed functional variants of these genes only in a minority of cases. A similar paucity of functional variants in ICP patients was observed in the canalicular transporter underlying two subtypes of congenital familial cholestasis, PFIC1 and BRIC1: ATP8B1 is a P-type ATPase that flips phosphatidylserine from the outer leaflet of the canalicular membrane to the inner leaflet, thereby maintaining membrane asymmetry. While very rare cases of

functional sequence variants in this gene have been reported for ICP patients, no evidence for a contribution of the locus towards general population risk of ICP could be demonstrated.

The next step towards the identification of as yet unknown contributors will be genome-wide association studies or next-generation sequencing of severe cases to identify causative functional variants. For the time being, studying the inheritance of risk in affected families provides novel clues to modifier loci and the combined effect of known genetic risk factors for ICP (7, 8).

Table 1: Association study in European ICP cohort (N = 563) (9)

Candidate gene	$P_{corr.}$	$P_{Haplotype}$
ABCB4 (MDR2)	3.4×10^{-7}	9.6×10^{-6}
ABCB11 (BSEP)	3.7×10^{-4}	3.6×10^{-3}
ABCC2 (MRP2)	n.s.	
ATP8B1 (FIC1)	n.s.	
NR1H4 (FXR)	n.s.	
FGF19	n.s.	

References:

1. Lee RH, Goodwin TM, Greenspoon J, Incerpi M. The prevalence of intrahepatic cholestasis of pregnancy in a primarily Latina Los Angeles population. J Perinatol 2006;26:527–32.
2. Jacquemin E, Cresteil D, Manouvrier S, Boute O, Hadchouel M. Heterozygous non-sense mutation of the MDR3 gene in familial intrahepatic cholestasis of pregnancy. Lancet 1999;353:210–1.
3. Savander M, Ropponen A, Avela K, et al. Genetic evidence of heterogeneity in intrahepatic cholestasis of pregnancy. Gut 2003;52:1025–9.
4. Wasmuth HE, Glantz A, Keppeler H, Simon E, Bartz C, Rath W, Mattsson LA, Marschall HU, Lammert F. Intrahepatic cholestasis of pregnancy: the severe form is associated with common variants of the hepatobiliary phospholipid transporter ABCB4 gene. Gut 2007;56:265–70.
5. Meier Y, Zodan T, Lang C, Zimmermann R, Kullak-Ublick GA, Meier PJ, Stieger B, Pauli-Magnus C. Increased susceptibility for intrahepatic cholestasis of pregnancy and contraceptive-induced cholestasis in carriers of the 1331T>C polymorphism in the bile salt export pump. World J Gastroenterol 2008;14:38–45.
6. Dixon PH, van Mil SW, Chambers J, Strautnieks S, Thompson RJ, Lammert F, Kubitz R, Keitel V, Glantz A, Mattsson LA, Marschall HU, Molokhia M, Moore GE, Linton KJ, Williamson C. Contribution of variant alleles of ABCB11 to susceptibility to intrahepatic cholestasis of pregnancy. Gut 2009;58:537–44.

7. Keitel V, Vogt C, Häussinger D, Kubitz R. Combined mutations of canalicular transporter proteins cause severe intrahepatic cholestasis of pregnancy. Gastroenterology 2006;131:624–9.
8. Zimmer V, Müllenbach R, Simon E, Bartz C, Matern S, Lammert F. Combined functional variants of hepatobiliary transporters and FXR aggravate intrahepatic cholestasis of pregnancy. Liver Int 2009;29:1286–8.
9. Dixon PH, Wadsworth CA, Chambers J, Donnelly J, Cooley SM, Jarvis S, Kubitz R, Lammert F, Marschall HU, Glantz A, Khan SA, Whittaker J, Geary M, Williamson C. The role of common genetic variation around six candidate loci for susceptibility to intrahepatic cholestasis of pregnancy. Hepatology 2010;52:479A.

Update on PSC genetics

Tom Hemming Karlsen
Professor of Gastroenterology, Clinic for Cancer Medicine, Surgery and Transplantation, Oslo University Hospital Rikshospitalet, Oslo, Norway

Primary sclerosing cholangitis (PSC) is a chronic inflammatory disease of the bile ducts which leads to cholestatic liver cirrhosis and the need for liver transplantation over a period of 7–12 years. Affected individuals are typically young (median age of onset 30–40 years) and suffer from an increased frequency of inflammatory bowel disease (IBD; 60–80% of the patients) and autoimmune diseases (AID; 25% of the patients). Over the last 5 years a series of genome-wide association studies (GWAS) have been performed in PSC and these associated conditions. The overall outcome of these studies is that of pronounced pleiotropy, i.e. most of the susceptibility genes found in immune-mediated conditions by means of GWAS are shared between one or more disease entities. To gain insight into the biological basis of this phenomenon, we have systematically interrogated the genetic relationship between PSC and 12 other autoinlammatory conditions including IBD. The overlap is equally pronounced with prototypical autoimmune diseases like type 1 diabetes as that for IBD. Genotypes at several of the susceptibility genes interact with the gut microbial community composition even under healthy condition (e.g. fucosyltransferase 2; *FUT2*), and further investigation of the relationship between genetic susceptibility in PSC and IBD on one side and the gut microbial influence on immune function and bile acid metabolism on the other side is ongoing and available data will be presented.

Carbon-11 labelled cholylsarcosine: A PET-tracer for conjugated bile acids

Kim Frisch[1], Steen Jakobsen[1], Michael Sørensen[1,2], Ole Lajord Munk[1], Aage K.O. Alstrup[1], Peter Ott[2], Alan F. Hofmann[3], and Susanne Keiding[1,2]
[1]Department of Nuclear Medicine and PET Centre, Aarhus University Hospital, Aarhus, Denmark;
[2]Department of Medicine V (Hepatology and Gastroenterology), Aarhus University Hospital, Aarhus, Denmark;
[3]Department of Medicine, University of California, San Diego, California, USA

Introduction: The trans-hepatocellular transport of bile acids and their enterohepatic circulation are essential for bile acids to perform their physiological function in digestion, detoxification and signaling. Due to their detergent property, however, bile acids are inherently cytotoxic. Indeed, accumulation of bile acids in hepatocytes caused by impaired transcellular transport can result in cell damage and cell death. We have shown that ^{11}C-cholylsarcosine, an analogue of endogenous cholylglycine, is a promising tracer for *in vivo* studies of the trans-hepatocellular transport and entero-hepatic circulation of conjugated bile acids by PET/CT (*J Nucl Med* 2012;53:772–778).

Methods: ^{11}C-Cholylsarcosine was prepared by ^{11}C-methylation of glycine followed by conjugation with cholic acid. Its blood-to-liver uptake and liver-to-bile excretion were investigated by dynamic PET/CT in anaesthetized 40-kg pigs. Possible formation of ^{11}C-metabolites was investigated in blood and bile samples. Possible inhibition of the trans-hepatocellular transport of ^{11}C-cholylsarcosine was investigated by dynamic PET/CT preceded by a high i.v. dose of cholyltaurine.

Results: ^{11}C-Cholylsarcosine was produced with a radiochemical yield of 13% (decay-corrected) and a radiochemical purity greater than 99%. The PET/CT studies showed rapid blood-to-liver uptake and liver-to-bile excretion of ^{11}C-cholylsarcosine, with radioactivity concentrations being more than 90 times higher in bile ducts than in liver tissue. ^{11}C-Cholylsarcosine underwent enterohepatic circulation and reappeared in liver tissue and bile ducts approximately 70 min after administration. Cholyltaurine inhibited the trans-hepatocellular transport of ^{11}C-cholylsarcosine. No ^{11}C-metabolites were detected in plasma or bile samples.

Discussion/Conclusion: We have synthesized a novel radiolabelled conjugated bile acid analogue, ^{11}C-cholylsarcosine. PET/CT studies in pigs show that hepatic uptake of ^{11}C-cholylsarcosine from blood and excretion into bile is comparable to that of endogenous cholyltaurine. ^{11}C-Cholylsarcosine may thus be valuable for PET/CT studies of the trans-hepatocellular transport and enterohepatic circulation of conjugated bile acids in patients with intra- and extra-hepatic cholestatic disorders.

Session V

Therapeutic potential of bile acids I

Effects of bile acid sequestrants on FXR and TGR5 signaling

Steven Kliewer[1,2] and David Mangelsdorf[2,3]
Departments of [1]Molecular Biology and [2]Pharmacology and the [3]Howard Hughes Medical Institute, University of Texas Southwestern Medical Centre, Dallas, TX, USA 75390-9041

Bile acids serve as important signaling molecules by acting on two distinct classes of receptors. First, bile acids activate the farnesoid X receptor (FXR), a nuclear receptor that regulates diverse aspects of lipid and carbohydrate homeostasis. Among its many actions, FXR induces the expression of fibroblast growth factor (FGF) 15/19 in enterocytes in the distal small intestine. FGF15/19 is subsequently released into the blood and binds to a receptor complex composed of FGF receptor 4 and β-Klotho on the surface of hepatocytes. Activation of this receptor complex results in the transcriptional repression of CYP7A1, which encodes the rate-limiting enzyme in the classic bile acid synthetic pathway. In addition to its role in the feedback repression of bile acid synthesis, FGF15/19 stimulates hepatic glycogen synthesis and represses gluconeogenesis, much like insulin. Thus, the FXR-FGF15/19 pathway regulates carbohydrate and lipid metabolism in the postprandial state. Bile acids also activate TGR5, a plasma membrane G-protein coupled receptor. In mice, TGR5 activation improves insulin sensitivity by increasing energy expenditure in skeletal muscle and brown adipose tissue, and improves glucose homeostasis by triggering secretion of the incretin hormone, glucagon-like peptide-1 (GLP-1), from intestinal L cells. In this presentation, we will provide evidence that bile acid sequestrants mediate their beneficial metabolic effects on cholesterol and glucose homeostasis by affecting both the FXR-FGF15/19 and TGR5-GLP-1 signaling pathways. The relative importance of these two pathways and the underlying mechanisms for sequestrant action will be discussed.

Ursodeoxycholic acid in primary sclerosing cholangitis: From evidence-based treatment to expert opinion

Ulrich Beuers
Department of Gastroenterology & Hepatology, Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Twenty seven years after the first anecdotal reports on beneficial effects of ursodeoxycholic acid (UDCA) on serum liver tests in patients with primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC), UDCA is today regarded as the standard treatment of PBC[1,2], whereas its routine use is controversially discussed in PSC[1] and even abandoned by some experts[3]. The EASL Clinical Practice Guidelines stated: "The available data base shows that UDCA (15–20 mg/kg/d) improves serum liver tests and surrogate markers of prognosis, but does not reveal a proven benefit on survival. The limited data base does not yet allow a specific recommendation for the general use of UDCA in PSC."[1] In contrast, the American Practice Guidelines stated, based on the same data: "In adult patients with PSC, we recommend against the use of UDCA as medical therapy."[2] How can UDCA be judged as useful and life-saving in PBC, but by some experts as even potentially harmful in PSC?

Disease pathogenesis: In PBC, the smallest interlobular and septal bile ductules up to 100 µM diameter are affected by a florid, non-purulent, destructive cholangitis. Impaired cholangiocyte HCO_3^- secretion due to impaired expression of the Cl^-/ HCO_3^- exchanger AE2[4–6] has more recently received increasing attention as a potentially crucial pathogenetic factor in PBC. UDCA stimulates biliary HCO_3^- secretion by posttranscriptional mechanisms[7] and, thereby, stabilizes a "biliary HCO_3^- umbrella"[8] which protects cholangiocytes against bile acid toxixity *in vitro*[9] and appears to be defective in the smallest bile ductules in PBC[5,6]. Thus, stimulation of defective HCO_3^- secretion at the level of the smallest intrahepatic bile ductules may be a key mechanism of action of UDCA in early stage PBC. In contrast to PBC, PSC affects not only small intrahepatic bile ductules, but mainly larger intrahepatic ducts and the extrahepatic biliary tree. Protective endogenous mechanisms against bile toxicity may differ between smallest bile ductules and larger intra- and extrahepatic bile ducts which are covered by mucins. The amount of biliary HCO_3^- secretion stimulated by UDCA at standard doses may be inadequate to protect the whole biliary tree against bile toxicity. Thus, more potent stimuli of biliary HCO_3^- secretion may be needed to effectively treat PSC and prevent periductular/periductal fibrosis at least at more extended stages[10,11].

The UDCA dose: At daily doses of 10–20 mg/kg, UDCA significantly improved[12–14] or tended to improve[15] serum liver tests in comparison to placebo in randomized trials pilot studies and trials and was well tolerated, but did not show profibrogenic effects similar to what was reported in early studies in PBC. The cohort sizes and/or follow-up periods of the available placebo-controlled studies in PSC performed at doses of 10–20 mg/kg daily, however, precluded clearcut judgement on effects of UDCA on long-term prognosis in PSC (as discussed for PBC a decade ago). High dose UDCA (30 mg/kg daily) was as effective as standard dose (20 mg/kg daily) in improving serum liver tests and was well tolerated during two years of treatment in mainly early stage PSC patients[16]. However, high dose UDCA was potentially harmful in a mixed cohort (> 40% cirrhosis, fibrosis) of patients treated for up to 5 years in that endpoints such as

development of varices, or listing for liver transplantation tended to be higher with high dose UDCA independent of disease stage at begin of treatment[17]. The obstruction of bile ductules/ducts due to fibrotic strictures in PSC may have contributed to high dose UDCA-induced disease progression and progression of portal hypertension. Similar harmful and potentially profibrotic effects of UDCA have not been observed at lower doses of 10–20 mg/kg in the past even after adequate follow-up[15].

Surrogate markers of prognosis: The long-term course and the low incidence of PBC and PSC make therapeutic studies with a primary endpoint 'survival free of liver transplantation' most difficult. In PBC, analyses of the predictive value of surrogate markers for treatment response and prognosis have unravelled in several independent patient cohorts that serum alkaline phosphatase, AST and bilirubin represent strong prognostic markers[18–22]. Thanks to these efforts, use of these surrogate markers as study endpoints in the evaluation of novel therapeutic approaches in PBC is meanwhile widely accepted by European and American authorities. In PSC, comparable analyses on the predictive value of surrogate markers for treatment response and prognosis are lacking and are urgently needed in order to identify those patients who may respond to UDCA and other novel medical interventions and those who may not.

Conclusion: UDCA (15–20 mg/kg/d) improves serum liver tests and surrogate markers of prognosis, but does not reveal a proven benefit on survival. This may be due to lack of studies of adequate size and follow-up and/or to limited efficacy. In adult patients with PSC, we recommend against the use of ***high dose*** UDCA (30 mg/kg/d) as medical therapy. Evaluation of surrogate markers of prognosis in PSC is urgently needed to effectively determine treatment response to UDCA and novel therapeutic approaches in PSC.

References:

1. EASL Clinical Practice Guidelines: Management of cholestatic liver diseases. J Hepatol 2009;51:237–67.
2. Lindor KD, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ. Primary biliary cirrhosis. Hepatology 2009;50:291–308.
3. Chapman R, Fevery J, Kalloo A, et al. Diagnosis and management of primary sclerosing cholangitis. Hepatology;51:660–78.
4. Prieto J, Qian C, Garcia N, Diez J, Medina JF. Abnormal expression of anion exchanger genes in primary biliary cirrhosis. Gastroenterology 1993;105:572–8.
5. Prieto J, Garcia N, Marti-Climent JM, Penuelas I, Richter JA, Medina JF. Assessment of biliary bicarbonate secretion in humans by positron emission tomography. Gastroenterology 1999;117:167–72.
6. Medina JF, Martinez A, Vazquez JJ, Prieto J. Decreased anion exchanger 2 immunoreactivity in the liver of patients with primary biliary cirrhosis. Hepatology 1997;25:12–7.
7. Beuers U. Drug insight: Mechanisms and sites of action of ursodeoxycholic acid in cholestasis. Nat CP Gastroent Hepatol 2006;3:318–28.
8. Beuers U, Hohenester S, de Buy Wenniger LJ, Kremer AE, Jansen PL, Elferink RP. The biliary HCO(3)(-) umbrella: a unifying hypothesis on pathogenetic and therapeutic aspects of fibrosing cholangiopathies. Hepatology;52:1489–96.

9. Hohenester S, Wenniger LM, Paulusma CC, et al. A biliary HCO3- umbrella constitutes a protective mechanism against bile acid-induced injury in human cholangiocytes. Hepatology 2012;55:173–83.
10. Hofmann AF, Zakko SF, Lira M, Clerici C, Hagey LR, Lambert KK, Steinbach JH, et al. Novel biotransformation and physiological properties of norursodeoxycholic acid in humans. Hepatology 2005;42:1391–1398.
11. Fickert P, Wagner M, Marschall HU, et al. 24-norUrsodeoxycholic acid is superior to ursodeoxycholic acid in the treatment of sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. Gastroenterology 2006;130:465–81.
12. Beuers U, Spengler U, Kruis W, et al. Ursodeoxycholic acid for treatment of primary sclerosing cholangitis: a placebo-controlled trial. Hepatology 1992;16: 707–14.
13. Lindor KD. Ursodiol for primary sclerosing cholangitis. Mayo Primary Sclerosing Cholangitis-Ursodeoxycholic Acid Study Group. N Engl J Med 1997;336:691–5.
14. Mitchell SA, Bansi DS, Hunt N, Von Bergmann K, Fleming KA, Chapman RW. A preliminary trial of high-dose ursodeoxycholic acid in primary sclerosing cholangitis. Gastroenterology 2001;121:900–7.
15. Olsson R, Boberg KM, de Muckadell OS, et al. High-dose ursodeoxycholic acid in primary sclerosing cholangitis: a 5-year multicenter, randomized, controlled study. Gastroenterology 2005;129:1464–72.
16. Cullen SN, Rust C, Fleming K, Edwards C, Beuers U, Chapman R. High dose ursodeoxycholic acid for the treatment of primary sclerosig cholangitis is safe and effective. J Hepatol 2008;48:792–800.
17. Lindor KD, Kowdley KV, Luketic VA, et al. High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. Hepatology 2009;50:808–14.
18. Pares A, Caballeria L, Rodes J. Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic Acid. Gastroenterology 2006;130:715–20.
19. Corpechot C, Abenavoli L, Rabahi N, et al. Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. Hepatology 2008;48:871–7.
20. Kuiper EM, Hansen BE, de Vries RA, et al. Improved prognosis of patients with primary biliary cirrhosis that have a biochemical response to ursodeoxycholic acid. Gastroenterology 2009;136:1281–7.
21. Kumagi T, Guindi M, Fischer SE, et al. Baseline ductopenia and treatment response predict long-term histological progression in primary biliary cirrhosis. Am J Gastroenterol;105:2186–94.
22. Corpechot C, Chazouilleres O, Poupon R. Early primary biliary cirrhosis: biochemical response to treatment and prediction of long-term outcome. J Hepatol 2011;55:1361–68.

Farnesoid X receptor exerts antisecretory actions on colonic epithelium – A new target for development of antidiarrhoeal drugs?

Magdalena Mroz[1], Niamh Keating[1], Joseph Ward[1], Silvie Amu[2], Padraic Fallon[2] and Stephen Keely[1]
[1]RCSI, Dublin, Ireland
[2]Trinity College, Dublin, Ireland

Introduction: Cl^- secretion, the main driving force for intestinal fluid secretion, can become dysregulated in conditions of disease, leading to the onset of diarrhoea. While such diseases, constitute a huge health and economic burden, available medications are unsatisfactory and more specific therapies are required. Here we investigated a role for the bile acid receptor, farnesoid X receptor (FXR), in regulation of intestinal secretory responses and as a target for development of antidiarrhoeal drugs.

Methods: GW4064 (5×10^{-3} M; 24 hours) was used to activate FXR. Cl^- secretion was measured as changes in short-circuit current across T_{84} cell monolayers or muscle-stripped mouse colonic tissues. Protein expression was measured by immunoblotting.

Results: GW4064 treatment significantly inhibited Cl^- secretory responses to the Ca^{2+}- and cAMP-dependent agonists, carbachol and forskolin, and to the naturally-occurring secretagogues, cholera toxin and deoxycholic acid. Intraperitoneal injection of GW4064 to mice (50mg/kg) attenuated Cl^- secretion in colonic tissues. However, jejunal sodium-dependent glucose co-transport and colonic ENaC currents were not decreased, suggesting the effects of FXR activation are specific for secretory processes. Furthermore, GW4064 treatment inhibited the severity of symptoms in a mouse model of diarrhoeal disease. We found that GW4064 decreased CFTR-mediated Cl^- currents in T_{84} cells and this was associated with a decrease in CFTR protein expression. GW4064 also inhibited Na^+/K^+-ATPase activity without altering its protein expression.

Discussion/Conclusion: These data reveal novel antidiarrhoeal actions of FXR in colonic epithelium. These effects are mediated by inhibition of multiple components of the Cl^- secretory pathway, without altering absorptive processes. Our data suggest that FXR agonists may be useful in treating secretory diarrhoeas associated with common intestinal disorders.

Session VI

Therapeutic potential of bile acids II

Therapeutic potential of *nor*UDCA and novel FXR/TGR5 agonists in PSC

Michael Trauner, M.D.
Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria

A major challenge in the current management of PSC is the lack of an effective and established medical treatment. Among the most promising future therapeutic options are 24-norursodeoxycholic acid (*nor*UDCA) and bile acid receptor/farnesoid X receptor (FXR) agonists (already sucessfully tested in PBC). Notably, both therapeutic approaches induce secretion of bicarbonate which counteracts biliary toxicity and improve sclerosing cholangitis in the *Mdr2(Abcb4)*$^{-/-}$ cholangiopathy model.

*nor*UDCA is a side chain-shortened C23 homologue of UDCA which possesses one less methylene group in its side chain and is more resistant to conjugation with taurine or glycine than UDCA, but instead is secreted into bile mostly in unchanged form. The secreted *nor*UDCA undergoes absorption by cholangiocytes, returns to the liver and is resecreted into bile. Such cholehepatic shunting leads to a bicarbonate-rich hypercholeresis and may also result in improved targeting to the liver. *nor*UDCA (but not "conventional" UDCA) reversed sclerosing cholangitis in the *Mdr2 (Abcb4)*$^{-/-}$ cholangiopathy model within 4 weeks of treatment. Its possible therapeutic mechanisms include (i) amelioration of bile hydrophobicity by biliary enrichement with hydrophilic *nor*UDCA and its metabolites, (ii) flushing of injured bile ducts by stimulation of bile flow and bicarbonate-rich choleresis, which dilutes toxic biliary content and reinforces the bicarbonate umbrella protecting against potentially toxic bile acids, (iii) induction of alternative bile acid detoxification (phase I and II enzymes) and elimination routes for bile acids, and (iv) direct anti-inflammatory and anti-fibrotic properties. Notably, tauro-*nor*UDCA which lacks cholehepatic hepatic shunting with stimulation of bicarbonate secretion also looses the therapeutics effects. A recent comprehensive gene expression and metabolomic profiling revealed - in addition to modulation of anti-inflammatory and anti-fibrotic genes - profound alterations in fatty acid and triglyceride metabolism, including a restoration of elevated short-chain and medium-chain fatty acids and reduced long-chain fatty acids in the *Mdr2*$^{-/-}$ cholangiopathy model by *nor*UDCA. This resulted in a less lipotoxic lipid profile with a higher saturation index and restoration of reduced triglyceride levels.

*nor*UDCA also targets the inflammatory cross talk between cells involved in inflammation and fibrogenesis in sclerosing cholangitis. This includes the interaction of a reactive cholangiocytes with components of the innate immune system such as neutrophils, Kupffer cells and natural killer cells, as well as portal myofibroblasts. As such, reactive cholangiocytes are not only victims, but also culprits in the pathogenesis of sclerosing cholangitis by secreting a range of proinflammatory and profibrogenic mediators. This includes the expression of VCAM, which has been linked to recruitment of lymphocytes to the inflammed bile duct epithelium and may also assist evasion of lymphocyte apoptosis, events which all may contribute to sustained bile duct inflammation. *nor*UDCA represents a multi-targeted therapeutic approach in sclerosing cholangitis, targeting hepatocytes, cholangiocytes and Kupffer cells, together with a profound

modulation of bile composition. Such a multi-targeted therapeutic approach may be essential for the treatment of a complex multifactorial disease such as PSC, as well as other cholangiopathies such as PBC. Currently *nor*UDCA is undergoing clinical development with phase 1 trials having been completed in 2011 and a phase 2 multicenter trials involving more than 30 European Centers about to be commence in the second half of 2012.

Another interesting therapeutic target for PSC are the bile acid receptors TGR5 and FXR. TGR5 is a G-protein coupled bile acid receptor at a plasma membrane, while FXR is a nuclear hormone receptor, and both receptors are involved in the regulation of metabolism and inflammation through bile acids. Notably, some TGR5 polymorphisms have recently been associated with pathogenesis of PSC and ulcerative colitis. A range of TGR5 and FXR are selective antagonists as well as dual TGR5/FXR ligands are now available and some of them have already been tested in sclerosing cholangitis models. It is importnat to emphasize, that neither UDCA nor *nor*UDCA are FXR or TGR5 ligands. We have recently tested some FXR and/or TGR5 ligands in the *Mdr2 (Abcb4)*$^{-/-}$ cholangiopathy model. A dual ligand with high affinity to FXR (INT-767, but not the clincial lead compound INT-747) was able to cure bile duct injury in these mice. Subsequent studies in FXR knock-out mice revealed that these effects were mediated exclusively by FXR and not by TGR5. The therapeutic mechanisms involved suppression of bile acid synthesis and direct anti-inflammatory and antifibrotic effects and silencing of the reactive cholangiocyte phenotype. Notably, this therapeutic effect was also linked to generation of a bicarbonate-rich choleresis which appears to be a common denominator for sucessful treatment of PSC and perhaps even (other) cholangiopathies in general. FXR activation by pharmacological ligands also ameliorates inflammation in a mouse model of DSS-induced colitis and impaired FXR signaling has recently been shown in IBD. Moreover, FXR is linked to the control of the gut flora and maintenance of gut integrity. Notably, intestinal overexpression of FXR was also able to cure biliary injury in *Mdr2 (Abcb4)*$^{-/-}$ mice, effects, which may also be linked to FGF-mediated supression of bile acid synthesis. This may call for design of intestine-specific FXR ligands which would (i) target only the gut without systemic side effects and (ii) should be able to cure both liver and associated/underlying IBD. Finally, It should be kept in mind that other gut arrived factors such as GLP1 (regulated by TGR5) could also have beneficial anti-apoptotic effects on cholangiocytes apart from their established metabolic effect.

References:

1: Fickert P, Wagner M, Marschall HU, Fuchsbichler A, Zollner G, Tsybrovskyy O, Zatloukal K, Liu J, Waalkes MP, Cover C, Denk H, Hofmann AF, Jaeschke H, Trauner M. 24-norUrsodeoxycholic acid is superior to ursodeoxycholic acid in the treatment of sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. Gastroenterology. 2006 Feb; 130(2):465–81.

2: Halilbasic E, Fiorotto R, Fickert P, Marschall HU, Moustafa T, Spirli C, Fuchsbichler A, Gumhold J, Silbert D, Zatloukal K, Langner C, Maitra U, Denk H, Hofmann AF, Strazzabosco M, Trauner M. Side chain structure determines unique physiologic and therapeutic properties of norursodeoxycholic acid in Mdr2-/- mice. Hepatology. 2009 Jun; 49(6):1972–81.

3: Moustafa T, Fickert P, Magnes C, Guelly C, Thueringer A, Frank S, Kratky D, Sattler W, Reicher H, Sinner F, Gumhold J, Silbert D, Fauler G, Höfler G, Lass A, Zechner R, Trauner M. Alterations in lipid metabolism mediate inflammation, fibrosis, and proliferation in a mouse model of chronic cholestatic liver injury. Gastroenterology. 2012 Jan; 142(1):140–51.

4: Baghdasaryan A, Claudel T, Gumhold J, Silbert D, Adorini L, Roda A, Vecchiotti S, Gonzalez FJ, Schoonjans K, Strazzabosco M, Fickert P, Trauner M. Dual farnesoid X receptor/TGR5 agonist INT-767 reduces liver injury in the Mdr2-/- (Abcb4-/-) mouse cholangiopathy model by promoting biliary HCO^{3-} output. Hepatology. 2011 Oct; 54(4):1303–12.

5. Modica S, Petruzzelli M, Bellafante E, Murzilli S, Salvatore L, Celli N, Di Tullio G, Palasciano G, Moustafa T, Halilbasic E, Trauner M, Moschetta A. Selective activation of nuclear bile acid receptor FXR in the intestine protects mice against cholestasis. Gastroenterology. 2012 Feb; 142(2):355–65.

6: Marzioni M, Alpini G, Saccomanno S, Candelaresi C, Venter J, Rychlicki C, Fava G, Francis H, Trozzi L, Benedetti A. Exendin-4, a glucagon-like peptide 1 receptor agonist, protects cholangiocytes from apoptosis. Gut. 2009 Jul; 58(7):990–7.

6α-ECDCA for NASH

Arun J. Sanyal M.D.
Charles Caravati Professor of Medicine, Chairman, Division of Gastroenterology, Hepatology and Nutrition, Virginia Commonwealth University School of Medicine, Richmond, VA, USA

6-Ethyl chenodeoxycholic acid (obeticholic acid, INT-747) is a is a semi-synthetic derivative of the primary human bile acid (BA) chenodeoxycholic acid (CDCA), the natural agonist of the farnesoid X receptor (FXR), a nuclear hormone receptor regulating glucose and lipid metabolism. In animal models, obeticholic acid decreases insulin resistance and hepatic steatosis. There are currently only limited data in humans. A phase 2a “proof of concept” clinical trial of obeticholic acid in type 2 diabetes mellitus with suspected or biopsy-proven NASH has been performed. This was sponsored by Intercept and was a prospective controlled trial involving a 6 week course of placebo versus one of two doses of obeticholic acid. The primary endpoint was improvement in insulin sensitivity as measured by a two-step euglycemic hyperinsulinemic clamp. The preliminary data have been presented at the annual meeting of the American Association for Study of Liver Diseases. Patients (n = 64) were randomized to receive placebo (n = 23), OCA 25 mg (n = 20) or 50 mg (n = 21) once daily for 6 weeks. Obeticholic acid treatment was associated with a significant increase in glucose infusion requirement at both doses studied while there were no significant changes with placebo. Obeticholic acid (25 mg) also produced a decrease in serum ALT and GGT and also caused weight loss compared to baseline. This was accompanied by increased FGF19. Based on these data, a phase 2b randomized prospective placebo-controlled clinical trial of obeticholic acid (25 mg/day) has been initiated by the NIDDK NASH Clinical Research Network. This trial will utilize a Vanguard plan of analysis and a futility analysis based on changes in liver enzymes will be performed in June 2012. Obeticholic acid causes a small decrease in high density lipoproteins (HDL). This may be due to increased clearance of HDL via increased scavenger receptor activity or decreased reverse cholesterol transport. Elucidation of these possibilities will be important in the final assessment of the value of obeticholic acid for the treatment of NASH.

Successful treatment of bile acid synthetic defects with oral cholic acid – Results from a 20+ year experience

Kenneth D.R. Setchell[1] and James E. Heubi[2]
Departments of Pathology and Laboratory Medicine[1] and Gastroenterology and Nutrition[2], Children's Hospital Medical Center, Cincinnati, Ohio 45229, USA

Introduction: Bile acid synthetic defects are a specific category of progressive familial intrahepatic cholestatic (PFIC) disorders associated with both early and late-onset chronic cholestasis. Thus far, 9 genetic defects in the complex 17 enzyme-catalyzed pathway of primary bile acid synthesis from cholesterol have been defined and mutations identified in the genes encoding these enzymes. These autosomal recessive disorders manifest as a broad phenotype, ranging from mild to severe liver disease, fat-soluble vitamin malabsorption, growth failure, and/or neurological disease. Biochemically, all share the common feature of an absence of hepatic synthesis of the primary bile acids, cholic and chenodeoxycholic acids, that are essential for the promotion of bile flow, concomitant with elevated levels of atypical bile acids that are cholestatic/hepatotoxic. The natural clinical history of these disorders is one of progression to cirrhosis and liver failure.

Methods: Patients were screened for a bile acid synthetic defect by mass spectrometric analysis of the urine and/or serum and confirmation was established by sequencing of the gene encoding the specific enzyme deficiency. Patients were administered cholic acid orally at a dose of 10–15 mg/kg bw/d and followed prospectively for biochemical and clinical response to therapy.

Results: Cholic acid therapy has been shown to be effective in most patients with bile acid synthetic defects and a number of patients have been treated for > 20 years, Long-term survival and an impressive clinical improvement of patients with bile acid synthetic defects is associated with down-regulation of bile acid synthesis and the provision of adequate levels of primary bile acids to generate bile flow. Both of these goals have been achieved with oral administration of the cholic acid, which led to a sustained reduction or disappearance of atypical bile acid metabolites concomitant with a consistent normalization in serum liver enzymes, and improvement in growth and liver histology. In > 20 years, no significant adverse effects have been associated with the use of cholic acid.

Discussion/Conclusion: Based on the successful application of this therapeutic strategy, cholic acid was recently granted orphan Status by the FDA and is in the final stages of marketing authorization in both the EU and US FDA.

List of Chairpersons, Speakers and Scientific Organizers

M. Ananthanarayanan, M.D., Ph.D.
Mount Sinai School of Medicine
Department of Pediatrics
Room 14-34 Annenberg Box 1664
One Gustave L. Levy Place
New York, NY 10029-6574
USA

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

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

John Y.L. Chiang, Ph.D.
Professor of Biochemistry
Biochemistry/Molecular Pathology
4209 SR 44
Rootstown, OH 44272
USA

Paul 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

Kim Frisch
Department of Nuclear Medicine and
PET Center
Aarhus University Hospital
Noerrebrogade 44, Building 10
8000 Aarhus
Denmark

Prof. Dr. Holger Gohlke
Heinrich-Heine-Universität
Universitätsstr. 1
40225 Düsseldorf
Germany

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

Dr. Gideon Hirschfield
University of Birmingham
Center of Liver Research
Institute of Biomedical Research
P.O. Box 363
Birmingham B15 2TT
Great Britain

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

Phillip B. Hylemon, Ph.D.
Professor of Microbiology & Medicine
Medical College of Virginia
Box 980678
Richmond, VA 23298
USA

Sama Islam
University of Gothenburg
Institute of Medicine
Sahlgrenska Academy
Gothenburg
Sweden

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

Dr. Milan Jirsa
Institute for Clinical and
Experimental Medicine
Laboratory of Experimental
Hepatology, Building Z1
Videnska 1958/9
140 21 Praha 4
Czech Republic

Dr. Tom Hemming Karlsen
University of Oslo
Rikshospitalet
Norwegian PSC Research Center
Medical Department
0027 Oslo
Norway

Saul J. Karpen, M.D., Ph.D.
Associate Professor of Pediatrics
Emory Children's Center
Pediatric Gastroenterology,
Hepatology and Nutrition
2015 Uppergate Drive, NE
Atlanta, GA 30022
USA

Dr. Stephen J. Keely
Beaumont Hospital
RCSI Education & Research Center
Smurfit Building
Beaumont Road
Dublin
Ireland

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

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

Steven Kliewer, M.D.
Professor of Molecular Biology
University of Texas
Southwestern Medical Center
Department of Moluecular Biology
5323 Harry Hines Blvd.
Dallas, TX 75390-9046
USA

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

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

David D. Moore, Ph.D.
Baylor College of Medicine
Texas Medical Center
One Baylor Plaza
Houston, TX 77030
USA

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

POSTER ABSTRACTS

Poster Numbers 1 – 104

Author Index to Poster Abstracts

Intrahepatic cholestasis of pregnancy levels of sulfated progesterone metabolites inhibit FXR resulting in a pro-cholestatic phenotype

Shadi Abu-Hayyeh[1], Georgia Papacleovoulou[1], Anita Lövgren-Sandblom[2], Sabiha Ravat[1], Vanya Nikolova[1], Jenny Chambers[1], Hanns-Ulrich Marschall[3], Malcolm G. Parker[1] and Catherine Williamson[1]
[1]Maternal and Fetal Disease Group, IRDB, Imperial College London, London, UK; [2]Department of Clinical Chemistry, Karolinska University Hospital Huddinge, Stockholm, Sweden; [3]Institute of Medicine, Department of Internal Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Introduction: Intrahepatic cholestasis of pregnancy (ICP) is the most prevalent pregnancy-specific liver disease and is associated with an increased risk of adverse fetal outcomes, including preterm labor and intrauterine death. The endocrine signals that cause cholestasis are not known but 3α-sulfated progesterone metabolites have been shown to be elevated in ICP leading us to study the impact of sulfated progesterone metabolites on farnesoid X receptor (FXR)-mediated bile acid homeostasis pathways.

Methods: Levels of the 3β-sulphated progesterone metabolite, epiallopregnanolone sulfate, were assayed in serum samples taken from fifteen 3rd trimester ICP pregnancies and fourteen matched controls. The impact of ICP-related concentrations of epiallopregnanolone sulfate on FXR function and co-factor recruitment was investigated in hepatoma cell lines and primary human hepatocytes.

Results: The 3β-sulphated progesterone metabolite, epiallopregnanolone sulfate is supraphysiologically raised in the serum of ICP patients ($P < 0.05$). We demonstrate that levels of epiallopregnanolone sulfate found in ICP can function as a partial agonist for the farnesoid X receptor (FXR), resulting in the aberrant expression of bile acid homeostasis genes. Furthermore, epiallopregnanolone sulfate inhibition of FXR results in reduced FXR-mediated bile acid efflux and secreted FGF19. Using co-factor recruitment assays, we show that epiallopregnanolone sulfate competitively inhibits bile acid-mediated recruitment of co-factor motifs to the FXR ligand binding domain.

Discussion/Conclusion: Our results reveal a novel molecular interaction between ICP-associated levels of the 3β-sulphated progesterone metabolite epiallopregnanolone sulfate and FXR that couples the endocrine component of pregnancy in ICP to abnormal bile acid homeostasis.

2

Bile acid modulation of brain microRNAs in the double transgenic APP/PS1 mouse model of Alzheimer's disease

J.D. Amaral[1,2], P.M. Borralho[1], A.F. Nunes[1], Y. Zeng[3], A.L. Sarver[3], C.J. Steer[3], C.M.P. Rodrigues[1,2]
[1]Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL) and [2]Department of Biochemistry and Human Biology, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal; [2]University of Minnesota Medical School, Minneapolis, Minnesota, USA

Introduction: MicroRNAs (miRNAs) are small, non-coding regulatory RNAs that post-transcriptionally regulate gene expression. These conserved molecules are highly expressed in the brain, being essential for neuronal function and survival. miRNA profiles are significantly altered in Alzheimer's disease (AD) patient brains. We have previously shown that tauroursodeoxycholic acid (TUDCA) is a strong neuroprotective agent in several AD experimental models. In addition, bile acids modulate both mRNA and miRNA expression in the rat liver. Nevertheless, the therapeutic role of TUDCA in AD pathology has not yet been ascertained. The present study was designed to identify miRNAs differentially expressed in wild-type and APP/PS1 transgenic mice, as well as elucidate the role of TUDCA in modulating miRNAs potentially associated with AD.

Methods: We carried out highly sensitive expression profiling of 1140 miRNAs on samples obtained from the hippocampus and frontal cortex of male APP/PS1 transgenic mice and wild-type littermates fed diets containing 0.4% TUDCA, or no bile acid, for 6 months. Validation was performed by qRT-PCR.

Results: APP/PS1 mice brains showed significant and large fold-change differential expression of 46 miRNAs including miR-295, -302c, -599, and -615, relative to wild-type mice. In addition, significant differences were also detected in 28 miRNAs in the direct comparison of untreated and TUDCA-treated APP/PS1 mice. Finally, in silico analysis identified putative target genes for specific miRNAs deregulated in APP/PS1 mice and modulated by TUDCA. Pathway enrichment within these putative targets revealed that miRNAs found altered in APP/PS1 mice are potentially involved in axon guidance mechanisms, long-term potentiation and/or depression, glutamate metabolism, and apoptosis. Interestingly, amyloid precursor protein (APP) 3'UTR is potentially targeted by miR-295, which expression was significantly decreased in the cortex of APP/PS1 mice. In contrast, miR-599 was upregulated in the brain of APP/PS1 mice and potentially targets the low-density lipoprotein (LDL) receptor-related protein 6 (LRP6) 3'UTR involved in Abeta clearance. Notably, miR-599 expression was decreased by TUDCA.

Discussion/Conclusion: miRNA profiling of APP/PS1 mice provides an appealing approach to address how changes in miRNA profiles translate into biological functions in AD pathological context. Modulation of AD-associated miRNAs by TUDCA may represent a potential therapeutic strategy for prevention and treatment of the disease.

(Supported by PTDC/SAU-NMC/117877/2010 and Pest-OE/SAU/UI4013/2011, and fellowships SFRH/BPD/47376/2008 (J.D.A), SFRH/BPD/65212/2009 (P.M.B) and SFRH/BPD/34603/2007 (A.F.N) from FCT, Portugal)

The expression of bile salt export pump (BSEP, ABCB11) and FIC1 ATPase (FIC1, ATP8B1) are regulated by microRNA-33 (mIR-33) which is downregulated in experimental cholestasis

Meena Ananthanarayanan[2], Ryan M. Allen[1], Tyler J. Marquart[1], Carolyn J. Albert[1], Frederick J. Suchy[3], David A. Ford[1] and Angel Baldan[1]
[1]Department of Molecular Biology and Biochemistry, St. Louis University School of Medicine, St. Louis, MO, [2]Section of Digestive Diseases, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT and Department of Pediatrics, University of Colorado Denver School of Medicine, Aurora, CO, USA

Introduction: MicroRNAs are small 20-25 nt noncoding RNAs shown to regulate a number of biological processes by binding to specific recognition motifs in the 3'-UTR of cognate mRNAs. We showed that the evolutionarily conserved mIR-33, downregulates the sterol transporter ABCA1 (PNAS 2010;107:12228). Whether mIR-33 binds to and regulate the expression of bile acid transporter mRNAs and their effects on bile secretion are unknown.

Methods: Functional analysis of mIRNA binding sites were evaluated by cloning the 3'-UTR of ABCB11 and ATP8B1 mRNAs downstream of luciferase reporter genes.

Results: Human and rodent *BSEP/Bsep* mRNAs contained mIR-33 recognition sequences overlapping the stop codon and 732- 751 nt after the stop codon respectively. Evolutionarily conserved mIR-33 binding sites were also found in the 3'-UTR of *ATP8B1/atp8b1* mRNAs. Cotransfection 3'-UTR of constructs into HEK293 cells in the presence or absence of a plasmid that overexpresses miR-33 confirmed that the 3'UTRs of these genes responded to miR-33. miR-33 overexpression resulted in ~40% decrease in luciferase activity when fused to the 3'UTR or the putative responsive elements of *ATP8B1/atp8b1* or *ABCB11/abcb11.* Mutations preventing the binding of the seed sequence of the miRNA abolished the response to miR-33. To verify whether mIR-33 regulates ABCB11 and ATP8B1 in human liver, Huh7 cell line was transfected with mIR-33 plasmid. qPCR analysis of ABCB11 and ATP8B1 message levels revealed that mIR-33 overexpression resulted in 24.8 and 25.7% decrease in levels respectively compared to a GFP control. To see whether mIR-33 expression levels are altered during cholestasis, mIR-33 was quantified post–CBDL. Expression of mIR-33 decreased to 35.1, 72.8 and 61% of sham-operated controls at 1, 3 and 7 days after CBDL suggesting adaptive regulation to allow maximal expression of BSEP and FIC1.

Discussion/Conclusion: Together, these results suggest that mIR-33 may play a major role in bile secretion by controlling the expression of BSEP/FIC1.

4

Cholesterol metabolism in gallstone disease: Analysis of circulating markers of sterol homeostasis

M. Bertolotti, P. Loria, E. Pellegrini, M. Del Puppo*, C. Galbusera*, S. Ognibene, E. Baldelli, C. Anzivino, L. Carulli, N. Carulli
Dipartimento di Medicina, Endocrinologia, Metabolismo e Geriatria, Università di Modena e Reggio Emilia, Modena, Italy
*Dipartimento di Medicina Sperimentale, Università degli Studi di Milano Bicocca, Monza, Italy

Introduction: Cholesterol gallstone disease has a high prevalence in developed countries, with a relevant impact in terms of complications and hospitalization. The pathophysiology is still incompletely unknown; in particular, it is still debated whether this condition associates with specific alterations of the different pathways regulating cholesterol homeostasis.
Aim of this study was to analyze the main pathways of cholesterol balance (synthesis, absorption, degradation) by analysis of circulating oxysterol levels, in a large population of gallstone patients.

Methods: Serum samples from 123 adult subjects (61 with and 62 without cholesterol gallstones) from the M.I.COL. (Multicentrica Italiana Colelitiasi) epidemiological study were analyzed. Plasma concentrations of hydroxylated sterols, considered as markers of cholesterol synthesis (lathosterol), absorption (campesterol, sitosterol) and degradation to bile acids (7alpha-hydroxy-4-cholesten-3-one, C4) were assayed by gas-chromatography mass spectrometry (GC-MS). Differences between groups were investigated by Mann-Whitney's U test.

Results: Circulating markers of cholesterol absorption (expressed as the ratio between plasma sitosterol, or campesterol, and total cholesterol) were not different in the two groups, and neither was the ratio plasma lathosterol to cholesterol (an index of cholesterol synthesis). Plasma levels of C4, a marker of the main metabolic pathway of bile acid synthesis, were significantly higher in gallstone patients (median, 0.82 microg/dl; 25–75% confidence limits: 0.48–1.42) compared to gallstone-free subjects (median, 0.44 microg/dl; 25–75% confidence limits: 0.31–0.95).

Discussion/Conclusion: These preliminary results show no evidence of "specific" defects in cholesterol synthesis and/or absorption underlying gallstone formation. The data on plasma C4 are in contrast with previous evidence linking reduced bile acid formation with increased biliary cholesterol saturation and might reflect some degree of bile acid malabsorption occurring in gallstone disease. The implications of this finding on gallstone management in individual patients are questionable; however, the possibility to identify a subpopulation at high risk for gallstone development must be considered.

Reduction of bile acid synthesis *in vivo* in patients receiving total parenteral nutrition

M. Bertolotti, L. Carulli, C. Anzivino, E. Baldelli, M. Del Puppo*, M.R. Odoardi, P. Loria, N. Carulli
Dipartimento di Medicina Endocrinologia Metabolismo e Geriatria, Università degli Studi di Modena e Reggio Emilia, Modena, Italy
*Dipartimento di Medicina Sperimentale, Università degli Studi di Milano Bicocca, Monza, Italy

Introduction: Bile acid synthesis, a key event in cholesterol homeostasis, is regulated via feedback inhibition exerted by hydrophobic bile acids recirculating to the liver; this control is believed to occur mainly on gene transcription of the limiting enzyme, cholesterol 7alpha-hydroxylase. The role of the alimentary tract and of gastrointestinal hormones is still to be defined.
Aim of the present study was to investigate the effect of artificial nutrition on bile acid synthesis.

Methods: 11 patients receiving artificial nutrition, either total parenteral nutrition (TPN, n = 6) or enteral nutrition (EN, n = 5) with no previous history of liver disease, underwent analysis of *in vivo* 7alpha-hydroxylation rates by isotope release analysis after i.v. injection of [7alpha-3H]cholesterol. The results were compared with those obtained in a population of 16 age-matched control subjects.

Results: Hydroxylation rates were lower in patients with artificial nutrition (TPN: 94 ± 13 mg/day; EN: 230 ± 39 mg/day, mean ± SEM) when compared to controls (385 ± 47 mg/day) ($P < 0.01$, one-way ANOVA). The finding was confirmed when using ANCOVA with age as the covariate. In a patient receiving EN hydroxylation rates increased 3.5-fold after treatment with the cholecystokinin analogue ceruletide (20 microg b.i.d. for 2 wk i.m.). Serum lathosterol/cholesterol ratio, a marker of cholesterol synthesis, was also significantly reduced in artificial nutrition. Serum levels of FGF-19 were increased in artificial nutrition, and inversely correlated with 7alpha-hydroxylation rates.

Discussion/Conclusion: *In vivo* 7alpha-hydroxylation is suppressed in artificial nutrition, particularly in TPN. The finding associates with reduced cholesterol production, possibly as a metabolic consequence. The findings, together with the preliminary results with the cholecystokinin analogue ceruletide, stress the importance of physiological alimentary function suggesting a regulatory role of gastrointestinal hormones on bile acid production and possibly cholesterol 7alpha-hydroxylase expression. FGF-19 is likely to play a relevant role in this regard.

6

Novel treatment options to improve protein folding and enhance pre-mRNA splicing in ATP8B1 deficiency

Ingrid T.G.W. Bijsmans[1], Wendy L. van der Woerd[1,2], Roderick H.J. Houwen[2] and Stan F.J. van de Graaf[1]
[1]Department of Metabolic Diseases, University Medical Center Utrecht, The Netherlands
[2]Department of Paediatric Gastroenterology, University Medical Center Utrecht, The Netherlands

Introduction: ATP8B1 deficiency is a severe hereditary disease characterized by intrahepatic cholestasis. The exact pathogenesis of the disease is elusive, and no effective pharmacological therapy is currently available. Many of the genetic defects in ATP8B1 are predicted to affect mRNA splicing and the most common missense mutation (p.I661T) leads to ATP8B1 protein misfolding. Pre-mRNA splicing might be improved by compounds that affect the cellular splicing machinery. Similarly, protein folding can be enhanced by small molecules that bind and stabilize partially misfolded proteins (pharmacologic chaperones) or by compounds that stimulate the cellular protein homeostasis (proteostasis) machinery (proteostasis regulators). We hypothesized that small molecules described to improve folding of mutant CFTR would include proteostasis regulators that could improve protein folding in ATP8B1 deficiency.

Methods: We investigated the potential of 13 proteostasis regulators to restore ATP8B1 plasma membrane expression by cell surface biotinylation in U2OS cells expressing wild-type ATP8B1 or the most common mutant ATP8B1 p.I661T. The splicing effect of all known predicted splice-site mutations in ATP8B1 was determined by RT-PCR using 9 minigenes.

Results: One regulator was excluded for further analysis due to excessive toxicity. Six proteostasis regulators caused a significant upregulation of ATP8B1 plasma membrane expression, 3 compounds resulted in a minor increase and 3 were ineffective. Two of the 13 ATP8B1 mutations resulted in incomplete exon skipping, in line with the relatively mild BRIC phenotype of the patients with these mutations. All other ATP8B1 splice-site mutations resulted in complete exon skipping or did not yield detectable RT-PCR products.

Discussion/Conclusion: Proteostasis regulators may provide novel therapeutic options for protein misfolding diseases including ATP8B1 deficiency. PFIC1 mutations lead to very low efficiency of exon-inclusion, whereas in BRIC1 residual correctly spliced ATP8B1 mRNA is detectable.

Targeting specific FXR isoforms in the bile acid biosynthetic pathway

M. Boesjes[1], J. Hageman[1], V.W. Bloks[1], H. Havinga[1], F. Kuipers[1,2], A.K. Groen[1,2]
Laboratory of [1]Pediatrics and [2]Laboratory Medicine, Center for Liver, Digestive and Metabolic Diseases, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

Introduction: Bile acids (BA) are synthesized from cholesterol in the liver and regulate lipid and carbohydrate metabolism. The nuclear receptor Farnesoid X Receptor (FXR) acts as an intracellular BA sensor and regulates the synthesis, secretion and uptake of BA. Four FXR splice variants are known, FXR alpha 1–4. Although these isoforms show differences in spatial and tissue specific expression as well as transcriptional activity, their physiological contribution is still unknown. In this study we aimed to define the specific role of FXR alpha 2 and FXR alpha 4 in control of BA and cholesterol metabolism.

Methods: Male FXR-/- mice were stably transduced with hepatic-specific scAAV expressing FXR alpha 2, FXR alpha 4 or GFP. Mice either received chow or chow supplemented with 0.5% cholic acid (CA).

Results: Plasma cholesterol levels were decreased in FXR alpha 2- and FXR alpha 4-treated mice compared to controls (by 50 and 25%, respectively), in correcting the pro-atherogenic phenotype of FXR-/- mice. Hepatic microarray analysis data and broad scale BA biosynthetic pathway analysis revealed distinct roles of the FXR isoforms. FXR alpha 2 appears to be involved in inhibiting the neutral branch of the primary BA biosynthesis and the CA production, while FXR alpha 4 appears to be involved in inhibiting the acidic branch and the chenodeoxycholic acid (CDCA) production. Fecal and biliary bile acid analysis indeed revealed an increased CDCA-derived BA pool in FXR alpha 2 treated mice, while FXR alpha 4 mice showed increased CA-derived BA. Furthermore, the FXR alpha 2 treated mice showed increased fecal cholesterol excretion due to a more hydrophilic BA pool.

Discussion/Conclusion: In conclusion, both FXR alpha 2 and FXR alpha 4 could rescue the plasma lipid profile in FXR-/- mice by normalizing the plasma cholesterol levels. A differential effect of FXR alpha 2 and FXR alpha 4 on BA biosynthetic genes was observed, indicating a role of the FXR isoforms in control of BA composition in mice.

8

Taurine pretreatment ameliorates liver injury and periportal Bsep expression following warm experimental ischemia and reperfusion

S. Braun[1], P. Cebula[2], T. Kolb[2], C. Strucks[1], P. van Caster[1], D. Herebian[3], E. Mayatepek[3], I. Bauer[1], B. Pannen[1], D. Häussinger[1], M.G. Donner[1]
[1]Department of Anesthesiology, [2]Department of Gastroenterology, Hepatology and Infectious Diseases, [3]Department of General Pediatrics and Neonatology, Heinrich Heine University Düsseldorf, Germany

Introduction: Ischemia and reperfusion (IR) injury is a crucial factor for developing cholestasis following liver resection. We investigated short-term regulation of hepatobiliary transporters, liver injury and the effect of preinduction with taurine and ischemic preconditioning in a rat model of warm IR.

Methods: Hepatobiliary function was studied by bile sampling, bile salt analysis, western blotting and quantitative immunofluorescence of hepatobiliary transporters in rat liver following warm IR (n = 4). Hepatocellular injury was investigated enzymatically and by TUNEL assay (n = 8). Further, the effect of taurine and ischemic preconditioning (IPC) on hepatic injury and hepatobiliary function was studied.

Results: Warm IR showed a steep down-regulation of Mrp2 and Bsep in pericentral hepatocytes (Mrp2 54.3 ± 4.3% of controls, Bsep 30.9 ± 6.8% of controls, $p < 0.05$). Bsep was also down-regulated in periportal hepatocytes to a lesser extent (62.6 ± 11.9% of controls, $p < 0.05$). Preconditioning with taurine and IPC significantly attenuated AST in ischemic reperfused rat liver (IR: 1936 ± 322 U/l; taurine + IR: 981 ± 131 U/l, $p < 0.05$ vs. IR, IPC-IR: 1105 ± 129 U/l, $p < 0.05$ vs. IR). Taurine preconditioning further reduced the number of TUNEL-positive cells (controls: 1.7 ± 0.6, IR: 18.4 ± 6.0, IR ± taurine: 2.5 ± 0.3 positive cells/ high power field, $p<0.05$ vs. IR). Taurine preconditioning significantly improved Bsep expression in periportal hepatocytes (87.6 ± 1.3%, $p < 0.05$ vs. IR), whereas IPC ameliorated Mrp2 expression in pericentral hepatocytes (81.3 ± 4.7%, $p < 0.05$ vs. IR). Reduced bile flow in the reperfusion period was not significantly increased by taurine or IPC. However, taurine modulated bile salt conjugation, as exemplified by an increased ratio of taurochenodeoxycholate/glycochenodeoxycholate (IR: 8.7 ± 2.6, taurine + IR: 233.0 ± 59.8, $p < 0.05$ vs. IR).

Discussion/Conclusion: IR results in rapid down-regulation of Mrp2 and Bsep by endocytic retrieval with a preponderance in pericentral hepatocytes. Ischemic preconditioning and taurine reduce hepatocellular injury and improve expression of canalicular transporters. Taurine further modulates bile salt conjugation and apoptosis and may therefore serve as a therapeutic target to ameliorate reperfusion injury.

ABCG2 plays an important role in the placental barrier for bile acids

Oscar Briz, Alba G. Blazquez, Marta R. Romero, Ruben Rosales, Maria J. Monte, Javier Vaquero, Rocio I.R. Macias, Doris Cassio, Jose J.G. Marin
Laboratory of Experimental Hepatology and Drug Targeting (HEVEFARM), National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd), IBSAL, University of Salamanca, Salamanca, Spain. (O.B., A.G.B., M.R.R., R.R., M.J.M., J.V., R.I.R.M., J.J.G.M.). INSERM, U757, University of Paris-Sud, Orsay, France (D.C.)

Introduction: ABCG2 is involved in several epithelial transport/barrier functions. Steroids, mainly sulphated derivatives, are considered to be among ABCG2 substrates. Here we have investigated the ability of ABCG2 to transport bile acids in liver and placenta.

Methods and Results: The fluorescent bile acid derivative cholylglycylamido fluorescein (CGamF) was exported into canaliculi-like structures formed by WIF-B9/R cells, which do not express BSEP, but displays high expression of ABCG2. Sensitivity to typical inhibitors suggested that CGamF export was mainly mediated by ABCG2. In CHO cells, co-expression of rat Oatp1a1 and human ABCG2 enhanced the uptake and efflux, respectively, of CGamF, cholic acid, glycocholic acid, taurocholic acid and taurolithocholic acid-3-sulfate. The efflux was inhibited by fumitremorgin C. The ability of ABCG2 to export these bile acids was confirmed by microinjecting them in Xenopus laevis oocytes expressing this pump. ABCG2-mediated bile acid transport was inhibited by oestradiol 17beta-D-glucuronide and fumitremorgin. In pregnant rats with obstructive cholestasis, the existence of an efficient placental barrier for bile acids accounted for < 2-fold increase in foetal cholanaemia in spite of > 14-fold increased maternal cholanaemia. In rat placenta, the expression of Abcg2, which was not affected by short-term cholestasis, was much higher than that of Bsep. In pregnant rats, fumitremorgin did not affect uptake/secretion of [^{14}C]-glycocholic acid by the liver but inhibited the transfer of this bile acid across the placenta. Obstructive cholestasis induced similar serum bile acid accumulation in wild-type and Abcg2$^{-/-}$ pregnant mice, but bile acid levels were markedly higher in placenta and foetal serum and liver of Abcg2$^{-/-}$ animals.

Discussion/Conclusion: ABCG2 is able to transport bile acids. The relevance of this transport depends on the relative expression in the same epithelium of other bile acid exporters. Thus, ABCG2 may play a key role in bile acid transport in placenta, as BSEP does in liver.

10

Bile acid sequestration increases transintestinal cholesterol excretion in mice

Gemma Brufau[1], Aycha Bleeker[1], Marleen Schonewille[1], Folkert Kuipers[1,2], Albert K. Groen[1,2]
Center for Liver, Digestive and Metabolic Diseases, Department of [1]Pediatrics and [2]Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Introduction: Colesevelam-HCl (COL) is a new generation bile acid sequestrant used in the treatment of dyslipidemias. It is generally accepted that COL reduces LDL- and total-cholesterol by increased cholesterol conversion into bile salts. In this study, we investigated the validity of this assumption and determined whether COL treatment in addition to reducing intestinal bile acid uptake, alters transtintestinal cholesterol excretion (TICE), the recently described novel route of cholesterol removal from the body. A second aim of this study was to investigate the role of LDL in this process.

Methods: Using D_5 and D_7-labeled cholesterol, whole body cholesterol fluxes and balance were determined in wild-type mice and in *Ldlr*$^{-/-}$ mice fed either COL (2%; wt/wt) or control diet for 2 weeks.

Results: As expected, COL treatment resulted in a massive induction of fecal bile acid loss. COL treatment resulted in a decrease in cholesterol absorption (around 40%) and induction of neutral sterol excretion (3.5-fold induction), which resulted in a 3-fold increase in cholesterol synthesis. Biliary cholesterol, phospholipids and bile acids were decreased in the COL-treated group. No differences were found in hepatic lipids. Interestingly, COL treatment induced a 10-fold increase in TICE. To investigate whether the strongly increased cholesterol flux across enterocytes was mediated via the LDL receptor we investigated the effect of COL in *Ldlr*$^{-/-}$ *mice*. Remarkably absence of the LDL receptor had no effect on TICE stimulation by COL.

Discussion/Conclusion: In conclusion, our data indicate that the cholesterol-lowering effect of COL is partly mediated via activation of TICE. Similarly, COL administration in *Ldlr*$^{-/-}$ mice resulted in an important induction in TICE, indicating that, at least in mice, The LDL receptor is is not important for transcellular cholesterol transport across the intestine.

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

Miriam Cantore*, Roland Reinehr*, Annika Sommerfeld, Martin Becker, and Dieter Häussinger
Clinic for Gastroenterology, Hepatology and Infectious Diseases, University Hospital Düsseldorf, Germany

Introduction: In perfused rat liver, hyperosmolarity induces Mrp2- and Bsep-retrieval from the canalicular membrane leading to cholestasis. The aim of this study was to elucidate the underlying signaling events.

Methods: Studies were performed in the perfused rat liver, rat hepatocyte couplets and in livers from $p47^{phox}$ *knockout* mice.

Results: Hyperosmolarity-induced retrieval of Mrp2 and Bsep from the canalicular membrane in perfused rat liver was accompanied by an activating phosphorylation of Src-kinases Fyn and Yes, but not of c-Src. Both, hyperosmotic transporter-retrieval and Src-kinase-activation were sensitive to apocynin (300 µmol/L), N-acetylcysteine (NAC; 10 mmol/L) and SU6656 (1 µmol/L). Also PP-2 (250 nmol/L), which inhibited hyperosmotic Fyn-, but not Yes-activation, prevented hyperosmotic transporter-retrieval from the canalicular membrane, suggesting that Fyn, but not Yes mediates hyperosmotic Bsep- and Mrp2-retrieval. Both, hyperosmotic Fyn-activation and Bsep-/Mrp2-retrieval were not observed in livers from $p47^{phox}$ *knockout* mice. Hyperosmotic activation of JNKs was sensitive to apocynin and NAC, but not to SU6656 and PP-2. This indicates that JNKs are not involved in transporter-retrieval, as also evidenced by experiments using the JNK-inhibitors L-JNKI-1 or SP6001255. Hyperosmotic transporter-retrieval was accompanied by a NAC- and Fyn-*knockdown*-sensitive inhibition of biliary excretion of the glutathione conjugate of 1-chloro-2,4-dinitro-benzene in perfused rat liver and of cholyl-L-lysyl-fluorescein secretion into the pseudocanaliculi formed by hepatocyte couplets. Hyperosmolarity triggered an association between Fyn and cortactin and increased the amount of phosphorylated cortactin underneath the canalicular membrane.

Discussion/Conclusion: Hyperosmotic cholestasis is triggered by NADPH oxidase-driven ROS-formation which mediates Fyn-dependent retrieval of Mrp2 and Bsep from the canalicular membrane, which involves an increased cortactin phosphory-lation.

12

Hyperosmolarity-induced retrieval of bile salt transport systems is mediated by oxidative stress and sensitive to cyclic AMP

Miriam Cantore*, Annika Sommerfeld*, Roland Reinehr, and Dieter Häussinger
Clinic for Gastroenterology, Hepatology and Infectious Diseases, University Hospital Düsseldorf

Introduction: In perfused rat liver, hyperosmolarity induces a ROS-/Fyn-mediated Mrp2-/Bsep-retrieval from the canalicular membrane, which leads to cholestasis. Cyclic AMP induces choleresis by increasing Na^+-taurocholate cotransport via Ntcp-insertion to hepatocyte plasma membranes.

Methods: Studies were performed in the perfused rat liver.

Results: In perfused rat liver, hyperosmolarity (385 mosmol/L) induced within 30min retrieval of Mrp2 and Bsep from the canalicular membrane as well as of Ntcp from the basolateral membrane which was accompanied by an activating phosphorylation of Src-kinases Fyn and Yes. Also the MAP kinase JNK, but not $p38^{MAPK}$ and Erk-1/-2, were activated upon hyperosmotic exposure. When DB-cAMP (50 µmom/L) was added for another 30 min, Mrp2, Bsep as well as Ntcp were inserted back in the respective membrane suggestive for an inhibition of otherwise observed hyper-osmolarity-induced cholestasis. DB-cAMP also inhibited hyperosmotic Fyn, Yes and JNK activation. As Fyn, but not Yes or JNK, was previously shown to mediate hyperosmolarity-induced Mrp2- and Bsep-retrieval, it is likely that cAMP mediates Mrp2-/Bsep-insertion via inhibition of Fyn. Inhibitor experiments suggest that the hyperosmolarity-induced Ntcp retrieval is triggered by oxidative stress. Preliminary experiments also point to a role of Src kinases such as Fyn or Yes in hyperosmolarity-induced Ntcp-retrieval from the basolateral membrane, because this process is sensitive to PP-2 (250 nmol/L), an inhibitor of both Src kinases.

Discussion/Conclusion: Hyperosmotic cholestasis is triggered by Fyn-dependent retrieval of Mrp2 and Bsep from the canalicular and by an Ntcp-retrieval from the basolateral membrane. All these processes are sensitive to cAMP which may involve cAMP-induced inhibition of Fyn-phosphorylation.

Slowed small intestinal transit: The second motility defect in cholesterol cholelithogenesis is caused by sterol enrichment of intestinal sarcolemmal membranes

MC Carey[1], M Xie[1], VR Kotecha[1], JD Andrade[1], JG Fox[2]
[1]Department of Medicine, Harvard Medical School, and Division of Gastroenterology, Brigham and Women's Hospital and Harvard Digestive Diseases Center, Boston, MA, USA
[2]Divisions of Comparative Medicine and Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

Introduction: In humans with cholesterol (CH) gallstones and during CH cholelithogenesis in animals, peristaltic activity of the small intestine is slowed. This creates a vicious cycle by promoting hyperabsorption of CH and hypersynthesis of the prolithogenic secondary bile salt deoxycholate.

Methods: We investigated in C57L/J mice whether a lithogenic diet (LD) and its individual components (1% CH, 0.5% cholic acid and 17% triglycerides), as well as intestinal infection with *Helicobacter hepaticus*, a prolithogenic agent in mice, would influence small intestinal transit. We quantified transit time from the distribution of ^{3}H-sitostanol along the length of the small intestine 20 minutes following intraduodenal instillation. We isolated proximal intestinal sarcolemmal membranes by density gradient centrifugation and measured their CH and phospholipid (PL) composition. We then blocked intestinal CH absorption by treating the mice with ezetimibe and repeated the motility, sarcolemmal isolation, and lipid composition studies.

Results: Dimensionless geometric centers, quantitative indices of intestinal propulsive activity, were slowed significantly ($P < 0.05$) by the LD but not slowed further by helicobacter infection (males, 9.4 ± 0.5 (uninfected), 9.6 ± 0.5 (infected) on LD, compared with 12.5 ± 0.4 and 11.4 ± 0.5 on chow. The LD effect was reproduced only by CH plus cholic acid. Sarcolemmal membranes became markedly enriched in CH (CH/PL ratio of 0.57 ± 0.03) compared to 0.45 ± 0.02 on chow ($P < 0.01$) and 0.38 ± 0.03 ($P < 0.0001$) in mice fed the LD plus ezetimibe, which also normalized motility.

Discussion/Conclusion: This work reveals the molecular origins of the second motility defect (in addition to the gallbladder) in CH cholelithogenesis. Although the primary source of intestinal CH in mice is the LD, the principal source of the sterol in most CH gallstone-prone humans is the liver, which hypersecretes CH molecules continuously into bile and the upper small intestine.

Reference:

Xie M, Kotecha VR, Andrade JD, Fox JG, Carey MC. Augmented cholesterol absorption and sarcolemmal sterol enrichment slow small intestinal transit in mice, contributing to cholesterol cholelithogenesis. J Physiol. 2012 Feb 13. [Epub ahead of print].

14

Hyperhomocysteinemia and -homocysteinbilia: Unrecognized signals and perhaps new perils of cholesterol cholelitho-genesis

MC Carey[1], J Zhang[1], DE Handy[2], Y Wang[3], G Bouchard[1], J Selhub[4], J Loscalzo[2]
[1]Department of Medicine, Gastroenterology Division and [2]Department of Medicine, Cardiovascular Division, Brigham & Women's Hospital, Harvard Medical School, Boston, MA, USA
[3]Molecular Pathology Unit, Center for Cancer Research, and Center for Systems Biology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA
[4]Vitamin Metabolism Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA

Introduction: Hyperhomocysteinemia has been reported with cholesterol (CH) gallstones and may explain the epidemiological link with coronary disease. Since CH hypersecretion is accompanied by hypersecretion of biliary phosphatidylcholine (PC), we hypothesized that trimethylation of hepatic phosphatidylethanolamine (PE) via the PE N-methyltransferase (PEMT) pathway would produce three molecules of homocysteine for every PC molecule synthesized.

Methods: We fed inbred mice a CH/cholic acid lithogenic diet (LD). We quantified total plasma and bile homocysteine (tHcy), folate and vitamin B_{12} (cofactors in Hcy's remethylation), as well as biliary cysteine, a reflection of Hcy's transsulfuration. Rate limiting enzymes in both the phosphocholine cytidylyltransferase (PCT) and PEMT pathways of PC synthesis were measured in liver homogenates. Other potential sources of tHcy production in the liver were assayed by gene expression.

Results: In C57L mice, plasma tHcy increased at 4 days (from 4.3 ± 0.2 µM to 8.7 ± 0.7 µM; $P = 0.001$) and bile tHcy increased as measured at 42 days (1.5 ± 0.1 to 5.3 ± 0.9 pmol/min/g liver; $P < 0.05$). Despite continuance on the LD, counter-regulation, which included remethylation to methionine with a decrease in folate and vitamin B_{12} levels and upregulation of betaine-homocysteine methyltransferase (*Bhmt*) and methylenetetrahydrofolate reductase (*Mthfr*), normalized plasma tHcy, except in the C57L strain with the greatest number (~6) of *Lith* genes. Trans-sulfuration of Hcy via cystathionine-β-synthase (*Cbs*) was also upregulated, as evidenced by increased bile cysteine. PEMT KO mice hypersecreted CH and PC and acquired gallstones similar to WT mice, but plasma tHcy levels remained normal.

Discussion/Conclusion: PEMT (but not PCT) activity is upregulated during CH cholelithogenesis and produces excess PC molecules for bile. If lithogenic humans behave similarly, then elevated plasma tHcy may be an early pre-stone marker, and plasma and bile tHcy levels may lead to pathological changes in the arterial system and gallbladder, respectively.

Reference:
Zhang J, Handy DE, Wang Y, Bouchard G, Selhub J, Loscalzo J, Carey MC. Hyperhomocysteinemia from trimethylation of hepatic phosphatidylethanolamine during cholesterol cholelithogenesis in inbred mice. Hepatology 2011; 54: 697–706.

The miRNA-21/PDCD4 pathway in the response of rat hepatocytes to bile acids: Deciding between cell death and survival

Rui E. Castro[1,2], Marta B. Afonso[1], Duarte M.S. Ferreira[1], Pedro M. Rodrigues[1], Pedro M. Borralho[1], Cecília M.P. Rodrigues[1,2]
[1]Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL) and [2]Department of Biochemistry and Human Biology, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

Introduction: Bile acids act as strong modulators of cell death and survival. In particular, deoxycholic acid (DCA) has been implicated in the pathogenesis of liver and gastrointestinal disorders, whereas ursodeoxycholic acid (UDCA) is strongly cytoprotective and approved in the treatment of primary biliary cirrhosis. In addition, we have recently shown that microRNAs (miRNAs or miRs) are significantly modulated by bile acids. Therefore, we aimed to clarify the mechanisms by which DCA and UDCA differentially modulate cell death and proliferation, particularly those involving the miR-21 signaling pathway, deregulated in several liver pathologies.

Methods: RNA and proteins were isolated from primary rat hepatocytes incubated with 25–400 μM DCA or UDCA from 4 to 48 h. miR-21 expression was evaluated by qRT-PCR. Pro-apoptotic programmed cell death 4 (PDCD4) protein, a miR-21 target, was analysed by immunoblotting. Cell death and proliferation were determined through the MTS, LDH and Hoechst assays.

Results: The results showed that, unlike UDCA, DCA modulated miR-21 and PDCD4 expression in a dose-dependent manner. While lower doses tended to activate this survival pathway, moderate and high doses were significantly inhibitory. In particular, 100 μM DCA decreased miR-21 between 10–50%, after 4 and 48 h of incubation, respectively, and increased PDCD4 expression. In contrast, UDCA only slightly induced the miR-21/PDCD4 pathway, while miR-21 overexpression inhibited PDCD4 and increased cell viability. In these conditions, augmented PDCD4 expression and apoptosis, as well as inhibition of cell proliferation induced by DCA were significantly attenuated. In agreement, DCA toxicity was similarly reduced after PDCD4 silencing.

Discussion/Conclusion: DCA-modulation of the miR-21/PDCD4 pathway correlates with its effects on cellular apoptosis. A better understanding of the mechanisms by which bile acids modulate proliferation and apoptosis, particularly those involving miRNAs, may have significant implications in the development of new therapeutic tools.

(Supported by grants PTDC/SAU-OSM/102099/2008, PTDC/SAU-ORG/111930/2009 and Ph.D. fellowship SFRH/BD/60521/2009 from FCT, Portugal).

16

Bile salt toxicity in sandwich-cultured rat hepatocytes (SCRH): Intracellular exposure to glycine conjugates appears to be the main determinant of bile salt toxicity

Sagnik Chatterjee, Patrick Augustijns and Pieter Annaert
Laboratory of Pharmacotechnology and Biopharmacy, Katholieke Universiteit Leuven, Leuven, Belgium

Introduction: Supraphysiological concentrations of bile salts are known to mediate apoptosis and (or) necrosis in hepatocytes. Bile salt secretion by hepatocytes into the canalicular space is primarily mediated by the Bile Salt Export Pump (BSEP; *ABCB11*). Development of bile canaliculi in SCRH takes several days. Therefore, we hypothesize that bile salt toxicity will decrease with culture time because of increased biliary excretion with culture time. SCRH expresses the enzymes and transporters required for bile salt disposition.

Methods: The *in vitro* bile salt toxicity was determined in day 1 and day 3 SCRH. Toxicity was assessed by reduced urea cycle function in SCRH. The intracellular concentrations of bile salts were measured after extracellular exposure to chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA) by UHPLC-MS/MS.

Results: Bile salts can be ranked in decreasing order of toxicity: glycine conjugated > unconjugated > taurine conjugated bile salts, as measured in day 1 cultures. CDCA, DCA, and glycochenodeoxycholic acid (GCDCA) exerted higher toxicity in day 1 SCRH than in day 3 SCRH. Intracellular concentrations of glycine conjugates of CDCA and DCA were much lower in day 3 than day 1 SCRH. However, the concentrations of unconjugated bile salts were found to be higher in day 3 cultures. The formation of glycine and taurine conjugates was found to depend on the time of incubation. The amount of glycine conjugates increased with incubation time (5 min–4 hr) while for taurine conjugates a reverse trend was observed.

Discussion/Conclusion: Decreased intracellular glycine conjugate concentration in day 3 compared to day 1 SCRH appears to play a role in the observed toxicity difference of bile salts between day 1 and day 3 SCRH. The decrease in intracellular concentration of glycine conjugates may be explained primarily by decreased conjugation and also by increased biliary excretion in day 3 SCRH versus day 1 SCRH.

Analysis of liver inflammation in mice invalidated for the vitamin D nuclear receptor

Chignard, N[1], Denaës T[1], Firrincieli, D[1], Housset, C[1],
[1]UPMC Univ Paris 06, UMR_S 938, CdR Saint-Antoine, Paris, France

Introduction: Nuclear receptors have been shown to control inflammation in the liver. Because, the vitamin D nuclear receptor (VDR) is expressed in cells from the myeloid lineage, including Kupffer cells, we investigated whether VDR is involved in the control of hepatic inflammation.

Methods: One-year old VDR knockout mice ($VDR^{-/-}$) were compared to wild type animals for systemic and liver inflammation by multiplex analysis, antibody array, western blot and RT-QPCR.

Results: Pro-inflammatory cytokines, such as IL-1β, IL-6 and TNFα, were increased in the plasma of $VDR^{-/-}$ mice compared to wild type animals. Although hepatic expression levels were low, these cytokines were also increased in the liver of $VDR^{-/-}$ when compared to wild type animals, as ascertained by an antibody array. Analysis of hepatic phosphorylated IκB protein levels by western blot did not reveal significant difference between $VDR^{-/-}$ and wild type mice. However, hepatic expression of Kupffer cell markers, analysed by real time PCR, indicated that Clec4f, F4/80 and cd68 were diminished in $VDR^{-/-}$ mice compared to wild type animals.

Discussion/Conclusion: In conclusion, one-year old $VDR^{-/-}$ mice display higher circulating inflammatory cytokines. These results also suggest that the number Kupffer cells and their activation status of are altered by the absence of VDR.

18

A randomized controlled trial of budesonide with ursodeoxycholic acid versus methotrexate plus ursodiol in primary biliary cirrhosis

I. Copaci, L. Micu, E. Mindrut, M. Voiculescu
Center of Internal Medicine, Fundeni Clinical Institute Bucharest, Romania

Introduction: Primary biliary cirrhosis (PBC) frequently progresses despite treatment with ursodeoxycholic acid (UDCA). The aim of the study was to determine if the addition of budesonide or methotrexate to UDCA would improve liver histology. Secondary endpoints included comparison of treatment arms with respect to overall survival and time to clinical decompensation.

Methods: PBC stage I to III patients (n = 52) were randomized into two treatment arms: UDCA 15 mg/kg/day and budesonide 6 mg/day (n = 28, group A) or UDCA 15 mg/kg/day plus methotrexate 15 mg/week (n = 24, group B). Patients were excluded if they had end-stage liver disease: bilirubin level greater than 10 mg/dl or albumin less than 3 g/dl; encephalopathy; hemorrhage from esophageal varices and/or portal gastropathy. Patients were followed-up for a median period of 5.5 years (range 4.2–7.6 years) or until treatment failure. Treatment failure was defined as: histological progression by at least two stages or to cirrhosis; death without liver transplantation; transplantation; variceal bleeding; development of ascites, encephalopathy or varices; doubling of serum bilirubin levels.

Results: Values for serum ALP, ALT and GGT improved in both groups. Bilirubin levels remained stable in group A and increased in group B ($p = 0.01$). Analysis of liver histology after 36 months of therapy revealed significant histological improvement in both groups when baseline was compared with the final biopsy. No patients in either group progressed to cirrhosis or extensive fibrosis. Overall, mean necro-inflammatory scores fell from 12.4 to 4.7 in group A and from 12.7 to 6.1 in group B. Fibrosis decreased 28% in group A ($p = 0.05$) and 13% in group B ($p = 0.08$).

Discussion/Conclusion: 1. Combination therapy might be beneficial for all PBC patients with pre-cirrhosis liver disease. 2. Inflammation decreased in both groups, but the histological stage was significantly decreased in the group with budesonide and UDCA.

Modulation of intestinal microflora affects hepatic bile acid synthesis in mice

Chiara Degirolamo[1], Stefania Rainaldi[1], Stefania Murzilli[1], Antonio Moschetta[1,2]
[1]Consorzio Mario Negri Sud, 66030 Santa Maria Imbaro, Chieti and [2]Clinica Medica "A. Murri", University of Bari, 70124 Bari, Italy

Introduction: The gastrointestinal tract is homing a highly dynamic microbial ecosystem that has metabolic and protective functions. Gut microbiota is crucial for bile acid (BA) biotransformation thus modulating BA pool composition. The aim of the study was to characterize the effects of probiotic-mediated modification of gut microbiota in regard to BA metabolism.

Methods: Mice were administered vehicle or probiotic mixture VSL#3 for 21 days. Fecal probiotic DNA composition and bacterial enzyme gene expression and activity were weekly monitored while serum, biliary, fecal BA levels and pool size were measured at the end of the treatment. A metabolic flux study by using [^{3}H]-taurocholic acid was performed to monitor ileal BA absorption. Finally, characterization of the bacterial lineages (phyla) present in the fecal microbiotas was obtained by metagenomic sequencing and analysis.

Results: VSL#3 modified fecal bacteria DNA composition and increased the expression and activity of BA-deconjugating enzymes along with a reduced fecal conjugated/unconjugated BA ratio. VSL#3-treated animals exhibited enhanced fecal BA excretion that was associated with changes in ileal BA absorption and compensated by an increased hepatic BA neo-synthesis due to repression of the entero-hepatic farnesoid X receptor-fibroblast growth factor 15 (FXR-FGF15) axis. Serum, biliary and BA pool size were unchanged upon VSL#3 treatment. When probiotic mixture was given to FXR-deficient animals, hepatic BA neo-synthesis appeared unchanged.

Discussion/Conclusion: Probiotic modification of gut microbiota is able to change BA metabolism via enhanced fecal excretion and hepatic neo-synthesis and may have a therapeutic potential in metabolic and inflammatory disorders of gut-liver axis where BA level modulation is desirable.

20

In vitro analysis of splicing mutations of *ABCB4* using minigene constructs

Peter H. Dixon, Mark Willsden, Catherine Williamson
IRDB, Imperial College Hammersmith Campus, Du Cane Road, London W12 0NN, UK

Introduction: Mutations of the phosphatidyl choline floppase *ABCB4* are implicated in a spectrum of cholestatic liver diseases, including intrahepatic cholestasis of pregnancy (ICP). Amongst the mutations reported are a number of presumed splicing mutations. However, liver biopsies are not clinically indicated in ICP, hence mRNA is not available to determine the effect of these mutations. We have therefore used an exon-trapping vector that contains partial splicing signals to investigate novel splicing mutants of *ABCB4*.

Methods: Genomic DNA was extracted according to standard protocols. Primers spanning the *ABCB4* coding region were used to amplify and sequence DNA from a cohort of 20 patients with ICP. Novel potential splicing mutations were analysed further by cloning into the pSPL3 vector and transfection of COS-1 cells. mRNA produced by the construct-transfected cells was isolated and analysed by RT-PCR, cloning and sequencing.

Results: DNA sequencing identified two possible splicing mutations in the patient cohort. The first was located at position -1, next to exon 1 which contains 6 bases of un-translated region prior to the initiation codon. In a second patient, a potential mutation was identified at exon 14+6. Exon trapping revealed the expected presence of the correct exons in the wild-type constructs and controls. However, the exon 1 variant resulted in much less product, which when cloned and sequenced was revealed to be one base-pair shorter than wild type, indicating a shift in the splicing site. The exon 14 variant construct produced identical results to wild-type.

Discussion/Conclusion: We have demonstrated the analysis of *ABCB4* splicing mutations by using minigene constructs in an exon-trapping vector; demonstrating aberrant splicing in a case of ICP and confirming the second observed variant as a neutral polymorphism. This approach enables characterisation of the effect of splicing mutations, where mRNA samples are not available.

Efficacy of ursodeoxycholic acid in the treatment of nonalcoholic fatty liver disease

O.P. Dudanova, I.A. Belavina
Petrozavodsk State University, Russia

Introduction: The aim of this study was to estimate the efficacy of ursodeoxycholic acid (UDCA - Ursofalk®) in the treatment of nonalcoholic fatty liver disease (NAFLD).

Methods: UDCA was used in 30 patients with HAFLD. Diagnosis was based on clinical and laboratory tests, combined with sonography and liver biopsy. The group comprised 18 (60.0%) females and 12 (40.0%) males, with the average age of 52.7 ± 6.9 (32–74) years, and average IBM of 32.6 ± 2.0 (28–40). UDCA was used at 11.7 ± 1.2 mg/kg (7.7–14.7 mg/kg) during 41.5 ± 6.4 weeks (12–72).

Results: Improvements of clinical and laboratory tests were observed after 12 weeks in 27 patients (90.0%). Alaninaminotransferase (ALAT) decreased by 33.1%, from 108.5 ± 44.5 IU/l to 72.6 ± 25.4 IU/l ($p < 0.05$); gammaglobulins decreased by 17.1%, from 18.7 ± 1.9 g/l to 15.5 ± 2.0 g/l ($p < 0.05$); and there was a reduction in cholesterol by 9.2%, from 6.5 ± 0.9 to 5.9 ± 0.8 mmol/l ($p < 0.05$). In patients with intrahepatic cholestasis alkaline phosphatase (AP) level was diminished by 18.9% and gammaglutamyltranspeptidase by 15.0%. ALAT level reached 58.2 ± 19.4 IU/l over 36 weeks, and 32.1 ± 0.9 IU/l ($p < 0.005$) over 50 weeks, while AP level became normal. Liver size decreased as well, right lobe from 145.4 ± 7.8 mm to 133.0 ± 4.9 mm ($p < 0.05$); left lobe from 77.2 ± 6.5 mm to 63.5 ± 10.3 mm ($p < 0.05$), longitudinal size of the spleen from 108.3 ± 9.2 mm to 97.8 ± 6.6 mm ($p < 0.05$), and transversal size from 45.9 ± 5.3 mm to 43.8 ± 4.3 mm ($p > 0.05$). Linear velocity of blood flow in the portal vein increased from 19.5 ± 2.6 sm/sek to 23.1 ± 2.8 sm/sek ($p < 0.05$).

Discussion/Conclusion: UDCA was effective in the treatment of 90.0% of patients with NAFLD, causing reliable clinical and laboratory test improvements when used at 11.7 ± 1.2 mg/kg. Positive effects were detected already after 12 weeks of treatment, whereas after 50 weeks we observed normalization of ALAT and AP, as well as blood flow improvement in the portal vein.

22

Cellular effects of disease-causing variations of the hepato-biliary transporter ABCB4

Thomas Falguières, Julien Gautherot, Jean-Louis Delaunay, Danièle Delautier, Anne-Marie Durand-Schneider, Chantal Housset, Michèle Maurice and Tounsia Aït-Slimane
INSERM and Université Pierre et Marie Curie Paris 6, UMR_S 938, Paris, France

Introduction: The ABC transporter ABCB4 (also called MDR3 or multi-drug resistance protein 3) is a phosphatidylcholine translocator responsible for biliary phospholipid secretion. Variations of the *ABCB4* gene have been recognized as causing progressive familial intrahepatic cholestasis type 3 (PFIC3) in children, low phospholipid associated cholelithiasis (LPAC) syndrome and intrahepatic cholestasis of pregnancy (ICP) in adults. For a better understanding of these pathologies, we investigated the effects of ABCB4 variations at the cellular level.

Methods: Missense mutations identified in patients with PFIC3 (I541F) and LPAC syndrome (T34M and R47G) were reproduced in the *ABCB4* cDNA. These constructs were expressed in the cell lines HepG2 and MDCK, and their expression and localization were analyzed. The functionality of the ectopically expressed proteins was studied by measuring the release of phosphatidylcholine into the culture medium of MDCK cells. Attempts to rescue trafficking-defective mutants were performed by environmental (low temperature) or pharmacological (drug treatment) means.

Results: In both cell models, the I541F ABCB4 mutant was ER-retained. However its plasma membrane targeting could be partially restored by lowering the temperature of cell culture or by treating the cells with cyclosporin A. On the other hand, the T34M and R47G mutants did not display expression or transport defect, but our preliminary results indicate that their phosphatidylcholine translocation activity is impaired.

Discussion/Conclusion: Point mutations of ABCB4 can lead to trafficking or activity defects, which may be linked to hepatobiliary diseases of different severities. Cyclosporin A rescue of a trafficking-defective mutant opens perspectives for treating certain forms of ABCB4-linked diseases by pharmacological means.

Ursodeoxycholic acid inhibits activation of miR-34a/SIRT1/p53, a pro-apoptotic pathway associated with disease severity in non-alcoholic fatty liver

Duarte M.S. Ferreira[1], Pedro M. Borralho[1,2], Marta B. Afonso[1], Pedro M. Rodrigues[1], Mariana V. Machado[3,4], Helena Cortez-Pinto[3,4], Cecília M.P. Rodrigues[1,2], Rui E. Castro[1,2]
[1]Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon; [2]Department of Biochemistry and Human Biology, Faculty of Pharmacy, University of Lisbon; [3]Department of Gastroenterology, Hospital de Santa Maria; [4]Instituto de Medicina Molecular, Faculty of Medicine, University of Lisbon, Lisbon, Portugal

Introduction: Non-alcoholic fatty liver disease (NAFLD) comprises a spectrum of stages ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), on which p53-mediated apoptosis appears to play a causative role. However, disease pathogenesis remains largely unknown. microRNA (miRNA or miR) expression is altered in human NASH liver, and modulated by ursodeoxycholic acid (UDCA) in rat liver. Here, we aimed to evaluate miR-34a/Sirtuin1(SIRT1)/p53 signaling in human NAFLD, and elucidate its function and modulation by UDCA in rat liver and primary rat hepatocytes.

Methods: Liver biopsies were obtained from NAFLD morbid obese patients undergoing bariatric surgery. Rat livers were collected from animals fed 0.4% UDCA diets. Primary rat hepatocytes were incubated with UDCA, and transfected with a specific miRNA-34a precursor and/or with a p53 overexpression plasmid. p53 transcriptional activity was assessed in nuclear extracts and by using target reporter constructs.

Results: Our results show that miR-34a, apoptosis and acetylated p53 increased with disease severity, while SIRT1 protein diminished in the liver of NAFLD patients. UDCA inhibited miR-34a/SIRT1/p53 signaling in the rat liver *in vivo* and in cultured primary rat hepatocytes. miR-34a overexpression confirmed its targeting by UDCA, which in turn prevented miR-34a-dependent repression of SIRT1, p53 acetylation and, ultimately, apoptosis. Modulation of SIRT1 by UDCA was mostly miR-34a-dependent. Significantly, p53 overexpression activated miR-34a/SIRT1/p53, which in turn was inhibited by UDCA. Furthermore, UDCA decreased general p53 activity, as well as specific transcriptional activation of PUMA, p21 and miR-34a.

Discussion/Conclusion: In conclusion, our results support a link between liver cell apoptosis and miR-34a/SIRT1/p53 signaling, specifically modulated by UDCA at the p53 transactivation level, and NAFLD severity. The miR-34a/SIRT1/p53 pro-apoptotic pathway may represent an attractive pharmacological target for the development of new drugs to arrest NAFLD progression.

(Supported by grants PTDC/SAU-OSM/102099/2008, PTDC/SAU-OSM/100878/2008, PTDC/SAU-ORG/111930/2009, and Pest-OE/SAU/UI4013/2011 and fellowship SFRH/BD/60521/2009 (D.M.S.F.) from FCT, Lisbon, Portugal).

24

Vitamin D nuclear receptor deficiency promotes E-cadherin cleavage and bile duct rupture in bile duct-ligated mice

Firrincieli, D[1], Zúñiga, S[1], Rey, C[1], Wendum, D[2], Lasnier, E[3], Rainteau, D[3], Cadoret, A[1], Boissan, M[1], Housset, C[1], Chignard, N[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: Alterations in bile duct cellular junctions have been reported in biliary-type liver diseases. These diseases were associated with vitamin D nuclear receptor (VDR) polymorphisms, a nuclear receptor known to regulate epithelial cellular junctions. Because VDR is predominantly expressed in biliary epithelial cells in the liver, we investigated its role in maintenance of bile duct integrity.

Methods: Biliary-type liver injury was induced by bile duct ligation (BDL) in VDR knockout mice ($VDR^{-/-}$) and wild-type littermates. Cellular junction integrity was investigated by E-cadherin expression analysis. Analysis of E-cadherin regulation by VDR in biliary epithelial cells was examined *in vitro*.

Results: After BDL, $VDR^{-/-}$ mice displayed increased liver damage as compared to wild-type BDL mice. Analysis of E-cadherin expression on liver sections demonstrated that $VDR^{-/-}$ BDL mice displayed altered E-cadherin staining that corresponded to an increase in bile duct rupture. Total liver protein analysis revealed that a truncated form of E-cadherin was present in higher amounts in $VDR^{-/-}$ mice submitted to BDL. Truncated E-cadherin also appeared in biliary epithelial cells downregulated for VDR and paralled calpain 1 activation. Truncated E-cadherin was not observed when calpain 1 expression was downregulated in VDR-silenced cells. Calpain 1-induced E-cadherin cleavage was dependent on EGFR signaling, a pathway known to be negatively regulated by VDR.

Discussion/Conclusion: In conclusion, $VDR^{-/-}$ mice display higher bile duct rupture after BDL, paralleling the cleavage of E-cadherin. Truncation of E-cadherin results from the activation of calpain 1, which was induced by the EGFR pathway. Altogether, these results indicate that VDR deficiency favors the loss of bile duct integrity.

Carbon-11 labelled cholylsarcosine: A PET-tracer for conjugated bile acids

Kim Frisch[1], Steen Jakobsen[1], Michael Sørensen[1,2], Ole Lajord Munk[1],
Aage K.O. Alstrup[1], Peter Ott[2], Alan F. Hofmann[3], and Susanne Keiding[1,2]
[1]Department of Nuclear Medicine and PET Centre, Aarhus University Hospital, Aarhus, Denmark
[2]Department of Medicine V (Hepatology and Gastroenterology), Aarhus University Hospital, Aarhus, Denmark
[3]Department of Medicine, University of California, San Diego, California, USA

Introduction: The trans-hepatocellular transport of bile acids and their enterohepatic circulation are essential for bile acids to perform their physiological function in digestion, detoxification and signaling. Due to their detergent property, however, bile acids are inherently cytotoxic. Indeed, accumulation of bile acids in hepatocytes caused by impaired transcellular transport can result in cell damage and cell death. We have shown that ^{11}C-cholylsarcosine, an analogue of endogenous cholylglycine, is a promising tracer for *in vivo* studies of the trans-hepatocellular transport and enterohepatic circulation of conjugated bile acids by PET/CT (*J Nucl Med* 2012;53: 772–778).

Methods: ^{11}C-Cholylsarcosine was prepared by ^{11}C-methylation of glycine followed by conjugation with cholic acid. Its blood-to-liver uptake and liver-to-bile excretion were investigated by dynamic PET/CT in anaesthetized 40-kg pigs. Possible formation of ^{11}C-metabolites was investigated in blood and bile samples. Possible inhibition of the trans-hepatocellular transport of ^{11}C-cholylsarcosine was investigated by dynamic PET/CT preceded by a high i.v. dose of cholyltaurine.

Results: ^{11}C-Cholylsarcosine was produced with a radiochemical yield of 13% (decay-corrected) and a radiochemical purity greater than 99%. The PET/CT studies showed rapid blood-to-liver uptake and liver-to-bile excretion of ^{11}C-cholylsarcosine, with radioactivity concentrations being more than 90 times higher in bile ducts than in liver tissue. ^{11}C-Cholylsarcosine underwent enterohepatic circulation and reappeared in liver tissue and bile ducts approximately 70 min after administration. Cholyltaurine inhibited the trans-hepatocellular transport of ^{11}C-cholylsarcosine. No ^{11}C-metabolites were detected in plasma or bile samples.

Discussion/Conclusion: We have synthesized a novel radiolabelled conjugated bile acid analogue, ^{11}C-cholylsarcosine. PET/CT studies in pigs show that hepatic uptake of ^{11}C-cholylsarcosine from blood and excretion into bile is comparable to that of endogenous cholyltaurine. ^{11}C-Cholylsarcosine may thus be valuable for PET/CT studies of the trans-hepatocellular transport and enterohepatic circulation of conjugated bile acids in patients with intra- and extra-hepatic cholestatic disorders.

26

Combined therapy with low-dose ursodeoxycholic acid in the treatment of nonalcoholic steatohepatitis in obese patients

Amelia Genunche-Dumitrescu, D. Badea, M. Badea, P. Mitrut, A. Badea
University of Medicine and Pharmacy, Clinical Hospital of Emergency, Craiova, Romania

Introduction: Aim of this study was to evaluate and compare the effectiveness of ursodeoxycholic acid (UDCA) monotherapy, simvastatinum and combination of UDCA and vitamin E in the treatment of NASH.

Methods: We studied 53 patients with NASH and obesity. We excluded patients with viral or autoimmune hepatitis, diabetes mellitus or drug abuse. The diagnosis was based on the correlation of histologic and clinical findings. Liver biopsy was performed before and after therapy.
A group composed of 18 normolipidemic cases, treated with UDCA 13–15 mg/kg/day, B group consist of 15 hyperlipidemic cases which received simvastatinum 20 mg/day and C group (20 patients) with UDCA and vitamin E (400 IU twice a day) therapy. We evaluated liver function tests, serum lipids and BMI at the begining of therapy, after 6 and 12 months.

Results: A number of 39 patients had elevated serum aminotransferase level, but 14 had normal values. In B group, lipide profile was: 7 cases with hypercholesterolemia, 4 cases with hypertriglyceridemia and 4 with both.
In A group, mean value of serum ALT-level was decreased from 88.3 ± 21.7 U/l at baseline, to 52.12 ± 17.5 U/l at 6 months. In B group, serum ALT was reduced (in mean with 19.3 ± 7.2 U/l) after 6 months and cholesterolemia was significantly improvement in 8 cases (72.7%). In 2 cases we increased simvastatinum dose at 40 mg/day. In C group mean ALT and AST levels was more decreased: in mean with 49.3 ± 5.2 U/l. After one year, aminotransferase levels reach normal range only in C group. Comparatively, in A and B groups the normalisation rates of ALT was lower (89.7% and 73.33%). Histopathologic examination was releved improvement the steatosis grade: 83.3% in A group, 73.3% in B group and 90.0% in C group.
We could not establish a correlation between the values of serum aminotransferases and others parameters, but multivariate analysis showed that the BMI > 28 kg/m² and elevation of serum ALT were associated with steatosis grade. Patients which associated combinated therapy with low caloric diet, had a good and rapid response.

Discussion/Conclusion: Combination of UDCA and vitamin E significantly improves aminotransferase levels and steatosis grade. The combinated therapy and low caloric diet still remains first line therapy in patients with NASH and obesity.

A helical structure of the N-terminal part of the TGR5 C-terminus is required for receptor membrane localization

Christoph G.W. Gertzen[1], Birte Schmitz[1], Britta Nisius[1], Verena Keitel[2], Holger Gohlke[1]
[1]Institut für Pharmazeutische und Medizinische Chemie, Heinrich-Heine-Universität, Universitätsstr. 1, 40225 Düsseldorf, Germany
[2]Klinik für Gastroenterologie, Hepatologie und Infektiologie, Heinrich-Heine-Universität, Moorenstr. 5, 40225 Düsseldorf, Germany

Introduction: TGR5 is a G-protein coupled bile acid receptor that is located in different non-parenchym cells of the liver. The structure of TGR5 is unknown. Previous studies demonstrated that the deletion of the 35 C-terminal amino acids results in ER retention of the mutated receptor. Here we aim at structurally characterizing the N-terminal part of the TGR5 C-terminus by means of molecular dynamics (MD) simulations.

Methods: The N-terminal part of the wildtype TGR5 C-terminus (aa284 to 302), the 285-290A and 291-297A variants, and the Δ291-297 variant were subjected to all-atom MD simulations in explicit solvent of up to 600 ns length. Conformations of the peptides were characterized and clustered by secondary structure content.

Results: The MD simulations reveal a significantly higher β-sheet proportion in the 285-290A variant. In contrast, the 291-297A, Δ291-297, and the wildtype peptides showed a higher amount of helicality. These findings agree with results of a hierarchical clustering that identified distinct clusters for the wildtype, the 291-297A variant together with the Δ291-297 variant, and the 285-290A variant. Visual examination of cluster representatives confirmed the formation of a short helical stretch at the N-terminal end of the peptides in all but the 285-290A variant.

Discussion/Conclusion: Immunofluorescence microscopy and FACS analysis shows that the N-terminal part of the TGR5 C-terminus (aa285-290) is required for plasma membrane localization. Since no known sorting signal was identified in the proximal C-terminus, these aa are most likely essential for the correct folding of the protein. Molecular dynamics simulations show that the proximal TGR5 C-terminus contains an α-helix. In contrast, the 285-290A variant shows β-sheet formation in this region, which likely leads to ER retention.

28

Fenofibrate, a peroxisome proliferator-activated receptor alpha (PPARalpha) agonist, up-regulates human MDR3/ABCB4

Nisanne S. Ghonem, Meena Ananthanarayanan, Carol J. Soroka, and
James L. Boyer
Department of Internal Medicine and Liver Center, Yale University School of Medicine, New Haven, Connecticut, USA

Introduction: The multi-drug resistance protein 3 (MDR3/ABCB4) determines phosphatidylcholine excretion in bile. MDR3/Mdr2 deficiency results in cholestasis in animals and man and is a potential therapeutic target. Fibrates (PPARalpha ligands) induce Mdr2 expression in mice dependent on PPARalpha (Biochem J 369:'539'03) and improve liver function in some patients with cholestatic liver disease.

Aim: To determine whether fenofibrate, a specific PPARalpha ligand, up-regulates human MDR3 expression and, if so, by what mechanism.

Methods: MDR3 mRNA and protein were assessed in primary cultures of human hepatocytes (PCHH) and HepG2 cells treated with fenofibrate (FF), bezafibrate (BF) and gemfibrozil (GF) (12.5–125 µM) for 6–72 hours. Wy-14,643 (10 µM), a PPARalpha agonist, served as a positive control. MDR3 localization was also assessed in HepG2 cells by confocal immunofluorescence microscopy. Finally, the 5'-upstream region of human MDR3 gene was analyzed in silico for potential PPAR response elements (PPRE) with MatInspector and cloned in 2 kb fragments spanning 10 kb upstream of the transcription start site to determine effects of FF on ABCB4-luciferase reporter constructs.

Results: In PCHH, Wy-14,643, FF, BF, and GF increased MDR3 mRNA expression by 4.1- 6.4-, 2.1-, and 1.3-fold, respectively ($p < 0.001$), compared to UDCA and CDCA (0.9- and 2-fold). FF and Wy-14,643 also increased MDR3 protein levels by 3-fold ($p < 0.01$) in PCHH and pseudo-canalicular MDR3 staining in HepG2 cells by 3- and 3.2-fold respectively ($p < 0.01$). FF did not stimulate mRNA expression of BSEP, MDR1, or MRP2, suggesting specificity of its actions. MatInspector analysis identified several PPREs throughout the 10kb ABCB4 promoter region. Co-transfection of the hMDR3 promoter with hPPARalpha/hRXRalpha expression plasmids in HepG2 cells increased luciferase activity by 2.6–11.7-fold in the 2kb promoter regions, when treated with FF.

Discussion/Conclusion: Activation of PPARalpha up-regulates MDR3 mRNA and protein expression in human hepatocytes providing a mechanistic basis for fenofibrate use in cholestatic liver disease.

Bile acids stabilize their receptor FXR by blocking ubiquitin-proteasome-mediated degradation

Gregorio Gil[1], William M. Pandak[2], Lin Zhang[1], Lingli Ouyang[1] and Zhumei X. Cronk[1]
Department of Biochemistry and Molecular Biology[1], Microbiology and Medicine[2], Medical College of Virginia at Virginia Commonwealth University, Richmond, VA, USA

Introduction: The farnesoid X receptor (FXR, NR1H4) is a member of the nuclear receptor superfamily that is expressed in liver, small intestine, kidney, adrenals, vascular smooth muscle, and adipose tissue. It is the major bile acid receptor and it is a master transcriptional regulator of cholesterol, bile acid and glucose homeostasis. Whereas its functions are well relatively known, very little is known how its expression is regulated.

Methods and Results: The FXR protein is very labile and its half-life is less than three hours in rat primary hepatocytes. However, bile acids increase its stability 3-fold. In vivo studies showed that FXR is also stabilized by its ligands in the entire animal. Proteasome inhibitors mimic the stability provided by its ligands. Mass spectrometric analysis identified 4 residues that are ubiquitinated. Immunoprecipitation studies show that FXR is indeed multi-ubiquitinated and ubiquination is dramatically diminished upon binding bile acids. Mutation of one of those four residues renders a protein that is not longer stabilized by its ligands. Preliminary studies have identified the E3 ubiquitin-protein ligase that catalizes the transfer of ubiquitin moieties to the FXR protein triggering its degradation by the ubiquitin-proteasome degradation pathway.

Discussion/Conclusion: The studies that will be presented strongly supports the idea that the bile acid receptor FXR is rapidly degraded by the ubiquitin-proteasome degradation pathway and that its ligands prevents the addition of the ubiquitin moieties making the protein more stable. We propose that therapeutic intervention targeted to prevent FXR ubiquitination might be used to control cholesterol, bile acids and glucose metabolism, which could lead to novel drugs for the treatment of metabolic disorders, such as hypercholesterolemia, diabetes and fatty liver disease.

30

Sulfonylurea receptor in myofibroblasts is a target for ursodeoxycholic acid: A new avenue for anti-arrhythmic therapy

Julia Gorelik[1], Francisca Schultz[1], Michele Miragoli[1], Max J. Lab[1]
Viacheslav O. Nikolaev[2], Catherine Williamson[3]
[1]National Heart and Lung Institute, Imperial Centre for Translational and Experimental Medicine, Imperial College London, Hammersmith campus, London, UK
[2]Emmy Noether Group of the DFG, Department of Cardiology and Pneumology, Heart Research Center Göttingen, Georg August University, Göttingen, Germany
[3]Institute of Reproductive and Developmental Biology, IRDB, Imperial College London, Hammersmith Campus, London, UK

Introduction: Intrahepatic cholestasis of pregnancy (ICP) is a disease characterised by raised maternal serum bile acids. It can be complicated by fetal distress and fetal arrhythmia (FA). The principal therapeutic agent used to treat ICP is ursodeoxycholic acid (UDCA). We recently found that myofibroblasts (MFB) which are present in fetal hearts play an important role in the development of FA. We demonstrated that UDCA significantly modulates cardiac excitability in an *in-vitro* model of the fetal heart (FHm). We also demonstrated that Glibenclamide, which binds to the sulfonylurea receptors and therefore blocks sarcolemmal K_{ATP} channels, completely abolished the hyperpolarization effect of UDCA on the MFBs membrane potential. Here we study the new properties of UDCA and the mechanism of its effect on the MFBs present in FHm.

Methods: The FHm consists of a defined combination of primary heterocellular culture of neonatal rat ventricular cardiomyocytes and MFBs. Calcium propagation characterstics were assessed optically. In parallel, MFB membranes were incubated for 2 h at 37˚C in 200 µl of the assay buffer containing (mmol/L) 20 tris, 100 NaCl, 1 EDTA (pH 7.6), [^{3}H]-Glibenclamide and increasing concentrations of unlabeled UDCA for radiolingand binding experiments.

Results: Optical recording of intracellular calcium duration (Ca_i^{2+}) on FHm showed that UDCA does not alter Ca_i^{2+} duration and therefore has no negative influence on cardiomyocytes, in accordance with the previous results where the resting membrane potential was also unaffected. Radioligand binding experiments demonstrated that UDCA can actively displace the [^{3}H]-glibenclamide in MFB membranes acting on sulfonylurea receptors, a protein subunit which contributes to the formation of KATP-channels in cardiac tissue, with an apparent half-maximal displacement at ~0.1 µmol/L.

Discussion/Conclusion: UDCA does not have any negative influence on cardiomyocyte function, however it binds directly to sulfonylurea receptors regulating potassium conductance. These data suggest a new pharmacological approach (UDCA) to a new biological therapeutic target (myofibroblasts) for fetal arrhythmia.

Are reduced levels of cholestenoic acids a cause of motor neuron disease?

W.J. Griffiths[1], Y. Wang[1], S. Theofilopoulos[2], E. Arenas[2], P.T. Clayton[3], M.T. Bassi[4]
[1]College of Medicine, Swansea University, UK, [2]Department of Medical Biochemistry & Biophysics, Karolinska Institutet, Sweden, [3]Institute of Child Health, UCL, London, UK, [4]E. Medica Scientific Institute, Lecco, Italy

Hereditary spastic paresis type 5 (SPG5) is a form of motor neuron disease. It is characterised by mutations in *CYP7B1*. Here we show that patients with SPG5 have greatly elevated plasma levels of 3β-hydroxycholestenoic acid but markedly reduced levels of 3β,7α-dihydroxycholestenoic acid. In vivo studies with zebrafish and in vitro studies with mouse brain primary cultures suggest that the latter acid has a protective effect towards motor neurons while the former acid is toxic.

Introduction: SPG5 is a disease resulting from mutations in a gene (*CYP7B1*) of the acidic pathway of bile acid biosynthesis. SPG5 presents as motor neuron disease, usually in the adult. CYP7B1 is the enzyme that 7α-hydroxylates oxysterols and also 3β-hydroxycholestenoic acid. This raises the question of whether a build up of CYP7B1 substrates or an absence of its products may be responsible for motor neuron disease.

Methods: Initially we profiled the oxysterol and cholestenoic acid content of plasma from SPG5 patients and healthy adults by LC-ESI-MS^n utilising a “charge-tagging” approach. We then investigated the identified compounds as ligands to the LXR, FXR, VDR and NURR1 nuclear receptors. Finally, we studied the in vivo and in vitro effects of compounds of differential abundance in plasma on zebrafish and mouse brain primary cultures.

Results: In SPG5 the plasma levels of 3β-hydroxycholestenoic acid more than double, while those of 3β,7α-dihydroxycholestenoic acid are markedly reduced. 3β-Hydroxycholestenoic acid was found to be toxic to zebrafish and also mouse primary brain cultures. On the contrary, 3β,7α-dihydroxycholestenoic acid increased the expression of Islet-1 protein in zebrafish, a marker protein for motor neurons, and also increased motor neuron survival in primary cultures.

Discussion/Conclusion: Our data indicates that 3β,7α-dihydroxycholestenoic acid, an LXR, ligand, has pro-survival properties towards motor neurons, while 3β-hydroxycholestenoic acid is toxic.

32

Human FXR regulates SHP expression through direct binding to an LRH-1 binding site, independent of an IR-1 and LRH-1

Janette Heegsma, Martijn O. Hoeke, Mark Hoekstra, Han Moshage and Klaas Nico Faber
Department of Gastroenterology and Hepatology, University Medical Center Groningen, Groningen, University of Groningen, The Netherlands

Introduction: FXR/RXRalpha is the master transcriptional regulator of bile salt homeostasis. FXR is activated by bile acids, RXRalpha by the vitamin A-derivative 9-cis retinoic acid (9cRA). Remarkably, 9cRA inhibits binding of FXR/RXRalpha to its response element, an inverted repeat-1 (IR-1). Still, most FXR/RXRalpha target genes are maximally expressed in the presence of both ligands, including the small heterodimer partner (SHP). Here, we revisited the FXR/RXRalpha-mediated regulation of human *SHP*.

Methods: Several *hSHP* promoter constructs were analyzed in FXR/RXRalpha-transfected DLD-1, HEK293 and HepG2 cells exposed to CDCA, GW4046 (synthetic FXR ligand) and/or 9cRA. FXR-DNA interactions were analyzed by *in vitro* pull down assays.

Results: *hSHP* promoter elements lacking the previously identified IR-1 (-291/-279) largely maintained their activation by FXR/CDCA, but were unresponsive to 9cRA. FXR-mediated activation of the *hSHP* promoter was primarily dependent on the -122/-69 region. Pull down assays revealed a direct binding of FXR to the -122/-69 sequence, which was abrogated by site-specific mutations in a binding site for the liver receptor homolog-1 (LRH-1) at -78/-70. These mutations strongly impaired the FXR/CDCA-mediated activation, even in the context of a *hSHP* promoter containing the IR-1. LRH-1 did not increase FXR/RXRalpha-mediated activation of *hSHP* promoter activity.

Discussion/Conclusion: FXR/CDCA-activated expression of *SHP* is primarily mediated through direct binding to an LRH-1 binding site, which is not modulated by LRH-1 and unresponsive to 9cRA. 9cRA-induced expression of *SHP* requires the IR-1. This establishes for the first time a co-stimulatory, but independent, action of FXR and RXRalpha agonists.

Protective effect of biliverdin and biliverdin reductase against bile acid-induced toxicity in liver cells

E. Herraez[1], E. Gonzalez Sanchez[1], M.J. Perez[2], N.S. Nytofte[3], O. Briz[1], M.A. Serrano[1], M.J. Monte[1], F. Jimenez[2], F. Gonzalez-San Martin[2], J.J.G. Marin[1]
[1]Laboratory of Experimental Hepatology and Drug Targeting (HEVEFARM). University of Salamanca, CIBERehd, Salamanca, Spain
[2]University Hospital of Salamanca, Institute of Health Sciences Studies of Castilla and Leon, Salamanca, Spain
[3]Queen Ingrid's Hospital, Nuuk, Greenland

Introduction: The accumulation in hepatocytes of bile acids, such as deoxycholic acid (DCA), induces oxidative stress, which may result in cell injury. Under these circumstances, several antioxidant mechanisms, such as biliverdin reductase alpha (BVRalpha)-mediated bilirrubin (BR)/biliverdin (BV) cycle may play a protective role. Homozygous patients for a recently described mutation in BVRalpha gene (BLVRA) (c.214C>A, p.Ser44X) suffer from episodes of green jaundice during cholestasis. The aim was to investigate whether, in these individuals, hepatocytes are less protected against potential bile acid-induced toxicity.

Methods: The open reading frame of BVRA was cloned in appropriate plasmids to express BVRalpha in mammalian cells and in Xenopus laevis oocytes. The c.214C>A mutation was reproduced using site-directed mutagenesis. BVRalpha expression was determined by quantitative RT-PCR and Western-blot. Mutated BVRalpha (mtBVRalpha) was analyzed by Western Blot and immunofluorescence and its enzymatic activity was determined by HPLC-MS/MS. ROS production and cell viability were measured by flow cytometry using dichlorofluorescein-diacetate and propidium iodide, respectively.

Results: The results indicated that mtBVRalpha was a truncated protein with no ability to transform BV into BR. In HepG2, PLC/PRF/5 and Huh-7 liver cells, with different expression levels of BVRalpha (HepG2 > PLC/PRF/5 > Huh-7) and ROS generation (Huh-7 > PLC/PRF/5 > HepG2), treatment with increasing concentrations of DCA or K2Cr2O7 (used here as a positive control of toxicity) resulted in enhanced ROS production and cell death. Administration of BV inhibited, in a dose-dependent manner, both effects induced by both compounds, without affecting the expression of BVRalpha and heme oxygenase-1. In contrast, BVRalpha expression was decreased by K2Cr2O7 and increased in response to DCA.

Discussion/Conclusion: In cholestasis, BV may play a role as a protective agent against bile acid accumulation, which is dependent on BVRalpha activity. This mechanism of defense is absent in individuals bearing homozygous inactivating mutations in BVRA.

34

Association of FXR polymorphisms with cholelithiasis

Satoko Hirobe-Jahn[1], Simone Harsch[1], Olga Renner[1], André Strohmeyer[1],
Silke Schimmel[1], Eduard F. Stange[2]
[1]Dr. Margarete Fischer-Bosch Institut für Klinische Pharmakologie, Stuttgart und Universität Tübingen, Germany
[2]Robert Bosch Krankenhaus, Stuttgart, Germany

Introduction: Risk factors for the multicausal disease cholelithiasis include a defect in bile acid homeostasis as well as ethnicity. Farnesoid X receptor (FXR) regulates intestinal and hepatic bile acid transporters. There is increasing evidence highlighting the importance of this nuclear receptor as a determinant for cholesterol gallstone development, yet the connection between gallstone pathogenesis and genetics of FXR is not clear.

Methods: The aim of the study was to determine an association between variants of the FXR gene and cholelithiasis. Minor allele frequencies (MAF) from HapMap databank between Caucasian and Asian populations were compared; two variants (rs11110385, rs11110386) with the greatest MAF deviation were selected for genotyping a Stuttgart cohort (gallstone patients n = 73, healthy controls n = 131). The subjects were subgrouped by gender and weight. Genomic DNA was obtained from blood leukocytes. Genotype frequencies were determined by MALDI-TOF MS analysis.

Results: The variants were included in a haplotype block of normal weight persons ($r^2 = 0.957$); homozygous carriers of the major allele featured an increased risk of cholelithiasis compared with persons carrying the homozygous minor allele (rs11110385 and rs11110386 $p = 0.0419$; OR = 4.444). The increase was also observed in the recessive model (rs11110385; $p = 0.0280$; OR = 3.611, rs11110386; $p = 0.032$; OR = 3.444).

Discussion/Conclusion: The SNPs rs11110385 and rs11110386 of the FXR gene are significantly associated with cholelithiasis, notably in a weight-specific manner. The results indicate a potential role of FXR-variants in the pathogenesis of gallstone disease.

Vitamin A deficiency (VAD) aggravates liver damage in obstructive cholestasis and is characterized by an altered bile acid profile and excessive bile duct damage

M. Hoekstra, M.O. Hoeke, J. Heegsma, H. Moshage and K.N. Faber
Department of Gastroenterology and Hepatology, University Medical Center Groningen, Groningen, University of Groningen, The Netherlands

Introduction: Vitamin A is a fat-soluble vitamin important for many biological process, i.e. eye sight, immunocompetence, cell differentiation and bile salt homeostasis. Mammals acquire vitamin A from the food and bile acids are essential for efficient absorption in the intestine. Most vitamin A is stored in hepatic stellate cells from which retinol homeostasis is controlled. The vitamin A-derivative, 9-cis retinoic acid, is the natural ligand for the retinoid X receptor and together with the farnesoid X receptor it controls bile acid synthesis and transport. Cholestatic liver diseases may lead to vitamin A deficiency (VAD) as intestinal uptake is impaired and activated stellate cells lose their vitamin A. Here, we investigated whether VAD contributes to liver damage in rats with obstructive cholestasis.

Methods: Rats were made VAD by omitting vitamin A from the diet. Control and VAD rats underwent bile duct ligation (BDL) and were terminated after 1, 2, 4 and 7 days. Blood samples were collected before surgery and at termination and analyzed for liver damage markers AST, ALT and gGT. Liver tissue was collected for analysis by Q-PCR and histology. Bile was collected and the composition was determined by GC/MS

Results: AST/ALT levels were 2-fold increased at 1 day BDL in VAD rats compared to BDL controls and remained high in the following days, whereas these liver damage markers clearly decreased in BDL control rats. Most pronounced was the exponential increase in gGT in VAD+BDL rats, while it was not elevated in BDL control rats. Expression of cytokeratin19 (cholangiocyte marker) was strongly increased in VAD+BDL rats and liver histology revealed excessive bile duct proliferation. Beta-muricholic acid, a protective bile acid, was strongly reduced in bile of VAD+BDL rats.

Discussion/Conclusion: In conclusion, VAD aggravates liver damage in obstructive cholestasis and is characterized by excessive bile duct damage that may be caused by toxic bile.

36

Dissecting the molecular pathways regulating FXR transactivation and transrepression

Danielle A.A. Hollman, Ellen C.L. Willemsen, Raffaella M. Gadaleta,
Alexandra Milona, Rogier van Gent, Vittoria Massafra, Arjan B. Brenkman,
Saskia W.C. van Mil
Department of Metabolic Diseases, University Medical Centre Utrecht and Netherlands Metabolomics Centre, The Netherlands

Introcution: The bile acid receptor FXR controls bile salt, lipid and glucose homeostasis by classical transactivation. Recently, we showed that FXR also inhibits the inflammatory response, probably via NF-κB transrepression. Different post-translational modifications and interacting proteins are thought to discriminate between transactivation and transrepression. Post-translational modifications have been described in FXR, but how these modifications contribute to transcriptional changes leading to different biological responses, is unknown. We aim to dissect the molecular pathways regulating FXR transactivation and transrepression.

Methods: HEK293T cells transfected with FLAG-FXRa2 were treated with or without GW4064 and TNFα for 24 hours. We performed FLAG-immunoprecipitations and ascertained post-translational modifications by Orbitrap mass-spectrometry.

Results: Phosporylation was detected at Serine-224. The S224A mutant (defective in phosphorylation) showed abrogated transcriptional activity of FXR on SHP, IBABP and BSEP promoters in reporter assays. In contrast, transactivation capacity of FXR-S224D (phospho-mimicking) was similar or higher than wt FXR. Electro-mobility shift assays show absence of binding of FXR-S224A to BSEP, SHP and IBABP oligos, in contrast to FXR wt and FXR-S224D. However, the ability of FXR-S224A to inhibit NF-κB signalling by transrepression is unaffected in reporter assays.

Discussion/Conclusion: Phosphorylation of FXR-S224 is important for transactivation of FXR target genes, but not NF-κB transrepression in vitro. Selective FXR ligands that do not result in S224 phosphorylation can be useful to treat intestinal inflammation without interfering with bile salt, glucose and fat metabolism.

Combination therapy of primary biliary cirrhosis with urso-deoxycholic acid and a dual PPARα/PXR agonist, bezafibrate

Akira Honda[1], Tadashi Ikegami[1], Teruo Miyazaki[1] Makoto Nakamuta[2], Hajime Takikawa[3] and 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: Combination therapy with ursodeoxycholic acid (UDCA) and bezafibrate is being recognized as a beneficial treatment for primary biliary cirrhosis (PBC) that is refractory to UDCA monotherapy. The current study was undertaken to explore the mechanisms of the remission of cholestasis by bezafibrate in PBC patients who failed to respond to UDCA monotherapy.

Methods: Patients with early-stage PBC and an incomplete biochemical response to UDCA (600 mg/day) monotherapy were treated with the same dose of UDCA plus bezafibrate (400 mg/day) for 3 months. Lipid metabolism before and after the additional treatment with bezafibrate was studied by analyzing serum lipid biomarkers and by cell-based enzymatic and gene expression assays.

Results: In addition to the significant improvement of serum biliary enzymes, IgM, cholesterol and triglyceride concentrations, reduction of 7α-hydroxy-4-cholesten-3-one (C4), a marker of bile acid synthesis, and increase of 4β-hydroxycholesterol, a marker of CYP3A4/5 activity, were observed in patients treated with bezafibrate. The reduction of bile acid synthesis caused the increase of serum UDCA proportion in the patients. *In vitro* experiments using cultured HepaRG cells demonstrated that bezafibrate controlled the target genes of peroxisome proliferator-activated receptor α (PPARα), as well as those of the pregnane X receptor (PXR); downregulating CYP7A1, CYP27A1 and sinusoidal Na^+/taurocholate cotransporting polypeptide (NTCP), and upregulating CYP3A4, canalicular multidrug resistance protein 3 (MDR3), MDR1 and multidrug resistance-associated protein 2 (MRP2).

Discussion/Conclusion: While UDCA replaces hydrophobic bile acids and activates canalicular BSEP and MDR3 and basolateral MRP4, bezafibrate inhibits hepatic synthesis and uptake of bile acids, enhances bile acid detoxification, and stimulates canalicular MDR3, MDR1 and MRP2 activities as a dual PPARα/PXR agonist. Furthermore, bezafibrate seems to augment the actions of UDCA by inhibiting bile acid synthesis and increasing the proportion of UDCA. On the other hand, UDCA appears to attenuate the adverse lithogenic effect of bezafibrate. These data lend support to the idea that the combination therapy with UDCA and bezafibrate is an excellent method for the treatment of early-stage PBC patients who exhibit an incomplete biochemical response to UDCA monotherapy.

38

Deoxycholic acid-FXR signalling is crucial for the bile acid metabolic phenotypes in Cyp8b1$^{-/-}$ mice

Xiaoli Hu[1], Ylva Bonde[2,3], Gösta Eggertsen[1], Mats Rudling[2,3]
[1]Division of Clinical Chemistry, Department of Laboratory Medicine,
[2]Metabolism Unit, Center for Endocrinology, Metabolism, and Diabetes, Department of Medicine,
[3]Molecular Nutrition Unit, Department of Biosciences and Nutrition, Karolinska Institute, Karolinska University Hospital Huddinge, Stockholm, Sweden

Introduction: Bile acid (BA) synthesis has a negative feedback regulation via FXR signalling, where the total BA content is important. BAs are FXR ligands, and the impact of specific BAs is debated. The role of secondary BAs on liver BA synthesis and enterohepatic circulation has only partially been unrevealed, partly due to their toxic effects.

Hypothesis/Aim: Absence of the powerful FXR ligand, the secondary BA deoxycholic acid (DCA), is important for the induction of BA synthesis in Cyp8b1$^{-/-}$ (KO) mice.

Methods: Wild type (WT) and KO mice were given ampicillin (AMP) for 3 days. Controls received saline (SAL).

Results: Cyp7a1 was induced 4.4-fold in WT-AMP mice, a level also seen in KO-SAL; whereas no subsequent effect on Cyp7a1 was found in KO-AMP. BA composition revealed detectable DCA only in WT-SAL, while CA was detected in both WT groups. An inverse correlation was obtained between ileal FGF15 and Cyp7a1 mRNA.
AMP-treatment dramatically increased ileal Asbt protein levels in WT-AMP and KO-AMP groups compared to respective controls. Accordingly, reduced faecal BA excretions (46-fold and 28-fold, respectively) were found in the two groups.

Conclusion/Discussion: Our results show that DCA is a much stronger suppressor of BA synthesis than CA in mice. The absence of DCA in Cyp8b1$^{-/-}$ mice may explain the induction of BA synthesis, resulting in a significant expansion of the BA pool enriched in muricholic acid with low affinity to FXR. AMP treatment also enhances the enterohepatic circulation by reducing faecal BA excretion through increasing Asbt expression in the ileum.

Resistance to diet-induced obesity conferred by dietary bile acid supplementation in mice is strain specific

Kristina Hüttinger, Tobias Fromme, Martin Klingenspor
Technische Universität München, Else Kröner-Fresenius Center, Freising-Weihenstephan, Germany

Introduction: Bile acids (BA) not only aid in the absorption of lipophilic nutrients but also function as signaling molecules in a number of peripheral target tissues. These BA effects are mediated by the G-protein coupled BA receptor (GPBAR1) formerly known as TGR5. In brown adipose tissue of C57BL6/J mice BA confers resistance to diet-induced obesity through GPBAR1 mediated induction of thermogenesis in a thyroid hormone dependant manner.

Methods: In an animal experiment, we employed purified low fat (LFD) and a high fat (HFD) diets either with or without cholic acid supplementation. Energy intake and assimilation, body mass development as well as body composition were monitored in two inbred mouse strains, C57BL6/J and 129Sv/Ev, fed either LFD or HFD.

Results: Mice of both strains displayed diet-induced obesity indicated by a larger increase in body mass and fat mass when fed HFD as compared to LFD. Cholic acid supplementation of LFD did not significantly alter this trajectory. Feeding HFD with cholic acid rendered C57BL6/J mice resistant to diet-induced obesity, as expected. Since energy assimilation was similar in all HFD fed C57BL6/J mice, cholic acid must exert its protective effect by increased energy expenditure. In contrast to C57BL6/J, in HFD fed 129Sv/Ev mice dietary cholic acid did not attenuate diet-induced obesity.

Discussion/Conclusion: We identified 129Sv/Ev as a mouse strain resistant to the effects of dietary cholic acid supplementation. In future, this animal model may serve to describe the underlying mechanisms of resistance to diet-induced obesity conferred by bile acids.

40

Increased serum liver X receptor ligand oxysterols in patients with non-alcoholic fatty liver disease

Tadashi Ikegami, Akira Honda, Teruo Miyazaki, Yasushi Matsuzaki, Tokyo Medical University Ibaraki Medical Center, Ibaraki, Japan
Hideyuki Hyogo, Susumu Tazuma, Hiroshima University, Hiroshima, Japan
Katutoshi Tokushige, Etsuko Hashimoto, Tokyo Women's Medical University, Tokyo, Japan
Kazuo Inui, Fujita Health University, Nagoya, Japan

Introduction: This study is a post-hoc analysis of a subset of patients who participated in our multi-institutional case control study that evaluated the effects of pitavastatin, a newly-developed statin, in NAFLD patients with dyslipidemia.

Methods: Serum samples of fifteen patients with biopsy-proven NAFLD with dyslipidemia were investigated. Serum markers of lipid metabolism were quantified by LC-MS/MS. These data were then compared with those of 36 sex- and age-matched healthy controls. In addition, changes in these markers produced by treatment with pitavastatin were evaluated.

Results: Serum plant sterols, reflecting intestinal cholesterol absorption, were significantly lower in NAFLD patients compared to controls, and cholesterol synthesis marker, the ratio of lathosterol to cholesterol, was not significantly different between the two groups. Serum proportions of LXRα ligand oxysterols (ratios to cholesterol) were significantly elevated in NAFLD patients compared to controls. The sum of oxysterols relative to cholesterol and HOMA-IR were significantly correlated. In NAFLD patients, marker of bile acid synthesis (ratio C4 to cholesterol) and marker of bile acid absorption (serum FGF19 concentration) were not significantly different from those in controls. By pitavastatin treatment, the marker representing cholesterol synthesis was significantly suppressed from 3 months after initiation of the treatment, and suppression remained significant during the observation period. Markers representing cholesterol absorption were unchanged at 3 months, but had significantly increased at 12 months. Serum oxysterol levels relative to cholesterol maintained high values and did not change significantly during the 12 months period of treatment.

Discussion/Conclusion: We speculate that serum LXRα ligand oxysterol levels (relative to cholesterol) could be surrogate markers of insulin resistance, and that high oxysterol levels in the circulation may play an important role in the development of hepatic and peripheral insulin resistance followed by NAFLD.

Identification of *S*-acyl glutathione conjugates of bile acids in human bile by means of LC/ESI-MS

Shigeo Ikegawa[1], Naohiro Hori[1], Takashi Iida[2], Alan F. Hofmann[3], 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
[3]Department of Medicine, University of California, San Diego, La Jolla, CA 92093-063, USA

Introduction: Previous work from this laboratory has reported the biotransformation of bile acids (BA) into the thioester-linked glutathione (GSH) conjugates via the intermediary metabolites formed by BA:CoA ligase and shown that such GSH conjugates are excreted into the bile in healthy rats as well as rats dosed with lithocholic acid or ursodeoxycholic acid. To examine whether such novel BA-GSH conjugates are present in human bile, we determined the concentration of the GSH conjugates of the five BA that predominate in human bile.

Methods: Bile was obtained from the three infants (age 4, 10, and 13 months) and the BA-GSH conjugates quantified by means of liquid chromatography (LC)/electrospray ionization (ESI)-linear ion trap mass spectrometry (MS) in negative-ion scan mode, monitoring characteristic transitions of the analytes.

Results: By LC/ESI-MS, only primary BA were present in biliary bile acid amidates, indicating that the dehydroxylation flora had not yet developed. Nonetheless, GSH conjugates of lithocholic acid as well as chenodeoxycholic acid were present in concentrations ranging from 27 to 1120 pmol, several orders of magnitude less than those of natural BA N-acylamidates. GSH conjugates were not present, however, in the ductal bile obtained from 10 adults (nine choledocholithiasis, one bile duct cancer).

Discussion/Conclusion: Our results indicate that BA-GSH conjugates are formed and excreted in human bile, at least in infants, although this novel mode of conjugation is a very minor pathway.

42

Gut microbiota regulates bile acid composition throughout the enterohepatic system

Sama Islam[1], Jenny Felin[1], Sirkku Jäntti[2], Tuulia Hyötyläinen[2], Annika Wahlström[1], Hanns-Ulrich Marschall[1], Bo Angelin[3], Matej Orešič[2], and Fredrik Bäckhed[1]
[1]Sahlgrenska Center for Cardiovascular and Metabolic Research/Wallenberg Laboratory, and Department of Molecular and Clinical Medicine, University of Gothenburg, S-413 45 Gothenburg, Sweden; [2]VTT Technical Research Centre of Finland, PO Box 1000, Espoo, FI-02044 VTT, Finland; [3]Department of Endocrinology, Metabolism & Diabetes C2-94, Karolinska Institute at Karolinska University Hospital Huddinge, S-14186 Stockholm, Sweden

Introduction: The gut microbiota has been implicated in deconjugation and further metabolism of primary bile acids in the distal intestine. Here we comprehensively profile bile acid composition throughout the enterohepatic system of germ-free (GF) and conventionally raised (CONV-R) mice. In addition, we study the role of the microbiota in the expression of genes involved in bile acid homeostasis.

Methods: Bile acids were measured using ultra performance liquid chromatography-mass spectrometry. Quantitative real-time PCR was used to determine expression of genes involved in bile acid homeostasis. Isotope dilution mass spectrometry was used to determine activity of bile acid synthesis enzymes.

Results: In the presence of gut microbiota, mice have a more chemically diverse bile acid pool throughout the enterohepatic system, with the greatest differences observed in the cecum, colon and feces. Furthermore, the total bile acid pool is smaller, with specific reductions in the primary bile acid muricholic acid rather than cholic acid. This difference can be attributed to reduced hepatic expression and activity of cholesterol-7α-hydroxylase (CYP7A1), and is associated with increased ileal fibroblast growth factor-15 (FGF15) expression. Finally, the gut microbiota regulates the expression of several genes involved in bile acid synthesis, reuptake, transport and conjugation in the liver and ileum.

Discussion/Conclusion: We have demonstrated that the gut microbiota has a profound *systemic* effect on bile acid metabolism. We demonstrate that the microbial suppression of biosynthetic genes in the liver is consistent with increased activation of FGF15 in the ileum and this suggests a mechanism of FXR-mediated microbial regulation of bile acid synthesis.

Intestinal bile acid transporter expression in inflammatory bowel disease

Jörg Jahnel[1,2], Peter Fickert[1], Christoph Högenauer[1], and Michael Trauner[1,3]
[1]Laboratory of Experimental and Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Medicine, [2]Department of Pediatrics and Adolescent Medicine, Medical University, Graz; [3]Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna; both Austria

Introduction: The enterohepatic circulation of bile acids (BA) includes the absorption of BA in the terminal ileum and colon, which are sites of inflammation in inflammatory bowel disease (IBD). In this study, we characterized changes in the mRNA expression levels of intestinal BA transporters, BA detoxifying systems, and their regulating nuclear receptors in patients suffering from different phenotypes and activity of IBD.

Methods: Mucosal biopsy specimens were taken from the terminal ileum in Crohn´s disease (CD; n = 21) and from the descending colon in ulcerative colitis (UC; n = 14). Levels of mRNA expression were measured by real-time PCR and compared with healthy controls (n = 9).

Results: Down-regulation of the main ileal BA uptake transporter (ASBT) was observed in active CD (36% vs. controls; $p < 0.05$) and persisted in remission of CD (53% vs. controls; $p < 0.05$), unlike other significant changes (BCRP 57%, SULT2A1 31%; both vs. controls and $p < 0.02$). Pancolitis but not left-sided colitis in UC altered expression of major BA transporters (MRP3 50%, MRP4 58%, MDR1 17%, OSTα 20%, OSTβ 29%; all vs. controls and $p < 0.05$) and nuclear receptors (PXR 80%, VDR 85%; $p < 0.05$). As positive marker served the inflammatory marker INOS.

Discussion/Conclusion: Alterations in intestinal BA transport and metabolism vary in IBD. In contrast with broad changes, possibly inflammation-associated, in UC pancolitis, effects on BA transporter and detoxifying systems seem selective in CD ileitis. Whether medical manipulation of the intestinal BA transport in IBD favorably affects symptoms like diarrhea requires further investigation.

44

Ezetimibe increases transintestinal cholesterol secretion in humans

Lily Jakulj[1], Theo van Dijk[2], Theo Boer[2], Renze Boverhof[2] , Erik G. Stroes[1] and Albert K. Groen[2]
[1]Departments of Pediatrics and Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[2]Department of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands

Introduction: Recently, we have shown that direct secretion of cholesterol from blood to the intestinal lumen is an active pathway for cholesterol removal from the body. In mouse models the capacity of this transintestinal cholesterol secretory pathway (TICE) is twofold higher than the hepatobiliary route and can be stimulated via diet but also pharmacologically. It was the aim of this study to quantify TICE in humans and investigate whether the pathway can be stimulated by pharmacological means.

Methods: Using a stable isotope based methodology cholesterol fluxes were assessed in 15 healthy males before and after treatment with the cholesterol absorption inhibitor ezetimibe. Biliary cholesterol secretion was calculated indirectly from bile acid fluxes.

Results: Compared to mice humans excrete less cholesterol via TICE; under control conditions the rate of TICE in 15 healthy males was about 25% of total fecal sterol output. Confirming earlier reports ezetimibe strongly increased neutral fecal sterol output in humans. Ezetimibe had no effect on biliary cholesterol output or dietary cholesterol input. Consequently, TICE increased about twofold but in addition ezetimibe increased de novo synthesis of cholesterol in enterocytes which effluxed directly from the enterocytes into the intestinal lumen.

Discussion/Conclusion: The TICE pathway is also active in humans albeit lees active compared to mice. Ezetimibe treatment stimulates the pathway suggesting that TICE is an attractive target to increase cholesterol excretion from the human body.

A novel major epimer of varanic acid from the bile of monitor lizards: Taurine-conjugated (24*R*, 25*S*)-3α,7α,12α,24-tetrahydroxy-5β-cholestan-27-oic acid

Narimi Kato[1], Takashi Iida[1], Shoujiro Ogawa[2], Mizuho Une[3], Kuniko Mitamura[4], Shigeo Ikegawa[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, Tokyo University of Science, Noda, Japan
[3]Faculty of Pharmaceutical Sciences, Hiroshima International University, Kure, Japan
[4]Faculty of Pharmaceutical Sciences, Kinki University, Higashi-Osaka, Japan
[5]Department of Medicine, University of California, San Diego, CA, USA

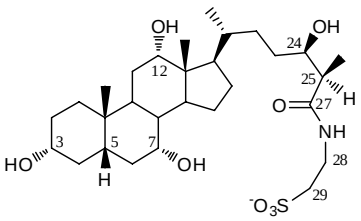
Introduction: C_{24} and C_{27} bile acids, together with C_{27} bile alcohols, are the predominant metabolites of cholesterol in most vertebrates. The species differences in bile acid and bile alcohol metabolism of vertebrates are of particular interest from the view point of their physiological functions, as well as phylogenetic relationships. As part of a program to clarify the chemical diversity of bile salts in vertebrates, and incidentally to verify the proposed pathways in bile acid biosynthesis, we report here the isolation and identification of major, unique bile C_{27} bile acids present in the gallbladder bile of (a) komodo dragon (*Varanus Komodoensis*) (b) gray's monitor (*Varanus olivaceus*), and (c) gila monster (*Heloderma suspectrum*), all of which are classified as monitor lizards.

Methods: The bile of the individual species was applied to a Sep-Pak tC_{18} cartridge and then individual bile acids were isolated by C_{18} reversed-phase (RP) preparative HPLC. Purity of isolated components was checked by RP-HPLC with an evaporative light-scattering detector (ELSD). The structures of the isolated components were elucidated by LC-MS/MS with an ESI probe and 2D-NMR techniques.

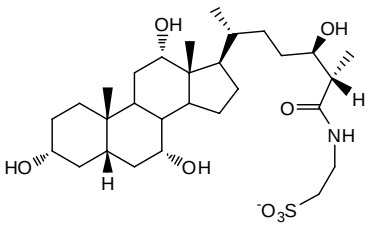
Results: RP-HPLC with ELSD profile of the bile acid fraction obtained from the gallbladder bile of komodo dragon showed two main peaks, which were designated as compounds **A** (8%) and **B** (92%). The biliary bile acid composition of gray's monitor showed at least four peaks, two of which were essentially corresponded to the peaks **A** (61%) and **B** (29%) in the komodo dragon, accompanied by two minor compounds **C** (1%) and **D** (9%). The biliary bile acid composition of gila monster was found to be similar to that of the komodo dragon, consisting of peaks **A** (3%) and **B** (97%).

Discussion/Conclusion: The retention time (RT) and NMR spectra of the peak **D** were completely in accord with that of an authentic sample of tauro-(25*R*)-3α,7α,12α-trihydroxy-5β-cholestan-27-oic acid. From the heteronuclear multiple quantum correlation (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra, the isolated compounds **A** and **B** were presumed to be tauro-3α,7α,12α,24-tetrahydroxy-5β-cholestan-27-oic acid (tauro-VA) stereoisomers. Then, the peaks **A** and **B** were compared with mixtures of four possible stereoisomers of tauro-VAs, which were previously synthesized in our laboratory. Peak A had the same RT of authentic

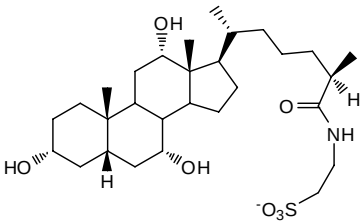
[tauro-(24*R*, 25*R*)-VA] whereas peak B had the same RT as the last-eluted component [tauro-(24*R*, 25*S*)-VA]. Although tauro-(24*R*, 25*R*)-VA has been reported previously, the remaining three stereoisomers have not as yet not identified as natural bile acids. Thus, this is the first report for the evidence of tauro-(24*R*, 25*S*)-VA from naturally occurring sources.



Tauro-(24*R*,25*R*)-VA (**A**)



Tauro-(24*R*,25*S*)-VA (**B**)



Tauro-(25*R*)-3α,7α,12α-trihydroxy-5β-cholestan-27-oic acid (**D**)

TGR5 (Gpbar-1) has anti-apoptotic and proliferative effects in isolated cholangiocytes and is overexpressed in human cholangiocarcinomas

Verena Keitel[1], Maria Reich[1], Roland Reinehr[1], Annika Sommerfeld[1], Kenko Cupisti[2], Wolfram Trudo Knoefel[2], Dieter Häussinger[1]
[1]Clinic for Gastroenterology, Hepatology and Infectious Diseases
[2]Department of General, Visceral, and Pediatric Surgery
Heinrich-Heine-University, Moorenstr. 5, 40225 Düsseldorf, Germany

Introduction: TGR5 is a bile acid receptor expressed in cholangiocytes. Bile acids mediate choleretic, proliferative and anti-apoptotic effects in biliary epithelial cells (1). Aim of the present study was to determine the role of TGR5 for cholangiocyte proliferation and apoptosis. Since elevated bile acid levels have been linked to cholangiocarcinoma (CCA) development the expression of TGR5 was studied in these tumors.

Methods: Cholangiocytes were isolated from TGR5 knockout and wildtype mice. Proliferation was studied using BrDU incorporation and PCNA western blotting. Apoptosis was analyzed by TUNEL. Liver tissue was collected from 15 patients undergoing surgery for CCA. Samples were taken from the macroscopically visible tumor and from the nontumorous margin. TGR5 expression was studied by realtime PCR, western blotting and immunofluorescence staining.

Results: Activation of TGR5 by taurolithocholic acid (TLC) increased cell proliferation as measured by BrDU incorporation and PCNA protein levels exclusively in wildtype-derived cholangiocytes. Stimulation of cholangiocytes with a TGR5 agonist or TLC induced serine phosphorylation of the CD95 receptor, which prevents apoptosis through internalization of the CD95 receptor. The number of apoptotic cells following CD95 ligand treatment was significantly higher in TGR5 knockout-derived cholangiocytes.
Since bile acids have been linked to CCA development TGR5 expression was studied in intrahepatic CCAs. TGR5 mRNA and protein were abundantly expressed in CCAs. Immunofluorescence staining revealed a significantly higher amount of TGR5-positive pixels/cell in the tumor tissue as compared to nontumorous cholangiocytes. CD95 receptor serine phosphorylation was significantly higher in the CCA samples as compared to the control samples.

Discussion/Conclusion: TGR5 mediates bile acid-induced cholangiocytes proliferation and inhibits CD95 ligand-dependent apoptosis through serine phosphorylation of the CD95 receptor. The high expression of TGR5 and the increased levels of CD95 serine phosphorylation in CCA tissue suggest that TGR5 may contribute to the apoptosis resistance of CCAs.

Reference:

1. Xia X, Francis H, Glaser S, Alpini G, LeSage G. Bile acid interactions with cholangiocytes. World J Gastroenterol 2006;12:3553–3563.

47

Ursodeoxycholic acid inhibits Toll-like receptor-2 and -4 driven cytokine release from human colonic epithelium

Orlaith Kelly[1,2], Joseph Ward[1], Caroline Jefferies[3], Frank E Murray[2], Stephen J Keely[1]
[1]Molecular Medicine, Royal College of Surgeons in Ireland/Beaumont Hospital, Dublin, Ireland
[2]Gastroenterology, Beaumont Hospital, Dublin, Ireland
[3]Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, Ireland

Introduction: Toll-like receptors (TLRs) are critical to intestinal immunity. In inflammatory bowel disease, epithelial TLR-2 and TLR-4 expression are increased and their activation integral cytokine release. Ursodeoxycholic acid (UDCA) can inhibit cytokine release. However its role in intestinal epithelial cytokine secretion is unknown. We assessed potential for UDCA in regulating colonic epithelial cytokine release under basal and TLR-stimulated conditions

Methods: T_{84} cells were pre-treated with UDCA (250 μM) before stimulation with TLR- specific ligands, PamCysK (TLR-2), polyinosinic:polycytidylic acid (Poly I: C; TLR-3), and lipopolysaccharide (LPS; TLR-4). IL-8/RANTES levels were measured. Resected human colon in Ussing chambers was pretreated with UDCA, stimulated and cytokine release into basolateral medium measured.

Results: In T_{84} cells, UDCA reduced basal RANTES release to 42 ± 19% of controls ($n = 4$, $p < 0.05$). Basal IL-8 release was unaffected. PamCysK, Poly I: C and LPS induced 2.01 ± 0.24, 3.0 ± 0.09 and 2.4 ± 0.31 fold increases in RANTES and 1.99 ± 0.5, 1.83±0.07 and 2.9± 0.24 fold increases in IL-8. UDCA inhibited PamCysK and LPS-induced RANTES release to 33.2 ± 7% and 39.1 ± 7.3% of controls, respectively ($p < 0.001$, $n = 4$) and LPS-induced IL-8 release to 55.7± 3.9% ($n = 3$, $p < 0.05$). In contrast, UDCA did not alter Poly I: C-induced RANTES or IL-8. LPS stimulation of human colonic mucosa increased IL-8 to 959 ± 22 pg/ml compared to 435 ± 46 pg/ml in controls ($n = 3$, $p < 0.001$). This response was abolished by UDCA (250 μM). A selective IκB kinase inhibitor mimicked effects of UDCA suggesting mediation by inhibiting NFκB activation through a MyD88-dependent pathway.

Discussion/Conclusion: Our findings show UDCA inhibits TLR-2 and TLR-4 induced cytokine release from cultured epithelia and intact human colonic tissue, suggesting that UDCA, or non-metabolisable derivatives thereof, may prevent TLR-driven intestinal inflammation

Ursodeoxycholic acid influences the expression of p27kip1 but not FoxO1 in patients with non-cirrhotic primary biliary cirrhosis (PBC)

A. Kempińska-Podhorodecka, J. Kopycińska, E. Elias, P. Milkiewicz, M. Milkiewicz
Pomeranian University Medical, Medical Biology Laboratory, Szczecin, Poland

Introduction: Enhanced expression of cyclin-dependent kinase inhibitor p27kip1, was reported to suppress proliferation in different cell types. Ursodeoxycholic acid (UDCA) has been suggested to delay progression to liver cirrhosis in primary biliary cirrhosis (PBC) provided it is introduced early in the course of the disease. Recent report suggests that UDCA influences expression of p27kip1 in murine models of colitis. Whether therapy with UDCA may modulate cell cycle via p27kip1 in patients with PBC remains to be elucidated.
The aim of the study was to analyze gene expression of transcription factor FoxO1 and its downstream target, p27kip1, in livers of patients with non-cirrhotic PBC, cirrhotic PBC and controls.

Methods: Liver tissues from 68 patients were included in this study. Total RNA was isolated from tissue obtained during routine percutaneous liver biopsies from patients with non-cirrhotic PBC (n = 27). These included 11 patients receiving UDCA 13–15 mg/kg/d and 16 patients naive to UDCA). Cirrhotic tissue was obtained from explanted livers. All patients were treated with UDCA, n = 22). Histologically normal liver tissues from large margin resections for HCC were used as controls (n=19). Total RNA was reverse transcribed into complementary DNA and quantitative TaqMan PCR analysis was used to evaluate mRNA expression of FoxO1 and p27kip1.

Results: Expression of FoxO1 mRNA showed significant increase only in cirrhotic PBC (10-fold increase; $p < 0.0001$ vs. controls). Expression of p27kip1 revealed significant increase only in cirrhotic PBC (9-fold increase; $p < 0.0001$ vs. controls). Non-cirrhotic patients treated with UDCA demonstrated decreased expression of p27kip1 mRNA when compared to UDCA naive subjects (1.4-fold vs. 2.3-fold increase respectively, $p = 0.03$).

Discussion/Conclusion: Expression of p27kip1 increases with the progression of PBC in a stage-dependent manner and may not be dependent of its upstream activator FoxO1. In non-cirrhotic PBC, UDCA may enhance cell proliferation exerting a positive effect on liver regeneration via p27kip1 dependent mechanism.

49

Ursodeoxycholic acid affects expression of CAR but not PXR mRNA in patients with non-cirrhotic primary biliary cirrhosis (PBC)

M. Klak[1], M. Milkiewicz[1], E. Wunsch[2], E. Elias[3], P. Milkiewicz[2]
[1]Medical Biology Laboratory, Pomeranian Medical University, Szczecin, Poland
[2]Liver Unit, Pomeranian Medical University, Szczecin, Poland
[3]Liver Unit, University of Birmingham, Birmingham, UK

Introduction: Constitutive androstane receptor (CAR) and pregnane X receptor (PXR) are both key regulators of numerous genes involved in detoxification and transport of various endo and xenobiotics. They were also suggested to be engaged in modulation of liver fibrosis. Expression of CAR and PXR has not yet been studied in the livers of patients with non-cirrhotic PBC. The aim of the study was to analyze gene expression of CAR and PXR in biopsies of patients with non-cirrhotic PBC

Methods: Liver tissues from 26 patients were included in this study. Ten patients were treated with UDCA (13–15 mg/kg b.w.) and 16 were not. Total RNA was isolated from tissue obtained during routine percutaneous liver biopsies. Histologically normal liver tissues from large margin resections for HCC were used as controls (n = 19). Total RNA was reverse transcribed into complementary DNA and quantitative real-time PCR analyses with TaqMan probes was used to evaluate mRNA expression of the analyzed nuclear receptors.

Results: Expression of CAR and PXR mRNA was significantly increased in patients with PBC when compared to controls (13-fold increase; $p < 0.0001$ and 18-fold increase, $p < 0.0001$ respectively). Patients treated with UDCA showed decreased expression of CAR but not PXR mRNA when compared to UDCA naive subjects (10-fold vs. 15-fold increase, $p < 0.03$ and 9-fold vs. 10-fold increase, $p = 0.5$, respectively).

Discussion/Conclusion: In non-cirrhotic patients with PBC the expression of analyzed orphan receptor genes showed a significant increase. UDCA treatment may partially improve CAR but not PXR expression.

Short-term feedback regulation of bile salt uptake by bile salts in rat liver

Stefanie Kluge[1], Olga Domanova[2,3,4], Thomas Berlage[2,3,4], Claudia Stross[1], Angelika Helmer[1], Verena Keitel[1], Dieter Häussinger[1] and Ralf Kubitz[1]
[1]Department of Gastroenterology, Hepatology and Infectious Diseases, Medical Faculty, Heinrich-Heine University, Düsseldorf, Germany
[2]Fraunhofer Institute for Applied Information Technology FIT, Schloss Birlinghoven, Sankt Augustin, Germany
[3]RWTH Aachen, Department of Computer Science, Ahornstr. 55, Aachen, Germany
[4]Bonn-Aachen International Center for Information Technology B-IT, Dahlmannstr. 2, Bonn, Germany

Introduction: The sodium taurocholate cotransporting polypeptide (Ntcp) is the major bile salt uptake transporter at the sinusoidal membrane of hepatocytes. *In vivo,* short-term feedback regulation of Ntcp by bile salts has not yet been investigated.

Methods: Subcellular localization of Ntcp and Bsep (bile salt export pump) was analyzed in immunofluorescence images from tissue sections by a new automated image analysis method. Net bile salt uptake was investigated by a pulse chase technique. Rat livers were continuously perfused with 100 µmol/l of TC. 25 µmol/l of TCDC, taurodeoxycholate (TDC), tauroursodeoxycholate (TUDC) or TC were added on top for 30 min, washed out, followed by a pulse of ^{3}[H]-TC.

Results: TCDC, but not TC, caused significant internalization of Ntcp in perfused rat livers, as shown by an increase of intracellular Ntcp-immunoreactivity in immunofluorescence images, while Bsep distribution remained unchanged. These results correlate with functional studies. TCDC, but not TDC, TUDC or TC, significantly increased the amount of ^{3}[H]-TC in the effluent, indicating a reduced sinusoidal net TC-uptake. This effect was sensitive to Chelerythrine (protein kinase C inhibitor) and Cypermethrin (protein phosphatase 2B inhibitor), whereas Phospho-inositide 3-kinase (PI3K) inhibitors had an additive effect.

Discussion/Conclusion: TCDC reduces sinusoidal bile salt transport by protein kinase C- and protein phosphatase 2B-mediated retrieval of Ntcp from the plasma membrane. Preliminary data point towards an involvement of further basolateral bile salt transporters. During an increased portal bile salt load differential short-term feedback regulation of sinusoidal transporters may adjust bile salt uptake and protect periportal hepatocytes from harmful bile salt concentrations.

51

Wild-type bile and “Byler bowel”: Diarrhoea and hepatic steatosis after liver transplantation in *ATP8B1* disease

AS Knisely, King’s College Hospital, London; LN Bull, University of California San Francisco, San Francisco; SA Kocoshis, Cincinnati Children’s Hospital Medical Center, Cincinnati

Introduction: *ATP8B1* is expressed in hepatocytes, cholangiocytes, and enterocytes. Its product, ATP8B1, modifies membrane-hemileaflet lipid composition at polarised-cell apices. Severe *ATP8B1* disease manifests in infancy as intrahepatic cholestasis and growth failure with malabsorption. Post-hepatic bile is markedly cholanopenic (deficient in primary bile acids). Liver transplantation (LTX) cures cholestasis but often leads to intractable, severe, even life-threatening diarrhoea. The allograft liver generally develops macrovesicular steatosis shortly after implantation. Diversion of wild-type bile flow, like bile-acid chelation, can relieve diarrhoea and reduce steatosis. Mechanisms of these phenomena are undefined. In addition, whilst bile cholanopenia in *ATP8B1* disease has been ascribed to bile salt export pump (BSEP) down-regulation or malfunction, severe BSEP deficiency (“giant-cell hepatitis”) differs phenotypically from severe ATP8B1 deficiency (small hepatocytes, tidily arrayed; BSEP normally expressed). Both *in vivo* hepatic bile-salt secretion and intestinal bile-salt absorption appear unaltered in Atp8b1-deficient mice. To assess bile-salt absorption we measured faecal bile-acid concentrations in an adolescent with persistent diarrhoea and hepatic steatosis after LTX for *ATP8B1* disease.

Methods: A 72-hour stool sample was evaluated for bile-acid concentrations and osmotic gap.

Results: Bile-acid concentrations were extremely low at 0.02 g/d (expected 0.25–0.45); osmotic gap was 74 mosm (< 0, secretory diarrhoea).

Discussion/Conclusion: Mouse/human liver/gut deficient in Atp8b1/ATP8B1 may handle bile salts differently, with, in human gut, not malabsorption but overabsorption. (Before LTX, similar overabsorption by cholangiocytes may contribute to cholanopenia in post-hepatic bile.) Biliary diversion at LTX may be indicated in *ATP8B1* disease as prophylaxis against diarrhoea. Post-LTX administration of fractionated allograft bile via jejunostomy will identify diarrhoeogenic components, permit titration of individual-patient susceptibility and patient-specific therapy, and give clues to mechanisms of both diarrhoeogenesis and allograft steatotic transformation.

*nor*UDCA protects CBDL mice from bile acid-induced collecting duct tubular injury

Elisabeth Krones[1], Marion J. Pollheimer[1], Franziska Durchschein[1], Michael Trauner[2], Peter Fickert[1]
[1]Research Unit for Experimental and Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University Graz, Austria
[2]Hans Popper Laboratory of Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Austria

Introduction: Renal failure commonly occurs in patients with advanced cholestatic and end-stage liver diseases and represents a high-risk situation with dismal prognosis. Tubular epithelial cell injury and cast formation at the level of collecting ducts followed by chronic tubulointerstitial injury and renal failure have recently been demonstrated in long-term common bile duct ligated (CBDL) mice, a mouse model of combined end-stage liver and kidney disease (Fickert P, J Hep 2011. 54 (Suppl 1): p. 244). In addition $FXR^{-/-}$ mice with a more hydrophilic bile acid pool were completely protected from kidney injury and fibrosis in response to CBDL suggesting a role for bile acids in mediating kidney injury. Accordingly we hypothesized, that hydrophilic *nor*UDCA, a side chain derivative of UDCA undergoing cholehepatic shunting and significant renal excretion, may protect against bile acid-induced tubular injury. Aim: To determine whether feeding of hydrophilic *nor*UDCA protects CBDL mice from bile acid-induced collecting duct tubular epithelial lesions. Moreover, we aimed to compare the *in vitro* effects of different bile acids in regard to cytotoxicity utilizing MDCK cells as well characterized collecting tubular duct epithelial cell line.

Methods: Kidneys of 7d *nor*UDCA-fed (0.5%) 3d CBDL mice and 7d chow-fed 3d CBDL mice were compared in regard to renal pathology using H&E and PAS stained kidney sections. Additionally, different bile acids (TCA, CDCA, GCDCA) were tested at increasing concentrations (100 µM, 500 µM, 1000 µM) on MDCK cells *in vitro*. Cell viability was assessed using WST-1 assay and morphometric analysis.

Results: Chow-fed 3d CBDL mice showed PAS-positive deposits in lumina of collecting ducts with severe epithelial injury. In contrast, *nor*UDCA prefeeding in CBDL mice prevented this specific type of tubular epithelial injury. Furthermore, CDCA, TCA, and GCDA induced collecting duct epithelial cell death *in vitro* in a dose- and time dependent manner.

Discussion/Conclusion: *nor*UDCA inhibits collecting duct tubular epithelial lesions in CBDL mice. Potentially toxic bile acids induce MDCK cell death in a dose dependent manner. Therefore we suggest that collecting duct tubular epithelial lesions in CBDL mice are, at least in part, bile acid-mediated and that urinary enrichment of hydrophilic bile acids (e.g. *nor*UDCA) may be protective.

53

Bile salt hydrophobicity causes cirrhosis and cholestasis in new mouse models for PFIC disease

Cindy Kunne[1], Marijke de Graaff[1], Suzanne Duijst[1], D. Rudi de Waart[1],
Ronald P.J. Oude Elferink[1], Coen C. Paulusma[1]
[1]Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, The Netherlands

Introduction: Progressive familial intrahepatic cholestasis types 1–3 are severe cholestatic liver diseases caused by deficiency of respectively ATB8B1, ABCB11 or ABCB4. Studies in corresponding mouse models show less severe phenotypes compared to human patients which, we hypothesize, is caused by a less toxic bile salt pool. Unlike humans, mice can efficicently (re)hydroxylate toxic bile salts, via a cytochrome p450 enzym-dependent pathway. Here, ATP8B1 and ABCB4 deficient mice were backcrossed with mice lacking hepatic cytochrome p450 enzyme activity, *Hrn* mice. These mice have a much more hydrophobic bile salt pool.

Methods: *Abcb4*$^{-/-}$*/Hrn* mice were fed 0.03% cholic acid (CA) or control diet for 9 weeks from weaning and *Atp8b1*$^{G308V/G308V}$*/Hrn* were fed CA (0.03%) 4 weeks. Bile salt metabolism and liver pathology were studied.

Results: CA-fed *Abcb4*$^{-/-}$*/Hrn* mice displayed liver damage and severe hepatic fibrosis/cirrhosis determined by picrosirius red staining and hydroxy-proline (OH-P) levels (2.35 ± 0.63 nmol/mg liver compared to 1.38 ± 0.6 nmol/mg liver in *Abcb4*$^{-/-}$ and 0.27 ± 0.02 nmol/mg liver in WT mice). Furthermore, CK19 and Ki67 stainings revealed strong bile duct and hepatocyte proliferation. *Atp8b1*$^{G308V/G308V}$*/Hrn* and *Atp8b1*$^{G308V/G308V}$ mice were cholestatic, evidenced by increased serum bilirubin and impaired bile flow. In addition, *Atp8b1*$^{G308V/G308V}$*/Hrn* mice showed severe weight loss upon CA feeding compared to WT and *Atp8b1*$^{G308V/G308V}$ mice. Stainings for fibrosis and proliferation, however did not show drastic changes.

Discussion/Conclusion: In conclusion, the pathology in these combined mouse models with more hydrophobic bile salt composition better resembles that in PFIC patients, indicating that the hydrophobicity of the bile salt pool is an important determinant in PFIC disease.

Bile acids regulate autophagy in colonic epithelial cells

Natalia Lajczak, Magdalena S. Mroz and Stephen J. Keely
Royal College of Surgeons in Ireland, Molecular Medicine Department, RCSI Education and Research Centre, Beaumont Hospital, Dublin 9, Ireland

Introduction: Colonic epithelial cells function as a physical barrier to luminal toxins and antigens and regulate the transport of fluid, nutrients and electrolytes to and from the gut lumen. Autophagy is an evolutionarily-conserved mechanism by which self-digestion of cellular proteins and organelles during periods of cellular stress can occur. The importance of autophagy in colonic epithelial cells in development of intestinal disorders, such as inflammatory bowel disease (IBD) and cancer, is becoming increasingly apparent. Bile acids are classically known for their roles in facilitating digestion and absorption of fats. The aim of this study was to investigate the effects of the common colonic bile acids, deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA), on autophagy in colonic epithelial cells.

Methods: T_{84} colonic adenocarcinoma cells were grown on 30 mm Millicell-HA inserts until they formed a polarized monolayer, mimicking the phenotype of native epithelial cells. Cells were then treated bilaterally with DCA (200 μM) or UDCA (500 μM) for 24h. Expression levels of LC3 protein, a reliable indicator of ongoing autophagy, were then investigated by western blotting.

Results: In cells treated with DCA for 24 h, expression levels of LC3 protein were increased $444 \pm 145\%$ ($n = 4$; $p < 0.05$) compared to untreated control cells. In contrast, UDCA exerted a relatively weak effect on autophagy that was not significantly different from untreated controls ($203 \pm 62\%$; $n = 4$).

Discussion/Conclusion: Our studies suggest that the composition of the colonic bile acid pool is likely to be important in regulating the extent of epithelial autophagy in the colon. Given the important roles that autophagy plays in regulating inflammatory responses and cell survival, these data are important for developing our understanding of the role that bile acids play in the pathogenesis such diseases.

55

Evolution of substrate specificity for the bile salt transporter ABST (SLC10A2)

Daniel Lionarons, James L. Boyer and Shi-Ying Cai
Yale University School of Medicine, New Haven CT, USA

Introduction: The apical Na^+-dependent bile salt transporter (ASBT/SLC10A2) is essential for maintaining the enterohepatic circulation of bile salts. It is not known when Slc10a2 evolved as a bile salt transporter, or how it adapted to substantial changes in bile salt structure during evolution. To address these questions we characterized ASBT orthologs from two primitive vertebrates, the sea lamprey (*Petromyzon marinus*), which utilizes early 5α-bile alcohols; and the marine skate (*Raja erinacea*) which utilizes structurally different 5β-bile alcohols; and compared substrate specificity with ASBT from humans which utilize modern 5β-bile acids.

Methods: Bile acid uptake and its sodium dependency was assessed with ^{3}H-taurocholic acid (^{3}H-TCA), in everted gut sacs from skate and lamprey. RACE-PCR was used to clone ASBT orthologues. Sequence alignment was performed with the ClustalW2 algorithm. A FXR-luciferase reporter assay was utilized to assess the ability of conjugated bile salts to be transported into cells transfected with ASBT/Asbt's.

Results: Everted gut sacs of skate but not the more primitive lamprey transported ^{3}H-TCA (a modern 5β-bile acid), and was sodium dependent. Molecular cloning identified ASBT orthologs from both species. Phylogenetic analysis placed lamprey Asbt and skate Asbt as the most primitive of known ASBT/SLC10A2 orthologs. Cell-based assays using recombinant ASBT/Asbt's indicate that lamprey Asbt has high affinity for 5α-bile alcohols, low affinity for 5β-bile alcohols, and lacks affinity for TCA; whereas skate Asbt showed high affinity for 5α- and 5β-bile alcohols, but low affinity for TCA. In contrast, human ASBT demonstrated high affinity for all three bile salt types.

Discussion/Conclusion: These findings suggest that human ASBT evolved from the earliest vertebrates by gaining affinity for modern bile salts while retaining affinity for older bile salts. Also, our results indicate that the bile salt enterohepatic circulation is conserved throughout vertebrate evolution.

Role of bile acids as cocarcinogenic agents in the development of cholangiocarcinoma

RIR Macias, E Lozano, MJ Monte, L Sanchez-Vicente, E Herraez, O Briz, JJG Marin
Laboratory of Experimental Hepatology and Drug Targeting (HEVEFARM), National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd), IBSAL, University of Salamanca, Spain

Introduction: Owing to its late diagnosis, the natural history of cholangiocarcinoma is usually poorly known. Although bile acid accumulation in the tumour area presumably occurs before overt cholestasis is detected, the role of this event in cholangiocarcinogenesis is unknown. The aim of this study was to elucidate whether bile acids are involved, as carcinogenic or cocarcinogenic agents, in the development of cholangiocarcinoma.

Methods and Results: Cholangiocarcinoma was induced in rats by administration of thioacetamide (TAA). Some animals underwent bile duct ligation (BDL) alone or together with TAA treatment. Liver samples were obtained 4 and 8 weeks after starting the treatment. Although TAA induced hypercholanaemia, this was more marked in BDL groups. Histological analysis and expression of specific markers CK-7, claudin-4 or GSTpi by real time RT-QPCR, western blot and immuno-fluorescence revealed higher bile duct proliferation in BDL > BDL+TAA >> TAA. Expression of epidermal growth factor receptor (ErbB2/Neu), involved in the pathogenesis of cholangiocarcinoma, was found in both groups of BDL, and less in TAA alone. These results suggest a promotor effect of bile acids rather than a carcinogenic activity. The SOS gene RecA from E. coli was used to investigate genotoxic effect. Luciferase activity in bacteria transformed with RecApromoter-Luc2 chimera was measured after incubation for 2.5 h with cisplatin (used as positive control), bile acids: deoxycholic acid, glycodeoxycholic acid, glycochenodeoxycholic acid (50–1000 µM) or litocholic acid (50–300 µM), TAA or rat hepatocyte lysates obtained after incubation with TAA for 24 h. Cisplatin and hepatocyte lysates containing TAA metabolites, but not pure TAA or bile acids were able to stimulate RecApr activity.

Conclusion: In cholangiocarcinogenesis, the accumulation of bile acids may play a promotor role due to the stimulation of ductular proliferation. This cocarcinogenic activity of bile acids is presumably also present in clinical situations in which partial cholestasis usually accompanies cholangiocarcinogenesis.

57

Development of an analytical method for focused metabolomics of bile acid conjugates

Masamitsu Maekawa[1], Takehiko Inoue[2], Kousaku Ohno[2], Hiroshi Nittono[3], Takashi Iida[4], Alan F. Hofmann[5], Junichi Goto[1], Miki Shimada[1], and Nariyasu Mano[1]
[1]Department of Pharmaceutical Sciences, Tohoku University Hospital, Sendai, Japan
[2]Faculty of Medicine, Tottori University, Yonago, Japan
[3]Junshin Clinic Bile Acid Institute, Tokyo, Japan
[4]College of Humanities and Sciences, Nihon University, Tokyo, Japan
[5]Department of Medicine, University of California, San Diego, CA, USA

Introduction: The total amount of bile acid in the body is strictly regulated by the nuclear receptor-mediated signaling network, and however, unusual bile acid conjugates appear in the urine and/or serum of the patient of cholestatic disorders and cholesterol-metabolizing enzyme-deficiency disorders. For finding disease-specific cholesterol metabolites in an efficient way, we developed a comprehensive method for analysis of bile acid conjugates as main cholesterol metabolites by LC/ESI-MS/MS using precursor ion scan and neutral loss scan.

Methods: LC/ESI-MS/MS was performed using an API 5000 mass spectrometer equipped with a Nanospace SI-2 LC system. LC separation was achieved on YMC-Pack Pro C18 (5 μm, 2.0 mm i.d. × 250 mm) by linear gradient elution using a mixture of 20 mM ammonium acetate (pH 7.0) and methanol as mobile phases. Heatmaps were drown by mass++ software and multivariate anlaysis were operated by SIMCA-P+.

Results: Glycine conjugates, taurine conjugates, and sulfates were readily found from the complex mixture of bile acid conjugates by precursor ion scan of *m/z* 74, *m/z* 124, and *m/z* 97, respectively. Neutral loss scan of 176 Da and 203 Da were effective in finding glucuronides and *N*-acetylglucosaminides, respectively. We analyzed bile acid conjugates in urine from the patients with Niemann-pick disease type C and 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase/isomerase deficiency. We found an unusually large variety of sulfated bile acids on both heatmaps focused by precursor ion scan of *m/z* 97. In addition, characteristic sulfated compounds were present in each heatmap.

Discussion/Conclusion: We demonstrated the efficiency of focused metabolomics of urinary bile acid conjugates in the patients with abnormality in the metabolism of cholesterol using LC/ESI-MS/MS. We want to apply the present method for various diseases to investigate the relationships between metabolome pattern and diseases.

An intact biliary glycocalyx adds to the barrier function of the apical membrane that protects cholangiocytes against toxic bile salts

Maillette de Buy Wenniger[1], Lucas; Hohenester, Simon[2]; van Vliet, Sandra J.[3]; Oude Elferink, Ronald[1]; Beuers, Ulrich[1]
[1]Academic Medical Center of the University of Amsterdam, Department of Gastroenterology and Hepatology, Tytgat Institute of Liver and Intestinal Research, Amsterdam, The Netherlands
[2]Aachen University, Department of Gastroenterology and Hepatology, Aachen, Germany
[3]VU University Medical Center, Department of Molecular Cell Biology and Immunology, Amsterdam, The Netherlands

Introduction: The biliary epithelium withstands millimolar concentrations of bile salt monomers dissolved in the bile it is exposed to. We recently reported that the 'biliary HCO_3^- umbrella' is crucial to prevent BEC damage inflicted by protonated bile acids. We hypothesized that an apical layer of glycosylated mucins and other proteins analogous to the glycocalyx on the luminal side of the intestinal epithelium helps BEC to stabilize this HCO_3^--umbrella on their apical surface. Here we studied the molecular structure of the cholangiocyte glycocalyx and the effect of glycocalyx disruption on cholangiocyte resistance to protonated, hydrophobic bile acids.

Methods: A human, non-malignant BEC line was evaluated by FACS for the expression of the mucin MUC1 and the surface glycan profile as investigated by a lectin panel. BEC were exposed to apical neuraminidase (1 U/ml) to test the effect of desialylation on BEC susceptibility to toxic bile salts. Chenodeoxycholate (CDC,pKa > 4) and its glycine- and taurine conjugates (GCDC,pKa > 4 and TCDC,pKa < 2) were administered at 0.25–2mM for 18h at pH7.1. WST-1 metabolic assays were performed to assess cell viability.

Results: Human BEC expressed significant levels of membrane-bound MUC1. O-glycans exposed on mucins and other membrane proteins were dominated by T-antigen (core1 glycan), but not Tn antigen. The main N-glycans were sialylated biantennary structures. The H-antigen (α1–2-fucose) was highly expressed, while the Lewis-X epitope was absent. Apical neuraminidase treatment induced marked desialylation without affecting viability. Pruning of the glycocalyx by neuraminidase treatment exacerbated GCDC toxicity and decreased metabolic activity by $45.2 \pm 20.9\%$ as compared to $11.2 \pm 18.4\%$ for cells not pretreated with neuraminidase (1mM GCDC, $p < 0.01$, $n = 6$). CDC but not TCDC toxicity was also significantly increased by neuraminidase pretreatment.

Conclusion: A biliary glycocalyx consisting of glycosylated mucins and other glycan-bearing proteins may stabilize the biliary HCO_3^- umbrella and thereby protect human cholangiocytes against pH-dependent toxicity of bile salt monomers.

59

High levels of ursodeoxycholic acid act as FXR antagonist and deplete liver cholesterol due to increased bile acid synthesis in morbidly obese patients

Marschall HU[1], Mueller M[2], Claudel T[3], Wagner M[2], Fickert P[2], Zollner G[2], Silbert D[2], Lackner K[4], Thorell A[4], Trauner M[2,3]
[1]Institute of Medicine, Department of Internal Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [2]Department of Medicine and [4]Institute of Pathology at Medical University of Graz, Graz, Austria, [3]Department of Medicine, Medical University of Vienna, Vienna, Austria, [5]Karolinska Institutet, Department of Surgery at Ersta Hospital, Stockholm, Sweden

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. We aimed to explore potential mechanism of UDCA action in patients with morbid obesity awaiting Roux-en-Y gastric bypass surgery.

Methods: Forty morbidly obese patients were randomized to UDCA (20 mg/kg/day three weeks before surgery) or no treatment (controls). Serum liver function tests, lipids, bile acids and markers of insulin resistance/diabetes (OGTT, HOMA) were obtained before and after treatment. During surgery, biopsies were taken from the liver for histology and gene as well as protein expression studies, and from omental and subcutaneous adipose tissues for gene expression studies and lipidomics.

Results: Three patients dropped out; UDCA 1 (diarrhea), controls 2 (pregnancy, bleeding). Completers of both groups were well matched by gender (female, 68.4 vs. 77.7%), age (42.8 $\pm$ 12.3 vs. 38.5 $\pm$ 10.1 years), BMI (41.9 $\pm$ 4.6 vs. 40.6 $\pm$ 3.9 kg/m^2), HOMA (5.1 $\pm$ 2.5 vs. 6.6 $\pm$ 3.9) and OGTT (IGT or T2DM, 37% vs. 50%). NAS scores were: no, 11 vs. 13; borderline, 4 vs. 4; definite, 4 vs. 1. UDCA despite significantly ($p < 0.05$) expanding the BA pool 10.6$\pm$7.6 fold (≤ 55.3 µmol/L; UDCA > 90%) increased bile acid synthesis as measured by serum C4 (7α-hydroxy-cholest-4-ene-3-one), CYP7A1 gene expression, and serum levels of primary bile acids CDCA and CA. Circulating FGF19 decreased by 18% ($p = 0.05$). Significant increases in gene expression levels of key regulators of lipid turnover (SREBP2, SCD, HMGCR) were reflected by significantly decreased serum LDL-cholesterol and increased triglycerides. SCD gene expression was also increased in omental but not subcutaneous adipose tissue of UDCA treated patients that in addition, had decreased apoptosis marker BAX expression in both adipose tissues. UDCA significantly decreased ALT, AST and gGT but did not affect HOMA, glucose tolerance, adiponectin and lectin.

Discussion/Conclusion: Changes in serum lipid and gene expression profiles in UDCA treated, morbidly obese patients indicate hepatic cholesterol depletion as a result of increased bile acid formation due to FXR-antagonistic effects of very high UDCA levels in noncholestatic livers.

The farnesoid X receptor (FXR) agonist obeticholic acid (OCA) increases plasma FGF-19 concentrations and decreases bile acid synthesis in primary biliary cirrhosis (PBC)

HU Marschall[1], V Luketic[2], A Lövgren-Sandblom[3], L Benthin[3], K Kowdley[4], G Hirschfield[5], A Mason[6], K Lindor[7], S Gordon[8], C Vincent[9], Roger Chapman[10], M Mayo[11], Andrew Burroughs[12], A Parés[13], D Jones[14], C Schramm[15], L Eliot[16], AF Hofmann[17], D Shapiro[16]

[1]Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Sweden; [2]Virginia Commonwealth University, Richmond, VA, USA; [3]Karolinska University Hospital Huddinge, Stockholm, Sweden; [4]Virginia Mason Medical Center, Seattle, WA, USA; [5]University of Toronto Western Hospital, Toronto, ON, Canada; [6]University of Alberta, Edmonton,USAAB, Canada; [7]Mayo Clinic, Rochester, MN, USA; [8]Henry Ford Health System, Detroit, MI, USA; [9]Centre Hospitalier de l'Université de Montréal, Montréal, QC, Canada; [10]John Radcliffe Hospital Headington, Oxford, UK; [11]UT South Western Medical Center, Dallas, TX, USA; [12]The Royal Free Sheila Sherlock Liver Centre, London, UK; [13]Digestive Diseases Institute, Barcelona, Spain; [14]Newcastle University, Newcastle, UK; [15]University Medical Center, Hamburg, Germany;[16]Intercept Pharmaceuticals, Inc, San Diego, CA, USA; [17]University of California San Diego, San Diego, CA, USA

Introduction: Obeticholic acid (OCA, 6-ethyl-chenodeoxycholic acid, 6-eCDCA) is a potent FXR agonist, derived from CDCA. FXR agonism controls bile acid (BA) synthesis from cholesterol via fibroblast growth factor 19 (FGF-19). A potent FXR agonist would therefore be expected to increase FGF-19 and decrease BA synthesis, which can be assessed by assaying the BA precursor C4 (7α-hydroxy-cholest-4-ene-3-one), as well as inferred from changes in endogenous BA levels.

Methods: Blood samples were taken on Day 0 and EOS to determine FGF-19 (ELISA), bile acid concentrations including OCA (HPLC-MSMS), and C4 levels (HPLC).

Results: OCA increased FGF-19 concentrations in a dose-related manner and decreased C4 concentrations to a similar extent for 10 and 50mg OCA doses. No significant changes were observed for total BA concentrations in either study. Lithocholic acid (LCA), a toxic BA, was not increased by OCA therapy. Significant decreases in endogenous BAs chenodeoxycholic acid, cholic acid, and deoxycholic acid, were observed in Study 202 ($p< 0.05$). In the smaller monotherapy study (Study 201) only decreases in deoxycholic acid were observed ($p< 0.05$).
In both studies, plasma OCA concentrations increased with dose. In the combination study (202), UDCA comprised the majority of total BA concentration (72%) but total OCA concentrations (including conjugates) < 5%. In the monotherapy study (201), total OCA concentrations comprised 7% and 20% of total BA concentrations for the 10 mg and 50 mg OCA doses, respectively.

Median (range)	Study 202 (UDCA combination)			Study 201 (Monotherapy)		
Change from Baseline to EOS	Placebo (N = 35)	OCA 10 mg (N = 29)	OCA 50 mg (N = 33)	Placebo (N = 15)	OCA 10 mg (N = 12)	OCA 50 mg (N = 9)
FGF-19 (pg/mL)	16 (-131 – +356)	115*** (-74 – +714)	213*** (-292 – +57005	11 (-43 – +220)	106 (-128 – +3788)	177* (9.3 – +38245)
C4 (ng/mL)	0.1 (-53 – +78)	-9.5 (-36 – +62)	-8.1* (-35 – +5.2)	0.2 (-14 – +35)	-6.8* (-48 – +6.1)	-6.5* (-30 – +4.3)
Total Bile Acids (µmol/L)	-1.3 (-57 – +69)	-5.9 (-66 – +107)	-4.2 (-301 – +473)	0.29 (-24 – +24)	-3.7 (-23 – +10)	6.2 (-7.0 – +91)
Total Endogenous Bile Acids (µmol/L)	0.0 (-26 – +27)	-2.3** (-25 – +20)	-3.7** (-145 – +157	0.29 (-24 – +25)	-4.2 (-24 – +9.9)	3.3 (-7.0 – +67)

Discussion/Conclusion: In patients with PBC, OCA shows potent FXR agonist properties, which likely contributes to the substantial improvement in liver enzymes seen in these trials. At the doses used, OCA comprises a relatively small fraction of the circulating bile acids.

Intrahepatic cholestasis of pregnancy is not associated with intrauterine fetal death but with gestational diabetes and preeclampsia

Hanns-Ulrich Marschall[1], Elisabeth Wikström Shemer[2], Jonas F. Ludvigsson[3,4], Olof Stephansson[3,5].
[1]Sahlgrenska Academy and University Hospital, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden; [2]Department of Obstetrics and Gynecology and Department of Clinical Sciences, Karolinska Institutet, Danderyd Hospital, Stockholm, Sweden; [3]Clinical Epidemiology Unit, Department of Medicine, Solna, Karolinska University Hospital and Institutet, Stockholm, Sweden. [4]Department of Pediatrics, Örebro University Hospital, Örebro, Sweden; [5]Department of Women's and Children's Health, Karolinska University Hospital and Institutet, Stockholm, Sweden

Introduction: Intrahepatic cholestasis of pregnancy (ICP) is characterized by severe pruritus in late pregnancy and elevated serum bile acids. Observational studies prior to 2000 indicated impaired fetal outcome including increased risk of intrauterine fetal death (IUFD). Since the introduction of the Tenth International Classification of Diseases (ICD-10) in 1997 diagnosis, management and outcome of ICP may have extensively changed. We aimed to determine whether (1) increased ICP awareness and treatment guidelines resulted in accurate diagnosis and improved management and (2) ICP is connected to other conditions before or diseases of pregnancy.

Methods: We studied 1,226,942 singleton deliveries in the Swedish Medical Birth Register between 1997 and 2009. Diagnosis of ICP (ICD-10: O26.6) was identified at discharge from the delivery hospital (n = 5,508).

Results: The diagnosis ICP was assigned to 0.32–0.58% of all deliveries with a significantly increasing trend until 2005 ($p < 0.0001$), when the incidence stabilized. Compared to non-ICP, women with ICP had increased rates of moderate preterm birth (32–36 weeks of gestation) (12.9% vs. 4.3%, $p < 0.0001$), and Cesarean section (19.6% vs. 15.6%, $p < 0.0001$). The rate of IUFD did not differ between women with and without ICP during pregnancy (0.31% and 0.34%, respectively, $p = 0.72$). In ICP, preeclampsia (1.1% vs. 0.4%, $p < 0.0001$) and gestational diabetes (1.2% vs. 0.4%, $p < 0.0001$) occurred more often as compared to non-ICP pregnancies. In general women with ICP were of higher maternal age (median 30.0 vs. 29.0 years, $p < 0.05$) and less likely to smoke cigarettes or overweight/obese (BMI ≥ 25.0 kg/m^2) in early pregnancy ($p < 0.0001$).

Discussion/Conclusion: Over time, a larger proportion of Swedish pregnant women have received a diagnosis of ICP, probably due to an increased awareness of this diagnosis. Our data confirm increased risk of preterm delivery but not for IUFD in ICP. The high rates of preeclampsia and gestational diabetes are new findings.

62

Gallstone disease in Swedish twins is linked to the Gilbert variant of *UGT1A1*

Marschall HU[1], Krawczyk M[2], Grünhage F[2], Stålberg Katsika D[3], Einarsson C[3], Lammert F[2]
[1]Institute of Medicine, Department of Internal Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [2]Department of Medicine II, Saarland University Medical Center, Homburg, Germany; [3]Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm

Introduction: The Gilbert syndrome-associated functional TATA box variant *UGT1A1*28* (A(TA)$_7$TAA) was found to increase susceptibility to pigment gallstone (GD) formation in patients with haemolytic anemia and cystic fibrosis. Further studies in extensive cohorts demonstrated that carriers of this variant carry an increased risk of cholesterol stones as well. We now aim to investigate this polymorphism as a determinant of GD in a unique cohort of Swedish Twins.

Methods: The Swedish Twin Registry was merged with the Hospital Discharge and Causes of Death Registries for gallstone disease-related diagnoses and screened for monozygotic (MZ) twins with GD living in the Stockholm area. Screening of the TwinGene database for gallstone disease resulted in additional concordant twins. Overall, we included 109 concordantly stone-free MZ and 126 stone-free independent DZ twins as controls. *UGT1A1*28* genotyping was performed using PCR-based assays with 5'-nuclease and fluorescence detection (TaqMan).

Results: Among twins with gallstones, the overall prevalence of *UGT1A1*28* was 61.1%. Overall, 58 and 8 out of 106 twins were hetero- and homozygous *UGT1A1* risk allele carriers, respectively. The case-control association tests showed a significantly ($P < 0.05$) increased risk of developing gallstones (OR = 1.62, 95% CI 1.00–2.63) in heterozygotes as compared to homozygous carriers of the common allele.

Discussion/Conclusion: These data from Swedish twins not suffering from hemolytic anemia confirm the Gilbert variant as risk factor for GD. Our observation is in line with the notion that supersaturation of bile with bilirubin may represent the initial step in gallstone formation.

FXR acetylation: What are the functional consequences on target gene expression?

Vittoria Massafra, Alexandra Milona, Danielle A.A. Hollman, Ellen C.L. Willemsen, Saskia W.C. van Mil
Department of Metabolic Diseases, University Medical Centre Utrecht, The Netherlands

Introduction: Nuclear receptors (NRs) are ligand-activated transcription factors whose activity can be regulated by post-translational modifications (PTMs). Particularly, acetylation at lysine residues is responsible for affecting NR function at multiple levels, e.g. activity, stability, DNA binding and heterodimerization. The Farnesoid X receptor (FXR) is activated by bile acids and belongs to the superfamily of NRs. FXR plays a pivotal role in maintaining bile acid homeostasis, and is further involved in lipid and glucose metabolism and inhibition of inflammation. FXR activates and represses transcription via multiple pathways (monomeric vs hetero-dimeric, transactivation vs transrepression etc) and different PTMs are likely to differentiate between these pathways. Here, we aim to identify at which sites FXR is acetylated and address the impact of these modifications on FXR function. This may open new perspectives to selectively control FXR function for therapeutic applications.

Methods: We overexpressed flag-FXR in 293T cells, performed flag-immunopre-cipitations and ascertained post-translational modifications by Orbitrap mass-spectrometry. Subsequently, using acetylation site mutants, we have addressed acetylation at these sites *in vivo* and analysed the effects of FXR acetylation by reporter assays, EMSA, GST pull down experiments and immunoprecipitation experiments.

Results: Four novel acetylation sites were identified, K122, K237, K339 and K460, all in phylogenetically conserved motives. Mutants defective for these sites have been screened for affecting FXR acetylation in 293T cells and were subsequently tested for their effect on DNA binding, heterodimerization to RXR, protein stability and transactivation and transrepression capacity.

Discussion/Conclusion: Here, we identify four novel acetylation sites in FXR. Understanding the exact post-translational regulatory mechanisms of FXR is of crucial importance for targeting FXR in clinic. Ultimately, unravelling the function of these modifications in FXR will give further insights for the development of drugs which selectively boost FXR ability to transactivate or transrepress specific subsets of FXR target genes.

64

Bile acid sequestration with or without voluntary wheel running dramatically reduces atherosclerosis in hypercholesterolemic mice

Maxi Meissner[1], Renze Boverhof[1], Henk Wolters[1], Rudolf A. de Boer[2], Rick Havinga[1], Vincent W. Bloks[1], Folkert Kuipers[1,3], Albert K. Groen[1,3]
Departments of [1]Pediatrics, [2]Cardiology, and [3]Laboratory Medicine, Center for Liver, Digestive, and Metabolic Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Introduction: Bile acid sequestrants (BAS) and physical activity (RUN) decrease incidence of cardiovascular events. Both treatments are often prescribed, yet it is not known whether their beneficial effects are additive. We assessed the effects of BAS treatment alone and in combination with RUN on cholesterol metabolism, heart function and atherosclerotic lesion size in hypercholesterolemic mice.

Methods and Results: Ldlr-deficient mice remained either sedentary (CONTROL), were treated with Colesevelam HCl (BAS), had access to a running wheel (RUN), or were exposed to BAS and RUN (BAS RUN). All groups were fed a high cholesterol diet for 12 weeks. Then, feces, bile and plasma were collected. Atherosclerotic lesion size was determined in the aortic arch and heart function by echocardiography.
BAS RUN ran more than RUN (6.4 ± 1.4 vs. 3.5 ± 1.0 km/day, $p < 0.05$). BAS and BAS RUN displayed ~3-fold reductions in plasma cholesterol levels ($p < 0.001$), ~2.5-fold increases in fecal neutral sterol ($p < 0.001$) and bile acid ($p = 0.01$) outputs, decreases in biliary secretions of cholesterol (~6-fold, $p < 0.0001$) and bile acids (~2-fold, $p < 0.001$) vs. CONROL while no significant effects were observed in RUN. Compared to CONTROL, lesion size decreased by 78% in both BAS and BAS RUN, ($p < 0.0001$).

Discussion/Conclusion: BAS reduce atherosclerosis in Ldlr-deficient mice, coinciding with a switch from body cholesterol accumulation to cholesterol loss. RUN slightly modulated atherosclerotic lesion formation but the combination of BAS and RUN had no clear additive effects in this respect.

Exploring a role for Cyp17a1 in the pathogenesis of cholestasis

Alexandra Milona[1], Ellen CL Willemsen[1], Catherine Williamson[2], Saskia WC van Mil[1]
[1]Department of Metabolic Diseases, UMC Utrecht, The Netherlands, [2]Department of Surgery and Cancer, Institute of Reproductive and Developmental Biology, Imperial College London, London, UK

Introduction: Because bile acids (BAs) are intrinsically toxic, their intrahepatic concentrations are tightly controlled. The nuclear receptor FXR regulates BA synthesis by transcriptional activation of *SHP*, which in turn represses transcription of the rate-limiting enzyme in BA-synthesis (*CYP7A1*). We previously showed that estrogens interfere with FXR function and may perturb BA homeostasis during pregnancy. Here, we present a novel hepatic target-gene of FXR/SHP that is estrogen-modulated and may contribute to the pathology of intrahepatic cholestasis of pregnancy.

Methods: *In vivo* experiments were conducted on age-matched wild-type and FXR-KO mice. Gene and protein expression was determined by qPCR and western blotting respectively. *In vitro* experiments including ChIP, EMSA, luciferase assays etc were conducted according to standard protocols.

Results: We find that BAs robustly repress the expression of the steroidogenic enzyme, *Cyp17a1*, in mouse liver. The effect of BAs on *Cyp17a1* is FXR-dependent and liver-specific. Extensive *In vitro* experiments demonstrate that *Cyp17a1* is regulated by a nuclear receptor cascade involving LRH-1, FXR and SHP. As such, the regulation of hepatic *Cyp17a1* is strikingly similar to that of the bile acid synthesis enzyme, *Cyp7a1*. Intriguingly, hepatic *Cyp17a1* expression is significantly higher in females than in males, is up-regulated during pregnancy and is induced upon estrogen treatment.

Discussion/Conclusion: 17α-hydroxyprogesterone (a product of Cyp17a1) was recently shown to cause cholestatic liver injury in mice. Our findings raise the possibility that estrogen-mediated dysregulation of FXR during pregnancy may induce hepatic *Cyp17a1* and expose cholestatic disease in pre-disposed individuals.

The function of Cyp17a1 in liver, and the relevance of its regulation by FXR, is currently under investigation.

66

Progressive familial intrahepatic cholestasis (PIFC) type II – 15 years treatment with UDCA

Miloshevski M., Serafimoski V., Calovska V., Genadieva-Dimitrova M.
University Clinic of Gastroenterohepatology, Skopje, Macedonia
O. Bernard, Hepatologie Pédiatrique, Hôpital Bicêtre, Paris, France

Introduction: PFIC II is a subtype of progressive familial intrahepatic cholestasis that is associated whit mutation in the ABCB11 gene encoding the bile export pump (BSEP).
BSEP (gene symbol Abcb11) is responsible for the canalicular excretion of bile acids. It has been now recognized that mutations in the gene encoding this protein (ABCB11) are responsible for a subgroup of infants, children and adults with progressive familiar cholestatic disorder causing jaundice, pruritus, growth failure, and progression to cirrhosis very often in the first decade of life.

Methods: BSEP gene mutation was studied in 4 patients, parents and two sons. The parents had a history of intermittent hiperbilirubinaemia, and slightly increasing of gamma-glutamyltransferase. Younger son had symptoms of jaundice, pruritus, and increasing of the level of AST, ALT and gamma-GT since nine-months age. The older son had occasional symptoms of hiperbilirubinaemia and elevation of gamma-GT.

Results: The familial gene analysis of BSEP show: one heterozygote mutation c.1445 A>G (p.Asp482 Gly) at the mother; one heterozygote mutation c.3382 T>C (p.Arg1128 Cys) at the father, one heterozygote mutation c.3382 T>C (p.Arg1128 Cys) at the older son, while the younger son had inherited these mutation from his parents: one heterozygote mutation c.1445 A>G (p.Asp482 Gly) and one heterozygote mutation c.3382 T>C (p.Arg1128 Cys). Liver biopsy was performed at the beginning of the investigation (16 years ago) and consequently every 4 years for the sons. All patients have been treated with ursodeoxycholic acid (UDCA), 20 mg/kg weight. Due to the complete disappearance of the symptoms and normalization of the laboratory analysis for all the patients except for the youngest son, the therapy was stopped after 5 years. After 15 years therapy with UDCA, the latest biopsy has shown a development of liver cirrhosis which is still well compensated.

Discussion/Conclusion: Ursodeoxycholic acid therapy is very useful for patients with mutations in the bile salt export pump (BSEP) gene. However, liver transplantation will be necessary in the near future at the youngest patient (today 30 years old) and it is a more definitive therapy.

Activation of FXR upregulates fatty acid β-oxidation and reduces triglyceride accumulation in human but not in mouse hepatocytes

Teruo Miyazaki[1], Akira Honda[1,2], Tadashi Ikegami[2], Yasushi Matsuzaki[2]
[1]Joint Research Center and [2]Department of Gastroenterology, Tokyo Medical University Ibaraki Medical Center, Japan

Introduction: The activation of FXR (nuclear bile acid receptor) downregulates fatty acid biosynthesis in liver through the inhibition of LXRα-SREBP1c pathway via SHP activation. Furthermore, the FXR activation is reported to upregulate the expression of PPARα that plays essential roles in the catabolism of fatty acids. However, it is not clear whether the elevation of PPARα protein itself stimulates the catabolism of fatty acids or not. This study purposed to explore the effects of FXR activation on fatty acid β-oxidation and triglyceride (TG) content in lipid accumulated cultured hepatocyte models.

Methods: Lipid accumulated cultured hepatocytes were established by exposing non-tumor hepatocyte cell lines (human Fa2N4 and mouse AML12 cells) to 1 μM synthetic LXR-ligand (To901317) for 4 days. Various endogenous and synthetic FXR-ligands (50 μM UDCA, 50 μM CDCA, 1 μM GW4064 or 1 μM INT-747 [6α-ethyl-CDCA]) were added together with To901317. Furthermore, effect of the FXR-ligands on the accumulated TG after To901317 exposure was also studied. Intracellular TG levels were evaluated by Oil-red stain and biochemical assay, and LXR/FXR-target mRNA levels were quantified by real-time PCR. Fatty acid β-oxidation was assayed by measuring acetylcarnitine (AcC) and 3-hydroxybutyrate (3HB) concentrations in cell culture medium by LC-MS/MS.

Results: To901317 caused marked accumulation of TG, which was significantly inhibited by simultaneous addition of CDCA, GW4064 or INT747, but not by UDCA in both human and mouse cell lines. SHP gene was markedly enhanced and genes of SREBP-1c and key enzymes in the fatty acid biosynthetic pathway were significantly inhibited by the addition of the FXR-agonists. Post To901317 exposure, the treatment with the FXR-agonists significantly decreased intracellular TG concentration in human but not in mouse cell line. Significantly increased PPARα and CPT-1α gene expressions and AcC and 3-HB concentrations in culture medium were observed in human but not in mouse cell line treated with the FXR-agonists.

Discussion/Conclusion: This study confirmed the activation of FXR significantly enhanced β-oxidation, in turn, improved lipid accumulation in human, but not mouse, hepatocytes. Therefore, FXR-ligands would be therapeutic agents for human NAFLD.

68

Farnesoid X receptor exerts antisecretory actions on colonic epithelium – A new target for development of antidiarrhoeal drugs?

Magdalena Mroz[1], Niamh Keating[1], Joseph Ward[1], Silvie Amu[2], Padraic Fallon[2] and Stephen Keely[1]
[1]RCSI, Dublin, Ireland
[2]Trinity College, Dublin, Ireland

Introduction: Cl^- secretion, the main driving force for intestinal fluid secretion, can become dysregulated in conditions of disease, leading to the onset of diarrhoea. While such diseases constitute a huge health and economic burden, available medications are unsatisfactory and more specific therapies are required. Here we investigated a role for the bile acid receptor, farnesoid X receptor (FXR), in regulation of intestinal secretory responses and as a target for development of antidiarrhoeal drugs.

Methods: GW4064 (5×10^{-3} M; 24 hours) was used to activate FXR. Cl^- secretion was measured as changes in short-circuit current across T_{84} cell monolayers or muscle-stripped mouse colonic tissues. Protein expression was measured by immunoblotting.

Results: GW4064 treatment significantly inhibited Cl^- secretory responses to the Ca^{2+}- and cAMP-dependent agonists, carbachol and forskolin, and to the naturally-occurring secretagogues, cholera toxin and deoxycholic acid. Intraperitoneal injection of GW4064 to mice (50 mg/kg) attenuated Cl^- secretion in colonic tissues. However, jejunal sodium-dependent glucose co-transport and colonic ENaC currents were not decreased, suggesting the effects of FXR activation are specific for secretory processes. Furthermore, GW4064 treatment inhibited the severity of symptoms in a mouse model of diarrhoeal disease. We found that GW4064 decreased CFTR-mediated Cl^- currents in T_{84} cells and this was associated with a decrease in CFTR protein expression. GW4064 also inhibited Na^+/K^+-ATPase activity without altering its protein expression.

Discussion/Conclusion: These data reveal novel antidiarrhoeal actions of FXR in colonic epithelium. These effects are mediated by inhibition of multiple components of the Cl^- secretory pathway, without altering absorptive processes. Our data suggest that FXR agonists may be useful in treating secretory diarrhoeas associated with common intestinal disorders.

Intrahepatic cholestasis of pregnancy levels of sulfated progesterone metabolites downregulate hepatic LXRα

Vanya Nikolova[1], Shadi Abu-Hayyeh[1], Georgia Papacleovoulou[1,] Malcolm G. Parker[1] and Catherine Williamson[1]
[1]Maternal and Fetal Disease Group, IRDB, Imperial College London, London, UK

Introduction: Women with intrahepatic cholestasis of pregnancy (ICP), a pregnancy-specific liver disorder, have in increased risk of developing cholesterol gallstones. ICP symptoms usually present in the third trimester of pregnancy when maternal sulphated progesterone metabolite (P4S) levels are at their highest. Liver X receptor α (LXRα) is a key regulator of cholesterol homeostasis. We aimed to investigate whether ICP levels of P4S could modulate the LXRα transcriptome and thus contribute to increased biliary cholesterol secretion.

Methods: The influence of ICP levels of P4S on LXRα as well as its target genes was assessed in human hepatoma cells using quantitative RT-PCR and Immunoblotting. LXRα reporter assays were employed to determine the domain of the nuclear receptor that mediates the impact of the sulphated progesterone metabolite.

Results: LXRα reporter assays demonstrated that the P4S epiallopregnanolone sulphate (PM5S), epiallo-pregnanediol 3-sodium sulphate (EPAS) and epipregnano-lone sulphate (EPS) attenuate the basal as well as the agonist-induced transactivity of LXRα in a ligand-binding-domain-dependent manner. Quantitative RT-PCT showed that PM5S, EPAS and EPS decreases the mRNA levels of the LXRα target ABCG1 in a dose-dependent manner while the mRNA expression of LXRα itself is reduced by PM5S only. Protein analysis also showed that PM5S, EPAS and EPS reduce the levels of LXRα in the nucleus.

Discussion/Conclusion: This is the first report of a functional interaction between progesterone metabolites, at concentrations found in ICP, and LXRα that reduces the expression of the ABCG1 cholesterol transporter. The importance of ABCG1 is exemplified by studies showing that ABCG1-deficient mice have elevated biliary cholesterol output. Therefore, this novel interaction between P4S and LXRα could explain the increased risk of cholesterol gallstone formation in women with ICP.

70

Induction of FGF19 but not ASBT by bile acids in short-term explant culture of human ileum

Nolan JD, Kennie SL, Zhang JH, Johnston IM, Dew T*, Dixon PH, Williamson C, Walters JR
Gastroenterology and Hepatology, Imperial College London, London, UK
* Kings College London, London, UK

Introduction: The bile acid (BA) pool size is heavily influenced by ileal FGF19 and ASBT expression. We investigated the responses of FGF19 and ASBT transcripts in human ileum to different BAs, including the synthetic BA obeticholic acid (OCA), a potent farnesoid X receptor (FXR) agonist.

Methods: Biopsies of normal tissue were obtained from patients attending colonoscopy. Ileal biopsies from 29 patients were incubated in parallel as primary explant cultures, in groups of 2 or 3, for up to 6 hours, with various BAs, including chenodeoxycholic (CDCA), glyco-CDCA (GCDCA) cholic (CA), deoxycholic (DCA), lithocholic (LCA) and OCA, alongside their paired BA-free controls. Real-time qRT-PCR was used to measure expression of FGF19 and ASBT. FGF19 protein in culture fluid was measured by specific ELISA.

Results: FGF19 RNA expression was induced in all ileal biopsies incubated with 50 µM CDCA or 50 µM GCDCA (median fold changes 342, $n = 22$, $p < 0.001$, and 114, $n = 15$, $p < 0.001$ respectively). Mean induction of expression of FGF19 RNA normalized to matched CDCA stimulated biopsies revealed 70% of the response with CA ($n = 4$), 30% with DCA ($n = 3$) and no induction with LCA ($n = 2$). Biopsies incubated with 1 µM CDCA ($n = 3$) had no induction but with 1 µM OCA ($n = 3$), a median 70-fold induction of FGF19 was found. FGF19 protein concentrations in culture fluid were significantly induced by BA and correlated with RNA ($r = 0.54$, $p < 0.005$). There was no change in expression of RNA for ASBT ($n = 4$) incubated with 50 µM CDCA, whereas the mean fold-change in FGF19 RNA from these biopsies was 285.

Discussion/Conclusion: FGF19 mRNA and protein expression in human ileum is very highly responsive to CDCA and GCDCA, but less so to CA and DCA and not to LCA. No change in ASBT expression was observed. OCA is more potent than CDCA at inducing FGF19 mRNA expression.

Chemical synthesis of 7α,12α-dihydroxy-cholest-4-en-3-one and its 12-deoxy analogue (C4): Key intermediates in the biosynthesis of bile acids from cholesterol

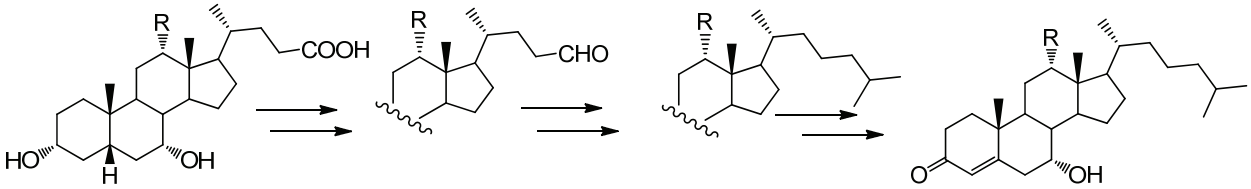
Kaoru Omura[1], Yuusuke Kimoto[1], Takashi Iida[1], Shoujiro Ogawa[2], Kuniko Mitamura[3], Shigeo Ikegawa[3], Lee R. Hagey[4], Alan F Hofmann[4]
[1]Department of Chemistry, College of Humanities and Sciences, Nihon University, Tokyo, Japan
[2]Faculty of Pharmaceutical Sciences, Tokyo University of Science, Noda, Japan
[3]Faculty of Pharmaceutical Sciences, Kinki University, Higashi-Osaka, Japan
[4]Department of Medicine, University of California, San Diego, CA, USA

Introduction: The conjugated enones in the title are key intermediates in the biosynthetic "neutral" pathway from cholesterol to the primary bile acids chenodeoxycholic acid (CDCA) and cholic acid (CA). Indeed, the level of C4 in plasma has been shown to be highly useful in patients as a biomarker whose concentration is directly proportional to the rate of bile acid biosynthesis. To our knowledge, the chemical synthesis of these 3-oxo-4-cholestene derivatives has not yet been attained, although C4 was synthesized enzymatically some years ago. We report herein the chemical synthesis of the two title compounds (**1a** and **1b**).

Methods: As shown in the Figure, our synthetic route starting from CA and CDCA began with the construction of the cholestane (*iso*-octane) side chain at C-17 in 5 steps. Subsequently, the 3-oxo-Δ^4 -steroid nucleus was prepared from the intermediary 3α,7α-dihydroxy-5β-cholestane derivatives in 6 steps. The principal reactions employed are (1) selective reduction of the carboxyl group at C-24 in CA and CDCA with triethylamine/ethylchloroformate/ $NaBH_4$, (2) Wittig reaction of the resulting C-24 aldehyde derivatives with isopropyltriphenylphosphonium iodide to generate the *iso*-octane side chain, and (3) oxidative-dehydrogenation of 3-oxo-5β-cholestane derivatives with iodoxybenzoic acid to afford the desired C_{27} 3-oxo-4-enes (**1a** and **1b**).

Results: All the reactions in the 11 step method proceeded cleanly and smoothly to give the desired compounds in good isolated yields. The structures of the target compounds [7α,12α-dihydroxy-cholest-4-en-3-one (**1a**) and 7α-hydroxy-cholest-4-en-3-one (**1b**) were established by LC-MS and ^{1}H- and ^{13}C-NMR techniques.

Discussion/Conclusion: The 3-oxo-4-cholestene derivatives prepared in this study are considered to be key intermediates in bile acid biosynthesis. Availability of these compounds will permit further studies on their plasma levels as biomarkers of bile acid synthesis as well as for metabolic studies.



R = OH (CA)
R = H (CDCA)

R = OHAc (**3a**)
R = H (**3b**)

R = OHAc (**5a**)
R = H (**5b**)

R = OH (**1a**)
R = H (**1b**)

Fig.

Chronic prednisolone treatment strongly influences bile salt and cholesterol fluxes in mice

Carolien Out[1], Anke Laskewitz[1], Rick Havinga[1],Theo van Dijk[2], Renze Boverhof[2], Henkjan Verkade [1], Folkert Kuipers[1,2] and Albert K. Groen[1,2]
[1]Departments of Pediatrics and [2]Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Introduction: Glucocorticoids are steroid hormones produced by the adrenal gland under control of the hypothalamic-pituitary-adrenal axis (HPA-axis). Related to their effects on the immune response, synthetic glucocorticoids, (*e.g.,* prednisolone) are widely used as anti-inflammatory and immunosuppressive drugs. Unfortunately, chronic glucocorticoid treatment is accompanied by many adverse effects in humans among which central obesity, hypertension, hyperlipidemia, hyperglycemia and insulin resistance. It was the aim of this study to investigate whether synthetic glucocorticoid influence bile acid and cholesterol homeostasis in mice.

Methods: 12-week old male BALB/c mice were continuously treated with prednisolone for 7 days by subcutaneous implantation of slow-release pellets, resulting in a calculated dose of 12.5 mg/kg/day.

Results: Sustained prednisolone treatment strongly influenced both bile salt and cholesterol homeostasis; fecal BA excretion decreased 75% due to stimulation of uptake resulting in fourfold increase in plasma concentration and double biliary output. Concomitantly, both biliary cholesterol and phospholipid secretion increased fourfold leading to a 50% increase in fecal neutral sterol output.

Discussion/Conclusion: Sustained prednisolone treatment strongly influences BA and cholesterol fluxes in mice. These changes may have multiple effects on energy homeostasis and reverse cholesterol transport in mice explaining part of the side effects induced by synthetic glucocorticoids.

73

Gut microbiota have profound effects on host bile acid and cholesterol metabolism

Carolien Out, Jurre Hageman, Trijnie Bos, Renze Boverhof, Rick Havinga,
Folkert Kuipers, Henkjan Verkade, and Albert K. Groen
Department of Pediatrics, Center for Liver, Digestive, and Metabolic Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Introduction: Intestinal bacteria play an important role in human metabolism. They extract energy from the diet and deliver substrate to the host mainly in the form of short chain fatty acids. In addition, intestinal microbiota are involved in bile acid metabolism, both deconjugation and dehydroxylation is carried out by bacteria. In this study we aimed to investigate the role of the intestinal microbiota in the dynamics of the enterohepatic cycle of bile acids and cholesterol.

Methods: 12-week old male C57Bl6 mice were treated with a mixture of non-absorbable antibiotics (bacitracin, neomycin, streptomycin 200 mg/kg) for 5 days.

Results: Treatment antibiotics dramatically increased the enterohepatic circulation of BA and cholesterol. Secretion of both BA and cholesterol into the bile increased threefold, also plasma BA increased but there was no effect on plasma cholesterol. Despite the strongly increased biliary BA and cholesterol output, fecal secretion of both BA and neutral sterols decreased, indicating enhanced absorption. This co-incited with increased expression of ASBT. Interestingly, expression of intestinal FGF15 decreased and liver CYP7A1 increased suggesting expansion of the body BA pool during the 5 days of treatment with antibiotics.

Discussion/Conclusion: Intestinal bacteria play a pivotal role in regulation of BA homeostasis. They inhibit BA and indirectly cholesterol absorption and thereby mediate a significant role in reverse cholesterol transport.

The cholesterol metabolite, 5-cholesten-3beta, 25-diol 3-sulfate, promotes hepatic proliferation in mice

Pandak WM[1], Xin Zhang[1,2], Qianming Bai[1,3], Genta Kakiyama[1], Leyuan Xu[1], Jin kyung Kim[1], and Shunlin Ren[1]
[1]Department of Veterans Affairs Medical Center & Virginia Commonwealth University Health Sciences Center, 1201 Broad Rock Boulevard, Richmond, VA, 23249, USA
[2]Department of Pathology, Fudan University Shanghai Medical College, 138 Yixueyuan Road, Shanghai 200032, China
[3]Department of Pathology, Fudan University Shanghai Cancer Center, 270 Dongan Road, Shanghai 200032, China

Introduction: The “acidic” pathway of bile acid biosynthesis, initiated by mitochondrial sterol 27alpha-hydroxylase (CYP27A1), has been proposed as a mediator of liver regeneration. We have previously shown that CYP27A1 catalyzes cholesterol to form not only 27-hydroxycholesterol, but 25-hydroxycholesterol (25HC) which can be sulfated via the oxysterol sulfotransferase, SULT2B1b. The product of 25HC sulfation, 5-cholesten-3beta, 25-diol 3-sulfate (25HC3S), has potent regulatory properties on lipid metabolism mediated via suppressing liver X receptor (LXRs) signaling. LXRs activation has been reported to be anti-proliferative. 25HC3S may promote hepatic proliferation via LXR signaling. Described are the effects of exogenous and endogenous 25HC3S on hepatic proliferation in vivo in C57BL/6 mice.

Results: Following exogenous administration, 25HC3S was found to have a 48h half-life in the circulation while becoming widely distributed in mouse tissues. Profiler™ PCR array and RTqPCR analysis showed that 25HC3S or overexpression of SULT2B1b plus administration of 25HC (formation of endogenous 25HC3S) significantly up-regulated proliferative gene expression of Wt1, PCNA, cMyc, cyclin A, FoxM1b, and CDC25b in a dose-dependent manner in the liver; and, down-regulated the expression of cell cycle arrest gene Chek2 by 5-fold and the apoptotic gene Apaf1 by 3-fold. Both exogenous and endogenous administration of 25HC3S significantly induced hepatic DNA replication as measured by the PCNA immunostaining labeling index. There was an associated reduction in expression of LXRs response genes, ABCA1 and SREBP1c. LXR activation by the synthetic agonist, T0901317, effectively blocked the 25HC3S-induced hepatic proliferation.

Conclusion: 25HC3S is a potent regulator of proliferation. Its formation may represent a novel regulatory pathway of liver proliferation by inactivating LXR signaling.

75

Programming of metabolic disease in mouse female offspring from cholestatic pregnancy

Georgia Papacleovoulou[1], Shadi Abu-Hayyeh[1], Evanthia Nikolopoulou[2], Bryn Owen[1], Vanya Nikolova[1], Eugene Jensen[3], AS Knisely[4] and Catherine Williamson[1]
[1]Maternal and Fetal Disease Group, IRDB, Imperial College London, London, UK; [2]Reproduction and Metabolism Group, IRDB, Imperial College London, London, UK; [3]Health Protection Research, Public Health and the Environment, The Netherlands; [4]Institute of Liver Studies, King's College Hospital, London, UK

Introduction: An increase in the incidence of obesity and type 2 diabetes over the past two decades constitutes a cardiovascular-metabolic disease epidemic that cannot be fully attributed to genetic predisposition and lifestyle. It is emerging that the intrauterine environment affects the developing baby, predisposing the unborn child to metabolic syndrome in later life. Intrahepatic cholestasis of pregnancy is a liver-specific disease of pregnancy that is characterised by elevated serum bile acid (BA) levels and dyslipidaemia in the mother. Therefore, we hypothesised that cholestatic pregnancy in mice can programme offspring to develop metabolic disease.

Methods: Using a mouse model, we studied the metabolic phenotype of 18-week-old adult female and male offspring from cholestatic mothers. The offspring were fed either normal chow (NC) or a Western diet (WD) for 6 weeks. Two cohorts with at least six animals/group were assessed.

Results: Cholestatic pregnancy affected female offspring only. WD feeding resulted in an increased BMI, impaired glucose tolerance, insulin resistance and inflammation ($p < 0.05$). Serum LDL-cholesterol, leptin and hepatic free fatty acids (FFA) were raised ($p < 0.05$). Macrophage infiltration was consistently observed in WAT and pancreatic islet size was increased. NC-fed females from cholestatic mothers developed mild hepatosteatosis and a pro-inflammatory profile, suggesting that these mice were predisposed to metabolic disease before WD challenge.
We also assessed the feto-placental unit to dissect underlying mechanisms of cholestatic programming. Fetuses developed a cholestatic phenotype (increased serum BA and induced hepatic Fxr pathways; $p < 0.05$). Hepatic cholesterol and FA biosynthetic pathways were increased ($p < 0.05$), without changes in serum cholesterol or FA levels. Cholestatic placentas had increased lipidic vacuolisation and cholesteryl esters, with increased expression of lipogenic pathways (*e.g.* Adrp, Acat-2; $p < 0.05$).

Discussion/Conclusion: Our results suggest that alterations of placental lipid handling in cholestatic pregnancy modify fetal metabolism, thereby increasing susceptibility of female adult offspring to metabolic disease.

TGR5 knockout mice are highly susceptible to lipopoly-saccharide treatment

Maria Reich[1], Verena Keitel[1] Dieter Häussinger[1]
[1]Clinic for Gastroenterology, Hepatology and Infectious Diseases, Heinrich-Heine-University, Moorenstr. 5, 40225 Düsseldorf, Germany

Introduction: TGR5 (Gpbar-1) is a membrane-bound bile acid receptor expressed in different nonparenchymal cells of the liver as well as in peripheral blood mononuclear cells. Activation of TGR5 decreased cytokine expression in isolated macrophages and Kupffer cells (1, 2). Aim of the present study was to determine the anti-inflammatory properties of TGR5 in a sepsis model.

Methods: TGR5 knockout (KO) and wildtype mice were intraperitoneally injected with lipopolysaccharide (LPS; 100 or 500 µg/animal) and sacrificed after 2–72 hours. Cytokine levels were analyzed in serum using ELISA-assays. Cytokine expression was determined in liver and spleen samples as well as in isolated bone-marrow derived macrophages by realtime PCR. HE staining was carried out for all liver samples.

Results: While all wildtype animals survived the treatment with high dose LPS (500 µg/animal, n = 11/group), 45% of the knockout mice died within the first 36 hours. AST/ALT and interleukin-1β concentrations were significantly elevated in serum from knockout mice as compared to their wildtype littermates. However, no difference in serum TNF-α levels was observed. Treatment with lower doses of LPS (100 µg/animal) resulted in significantly raised IL-1β and TNF-α levels in TGR5 KO mice. Furthermore, HE staining revealed significantly more inflammatory infiltrates in livers from TGR5 KO mice as compared to controls.

Discussion/Conclusion: TGR5 knockout mice are more susceptible towards LPS treatment as demonstrated by elevated serum levels of inflammatory cytokines, increased liver inflammation and raised AST/ALT levels. Using a high dose LPS sepsis model our study confirms and extends recently published data (3, 4) in describing a significantly higher mortality rate in the TGR5 knockout mice.

References:

1. Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, et al. A G protein-coupled receptor responsive to bile acids. J Biol Chem 2003;278:9435–9440.
2. Keitel V, Donner M, Winandy S, Kubitz R, Häussinger D. Expression and function of the bile acid receptor TGR5 in Kupffer cells. Biochem Biophys Res Commun 2008;372:78–84.
3. Wang YD, Chen WD, Yu D, Forman BM, Huang W. The G-Protein-coupled bile acid receptor, Gpbar1 (TGR5), negatively regulates hepatic inflammatory response through antagonizing nuclear factor kappa light-chain enhancer of activated B cells (NF-kappaB) in mice. Hepatology 2011;54:1421–1432.
4. Pols TW, Nomura M, Harach T, Lo SG, Oosterveer MH, Thomas C, et al. TGR5 Activation Inhibits Atherosclerosis by Reducing Macrophage Inflammation and Lipid Loading. Cell Metab 2011;14:747–757.

77

Involvement of the peroxisomal ABC-transporter Pmp70 in the intracellular transport of bile salts in rat hepatocytes

Krzysztof P. Rembacz[1], Jannes Woudenberg[1], Zhengyu Du[1],
Fiona A.J. van den Heuvel[1], Elles Z. Jonkers[2], Frans Stellaard[2], Han Moshage[1] and Klaas Nico Faber[1]
University of Groningen, University Medical Center Groningen, Departments of [1]Gastroenterology and Hepatology and [2]Laboratory Medicine, Groningen, The Netherlands.

Introduction: Peroxisomes are organelles highly enriched in the liver, which play a central role in bile salt homeostasis. The final steps of bile salt biosynthesis, including side chain shortening and conjugation to taurine or glycine, occur inside peroxisomes after which they enter the enterohepatic cycle. In this cycle, significant amounts of bile salts are deconjugated in the intestine and after recycling to the liver need to pass through peroxisomes again for reconjugation. In contrast to our knowledge about bile salt transporters in plasmamembranes, very little is known about transport of bile salt (intermediate)s across peroxisomal membranes. Here, we analyzed the putative involvement of the peroxisomal ABC-transporter Pmp70 (*Abcd3*) in this process.

Methods: Primary rat hepatocytes were exposed to the PPARalpha ligand fenofibric acid (FFA) to induce expression of Pmp70. Small interfering RNA was used to prevent FFA-induced expression of Pmp70. FFA- and/or siRNA-treated hepatocytes were exposed for 3 h to deuterium-labeled cholic acid (D_4CA) and conversion to, and accumulation of, D_4-tauro cholic acid (D4-TCA) in hepatocytes and medium were quantified by LC/MS/MS mass spectrometry.

Results: FFA treatment of rat hepatocytes strongly induced Pmp70 expression (5–10-fold) and increased the accumulation of D_4TCA in the medium (+15%), while reducing the intracellular levels of D_4CA (-44%) and D_4TCA (-15%). SiRNA treatment effectively prevented the FFA-induced expression of Pmp70 in rat hepatocytes and concomitantly increased the intracellular levels of D_4CA and D_4TCA and decreased D_4TCA levels in the medium.

Discussion/Conclusion: These data suggest a role for Pmp70 in the transport of unconjugated bile salts into peroxisomes to become conjugated to taurine and efficiently exported from hepatocytes.

The acidic pathway of bile acid biosynthesis: Role in oxysterol sulfation, lipid metabolism and inflammatory responses

Shunlin Ren, Leyuan Xu, Qianming Bai, Jin Koung Kim, Shijian Chu, Philip Hylemon, Douglas M. Heuman, and Williams M. Pandak
Department of Internal Medicine, Virginia Commonwealth University/McGuire VA Medical Center, Richmond, VA 23249, USA

Introduction: We previously reported that the rate limiting step in the acidic pathway of bile acid synthesis is mitochondrial cholesterol uptake. This barrier can be overcome by increasing expression of the intracellular cholesterol transporter, StarD1. Overexpression of StarD1 was shown to down-regulate cholesterol and fatty acid biosynthesis. A search for the possible mechanism of these regulatory effects led to the discovery of of 5-cholesten 3,25-diol 3-sulfate (25HC3S), which was shown to be synthesized from 25-hydroxycholesterol (25-OHC) by oxysterol sulfotransferase 2B1b (SULT2B1b). Functional studies showed that 25HC3S and 25-OHC co-ordinately regulate lipid metabolism and inflammatory responses via nuclear receptor signalling.

Methods: Following addition of oxysterols, gene expressions were analyzed by qRT-PCR and Western Blot; lipid levels and inflammatory responses, by biochemical methods.

Results: Addition of 25HC3S to culture media of primary hepatocytes or macrophages decreased nuclear protein levels of LXR, SREBP mRNA levels, inhibited SREBP-1 and 2 maturation, and subsequently down-regulated key enzymes of lipid biosynthesis, leading to decreased intracellular lipid levels. 25HC3S increased expression of PPARγ and its nuclear protein levels, increased cytosol IkB levels, decreased nuclear NFkB levels, and subsequently reduced pro-inflammatory cytokine expression and secretion. PolarScreen^{TM} PPARγ-competitor assay showed that the IC_{50} for 25HC3S is similar to those of the natural ligands, ~1 μM. Molecular docking and molecular dynamic simulation studies showed that PPARγ contains a single ligand binding pocket, which gave a perfect fit for 25HC3S by forming two hydrogen bonds. Following binding, 25HC3S is hypothesized to change the three dimensional structure, allowing activation of PPARγ. In contrast to 25HC3S, 25-OHC, a known LXR ligand, increased nuclear LXR and decreased nuclear PPARγ and cytosol IkB levels. Overexpression of the gene encoding SULT2B1b decreases hepatic lipid levels in a NAFLD animal model.

Discussion/Conclusion: The acidic pathway plays an important role in regulating lipid metabolism and inflammatory responses.

79

Understanding human metabolic effects of resins from monitoring responses following acute or chronic treatment

Mats Rudling, Sara Straniero, Bo Angelin, Beatrice Sjöberg
Department of Medicine, Karolinska Institute, Huddinge Stockholm, Sweden

Introduction: Resins may increase plasma TGs and reduce blood glucose. Bile acid (BA) synthesis is regulated via hepatic FXR. BAs also bind intestinal FXR initiating synthesis and secretion of FGF19 that inhibits hepatic BA synthesis.

Methods: To study responses during the initiation and after termination of resin treatment to get insight in why resins alter plasma TG and glucose and how long their effects last.

Results: 10 normals received (cholestyramine) (4 g to 16 g/d for 3 wks with weekly doubled dose) At 16 g/d BA synthesis was induced 13-fold, cholesterol synthesis 2.7-fold, serum TGs and total BAs were stable. Total cholesterol decreased 28% while glucose and insulin tended to decrease. After treatment BA and cholesterol synthesis remained increased one week. Serum BAs and FGF19 increased 2–3-fold after resin seponation while glucose, TGs and PCSK9 tended to decrease. Also the effects of a single day resin-treatment (4 g x 4) was studied. BA synthesis was induced 3.5-fold and cholesterol synthesis 0.6-fold. These responses persisted 36 hrs after the last resin dose. In contrast to the chronic resin experiment plasma TGs increased 100% overnight and serum glucose and insulin levels were significantly elevated during night as was plasma BAs.

Discussion/Conclusion: Resin-induced changes of plasma TGs and glucose are independent of BA synthesis. Rather, altered BA levels and composition appear important. Acute treatment rapidly drains BAs whereas chronic treatment results in a constant or increased pool of BAs caused by a strong >10-fold stimulation of BA synthesis. The stimulation of BA and cholesterol synthesis appears within hours after resin intake and lasts up to a week after seponation.

Circulating FGF19 is strongly increased in human extrahepatic cholestasis

Mats Rudling, Rolf Hultcrantz, Annika Bergqvist, Bo Angelin
Department of Gastroenterology and Endocrinology, Karolinska Hospital/Institute, Huddinge Stockholm, Sweden

Introduction: FGF15/19 is a FXR-driven inhibitor of bile acid (BA) synthesis released from the small intestine. In mouse models extrahepatic cholestasis induces BA synthesis presumably due to reduced intestinal FGF15 expression. However, in man BA synthesis is reduced in extrahepatic cholestasis. We investigated how circulating levels of FGF19 and BA synthesis relate to 1) cholestasis in humans due to stricture, 2) cirrhosis, 3) fatty liver, 4) hemochromatosis studying a total of 102 patients and 435 controls.

Methods: Overnight fasting serum was obtained and BA synthesis monitored using the serum marker C4c. Total serum BAs were determined enzymatically and FGF19 monitored by ELISA.

Results: BA synthesis is induced 50% in steatosis and 2.5 fold in hemochromatosis, while a 30% reduction occurs in liver cirrhosis and in cholestatic patients. FGF19 is increased 8-fold in cholestasis, and correlates strongly to total BAs both in the whole series and in cholestatic subjects only ($r^2 = 0.6$)

Discussion/Conclusion: The results show that in response to extrahepatic cholestasis in humans, circulating levels of FGF19 are increased about 10-fold thereby contributing to a limited but significant 34% reduction of the level of BA synthesis in this situation. The results are in line with findings demonstrating that FGF19 mRNA levels are detected in human liver samples from patients with cholestasis.

81

Liver HMG-CoA reductase is contributing to transintestinal cholesterol excretion

Marleen Schonewille[1], Jurre Hageman[1], Albert K. Groen[1,2] and Gemma Brufau[1]
Centre for Liver, Digestive and Metabolic Diseases, Departments of [1]Pediatrics and [2]Laboratory Medicine, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands

Introduction: Transintestinal cholesterol excretion (TICE) pathway is one of the main contributors of cholesterol excretion in mice. To date, the origin of cholesterol excreted via TICE remains elusive. Previous data from our group have shown a clear association between increased TICE and increased cholesterol synthesis. Therefore, the aim of this study was to investigate the contribution of cholesterol synthesis in TICE.

Methods: To do so, we studied whole body cholesterol fluxes in wild-type mice receiving either liver specific adeno-associated virus (AAV) containing a short hairpin against 3-hydroxy-3-methylglutaryl coenzyme A reductase (shHmgcr), or AAV containing shGFP (control group).

Results: As expected, mice receiving AAV-shHmgcr showed a 5.2 fold decrease in Hmgcr mRNA levels in the liver compared to control mice, whereas no differences in Hmgcr mRNA expression in the small intestine were found between the groups. No signs of illness were associated with the administration of the viruses: no differences in body weight, food intake, fecal output or liver weight were found between the groups. Total cholesterol levels in liver and plasma were decreased in the shHmgcr mice (2.7-fold and 1.3-fold, resp.). Similarly, biliary cholesterol secretion was 2-fold decreased in the shHmgcr group. No differences in biliary bile acid secretion were found whereas total bile acids in feces were 1.5-fold increased in shHmgcr mice compared to the control mice. Remarkably, the decrease in cholesterol synthesis was positively associated with a 2.1-fold decrease in neutral sterols and 2.9-fold decrease in TICE.

Discussion/Conclusion: In conclusion, our results indicate that hepatic Hmgcr play an important role in the TICE pathway.

Nor-UDCA and UDCA protect against arrhythmia in a model of the fetal heart

Francisca Schultz[1,2], Michele Miragoli[1], Anamika Bhargava[1], Michael Trauner[3], Catherine Williamson[2] and Julia Gorelik[1]
[1]National Heart and Lung Institute, Imperial Centre for Translational and Experimental Medicine, Imperial College London, Hammersmith campus, London, UK
[2]Institute of Reproductive and Developmental Biology, IRDB, Imperial College London, Hammersmith Campus, London, UK
[3]Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria

Introduction: Intrahepatic cholestasis of pregnancy (ICP) can be complicated by fetal distress and fetal arrhythmia (FA). We recently found that elevated bile acids in the maternal blood act upon myofibroblasts (MFB), which are present in the fetal heart but not in the maternal heart, and that triggers FA. In a model of the liver disease sclerosing cholangitis, a chemically modified version of UDCA, called NorUDCA, showed a better protective effect against the toxic effects of bile acids. NorUDCA contains one less methylene group in its side chain. We explored the potential protective effect of UDCA and norUDCA in an *in vitro* model of the fetal heart (FHM).

Methods: The FHM consists of a heterocellular culture of neonatal rat ventricular cardiomyocytes and MFBs. Calcium wave duration and amplitude after treatment with either bile acids, UDCA or NorUDCA were assessed optically. Cardiomyocyte contraction was scored. Membrane potential changes in MFB were assessed using sensitive dye (DiBAC4).

Results: Optical recording of intracellular calcium duration in the FHM showed that UDCA (control: 382.7 ± 63.6 ms; 10nM: 394.3 ± 44.1 ms; 100nM: 337.4 ± 64.8 ms; 1 µM: 407.6 ± 24.7 ms) and NorUDCA (control: 402.9 ± 26.5 ms; 10 nM: 385.0 ± 28.54 ms; 100nM: 393.7 ± 36.0 ms; 1 µM: 402.2 ± 26.87 ms) do not alter Ca_i^{2+} duration and therefore have no negative influence on cardiomyocytes. This is in accordance with our previous results where the resting membrane potential was also unaffected. Contraction experiments show that UDCA and norUDCA protect against arrhythmia induced by taurocholate (TC). Measurements of the membrane potential show that both UDCA and NorUDCA hyperpolarise MFB, while TC depolarises them.

Discussion/Conclusion: Depolarisation of MFB may lead to depolarisation and arrhythmia in cardiomyocytes, thereby the hyperpolarising effect of UDCA and norUDCA may provide protection against arrhythmia.

83

Successful treatment of bile acid synthetic defects with oral cholic acid – Results from a 20+ year experience

Kenneth DR Setchell[1] and James E. Heubi[2]
Departments of Pathology and Laboratory Medicine[1] and Gastroenterology and Nutrition[2], Children's Hospital Medical Center, Cincinnati, Ohio 45229, USA

Introduction: Bile acid synthetic defects are a specific category of progressive familial intrahepatic cholestatic (PFIC) disorders associated with both early and late-onset chronic cholestasis. Thus far, 9 genetic defects in the complex 17 enzyme-catalyzed pathway of primary bile acid synthesis from cholesterol have been defined and mutations identified in the genes encoding these enzymes. These autosomal recessive disorders manifest as a broad phenotype, ranging from mild to severe liver disease, fat-soluble vitamin malabsorption, growth failure, and/or neurological disease. Biochemically, all share the common feature of an absence of hepatic synthesis of the primary bile acids, cholic and chenodeoxycholic acids, that are essential for the promotion of bile flow, concomitant with elevated levels of atypical bile acids that are cholestatic/hepatotoxic. The natural clinical history of these disorders is one of progression to cirrhosis and liver failure.

Methods: Patients were screened for a bile acid synthetic defect by mass spectrometric analysis of the urine and/or serum and confirmation was established by sequencing of the gene encoding the specific enzyme deficiency. Patients were administered cholic acid orally at a dose of 10–15 mg/kg bw/d and followed prospectively for biochemical and clinical response to therapy.

Results: Cholic acid therapy has been shown to be effective in most patients with bile acid synthetic defects and a number of patients have been treated for > 20 years, Long-term survival and an impressive clinical improvement of patients with bile acid synthetic defects is associated with down-regulation of bile acid synthesis and the provision of adequate levels of primary bile acids to generate bile flow. Both of these goals have been achieved with oral administration of the cholic acid, which led to a sustained reduction or disappearance of atypical bile acid metabolites concomitant with a consistent normalization in serum liver enzymes, and improvement in growth and liver histology. In > 20 years, no significant adverse effects have been associated with the use of cholic acid.

Discussion/Conclusion: Based on the successful application of this therapeutic strategy, cholic acid was recently granted orphan Status by the FDA and is in the final stages of marketing authorization in both the EU and US FDA.

Use of novel fluorescent variants of cholic acid to study intracellular sorting of bile acids

Shiva Shajari[1], Krzysztof P. Rembacz[1], Mark Hoekstra[1], Elles Z. Jonkers[2], Miguel A. Miranda[3], Frans Stellaard[2], Han Moshage[1] and Klaas Nico Faber[1]
[1]Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; [2]Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; [3]Instituto de Tecnología Química, Departamento de Química (UPV-CSIC), Valencia, Spain

Introduction: Unconjugated bile acids, like cholic acid (CA) and ursodeoxycholic acid (UDCA), are efficiently conjugated to glycine and taurine in hepatocytes, thereby becoming high-affinity substrates for dedicated transporters in the enterohepatic circulation. Bile acid-CoA:amino acid N-acyltransferase (BAAT) is the sole enzyme responsible for bile acid conjugation. Most, if not all, BAAT is localized in peroxisomes. After exposing primary hepatocytes to deuterium-labeled CA, we recently showed that its taurine conjugate indeed can be detected in peroxisomes, suggesting the shuttling of bile acids through these organelles. Fluorescent bile acids have been used to visualize bile acid transport, however, most of these are not suitable to study intracellular trafficking, because 1) the fluorescent group is present at the conjugation site or 2) the fluorescent group impairs transport by bile acid transporters. Here, we analysed the conjugation and sorting of novel variants of fluorescently-tagged CA.

Methods: Primary rat hepatocytes were exposed for various periods (5 minutes-24 hours) to 3alpha-nitrobenzoxadiazole(NBD)-CA, 3beta-NBD-CA, 7alpha-NBD-CA, 7beta-NBD-CA, 3alpha-bimane(BM)-CA, 3alpha-aminophtalimide(aFT)-CA or 3alpha-Dansyl(Dns)-CA. The subcellular location of fluorescently-tagged CA was analysed by confocal laser scanning microscopy. Conjugation of NBD-CA was analysed by liquid chromatography-tandem mass spectrometry.

Results: All fluorescent variants of CA were rapidly (within minutes) taken up by primary hepatocytes and revealed a transient, but highly variable, accumulation in subcellular structures. Minor, but significant amounts of 3alpha-NBD-TCA were detected in the medium of primary hepatocytes exposed to 25 uM 3alpha-NBD-CA. The other NBD-CA variants were not conjugated at all. Mass spectromical and life-time imaging of the various 3alpha-tagged CA forms is currently being performed.

Discussion/Conclusion: 3alpha-NBD-CA is a (weak) substrate for taurine/glycine-conjugation by primary rat hepatocytes. Testing different fluorescent tags at this position in CA may allow the identification of a fluorescent bile acid that is suitable to analyse intracellular trafficking of bile acids in hepatocytes.

85

Bile acid flux is a more powerful signal than FGF-19 in the regulation of CYP7A1 in rabbits

Quan Shang, Monica Saumoy, Gerald Salen, Guorong Xu
Department of Medicine, University of Medicine & Dentistry of New Jersey, Newark, NJ, USA; and GI Lab, VA Medical Center, East Orange, NJ, USA

Introduction: It was proposed that CYP7A1 expression is regulated through a gut-liver signaling pathway FGF15/19-FGFR4 which is initiated by activation of FXR in the intestine rather than in the liver. This study evaluated the role of intestinal FGF19 in the regulation of CYP7A1 in the rabbit and determined whether its role has been overstated when compared with the bile acid flux returning to the liver.

Methods: New Zealand White rabbits were used (n = 6/each group). Study 1 consisted of rabbits fed 0.5% or 2% cholesterol (Ch), 0.3% cholic acid (CA) and controls. Study 2: controls and rabbits with bile fistula alone (BF), and after 6 hours with glycodeoxycholic acid (GDCA) perfusion through the splenic vein to bypass the ileum with (GDCA+BF) or without (GDCA) bile fistula.

Results: In Study 1, portal blood bile acid concentrations, measured to represent the bile acid flux returning to the liver, increased 93% ($p < 0.001$) in the CA group, 79% ($p < 0.05$) in the rabbits fed 2% Ch but remained unchanged in those rabbits fed 0.5% Ch. Ileal FGF19 mRNA increased 270-fold ($p < 0.001$) in the CA group and 28-fold in the 2% Ch group as compared to the controls with suppression of hepatic CYP7A1 mRNA levels in the CA (-83%, $p < 0.001$) and 2% Ch (-52%, $p < 0.05$) but not the 0.5% Ch group. For the first time, we measured FGF19 protein “signal” levels in the portal blood which were 105 ± 28 (control), 73 ± 15 (0.5% Ch), 61 ± 20 (2% Ch) and 89 ± 15 (CA) pg/250µl respectively. Note that portal blood FGF19 levels were not elevated in the CA group, although FGF gene expression in the ileum was extremely high.Portal blood FGF19 levels in the 2% Ch group decreased 42% ($p < 0.01$) compared to the controls though ileal FGF19 gene expression in this group was enhanced and CYP7A1 expression in the liver was suppressed. In Study 2 where GDCA was perfused directly into splenic vein to bypass the ileum, portal bile acid concentrations in the GDCA+BF and GDCA groups increased 2-fold as compared to the controls. Ileal FGF19 mRNA was decreased ($p < 0.01$) 83% in the BF, 98% in the GDCA+BF and 90% in the GDCA groups respectively while hepatic CYP7A1 expression was suppressed ($p < 0.05$) 87% and 82% in the GDCA+BF and GDCA groups and unchanged in the BF group. Thus, the suppression of CYP7A1 in the liver was solely resulted from the perfused GDCA whereas the massive reduction of ileal FGF19 expression in the BF, GDCA+BF and GDCA groups did not induce hepatic CYP7A1 expression. Despite the fact that FGF19 expression in the ileum was sharply repressed in the BF, GDCA+BF and GDCA groups, it did not reflect the signal (FGF19 protein) levels in the portal blood which is the pathway for FGF19 to reach the liver. Portal blood FGF19 protein concentrations were 109 ± 27 (Controls), 108 ± 15 (BF), 89 ± 22 (GDCA+BF) and 80 ± 50 pg/250µl (GDCA) respectively without significant differences. Although the signal FGF19 levels did not increase, CYP7A1 expression remained inhibited in the GDCA+BF and GDCA groups where bile acid flux returning to the liver was increased by perfusion.

Discussion/Conclusion: As a target gene of FXR, FGF19 expression in the ileum reflects the changes in the circulating bile acid flux through the ileum. However, FGF19 gene expression in the ileum does not always reflect CYP7A1 expression whereas the bile acid flux returning to the liver via portal blood determines the expression of CYP7A1 under different physiological conditions. The signal FGF19 protein concentrations in the portal blood do not mirror the changes in the expression of FGF19 in the ileum nor correlate with the expression of CYP7A1 in the liver. Our results suggest that in rabbits, the enterohepatic circulating bile acid flux represents a more accurate signal than FGF19 for the regulation of CYP7A1.

86

The N-terminal part of the TGR5 C-terminus is essential for receptor membrane localization

Lina Spomer[1], Verena Keitel[1], Dieter Häussinger[1]
[1]Klinik für Gastroenterologie, Hepatologie und Infektiologie, Heinrich-Heine-Universität, Moorenstr. 5, 40225 Düsseldorf, Germany

Introduction: TGR5 is a G-protein coupled bile acid receptor, which has been linked to bile acid and glucose homeostasis, energy expenditure and atherosclerosis. Thus TGR5 represents an interesting drug target for liver and metabolic diseases. The structure of TGR5 is unknown. We have previously demonstrated that the deletion of the 35 C-terminal amino acids results in ER retention of the mutated protein. Aim of the present study was to characterize the role of the TGR5 C-terminus for receptor localization.

Methods: TGR5 mutations were introduced by site-directed-mutagenesis. Impact on receptor localization was determined by immunofluorescence microscopy and FACS analysis. TGR5 function was studied using a cAMP sensitive luciferase reporter gene.

Results: Truncation of the TGR5 C-terminus at amino acids (aa) 284 to 297 resulted in an accumulation of the mutated proteins in the ER and loss of function. Truncation at aa 300 had only minor effects on localization. To elucidate the role of the proximal TGR5 C-terminus deletion variants (Δ285-290; Δ291-297) as well as alanine, glycine or proline substitutions (285-290A/G/P; 291-297A/G/P) were generated. Deletion or substitution of aa 285-290 led to ER retention and almost complete loss of function. The deletion/substitution variants 291-297 were detected both in the ER and in the plasma membrane. Stimulation with taurolithocholic acid resulted in increased cAMP generation by the 291-297 variants.

Discussion/Conclusion: The N-terminal part of the TGR5 C-terminus (aa285-290) is required for plasma membrane localization. Since no known sorting signal was identified in the proximal C-terminus, these aa are most likely essential for correct folding of the protein. Computational analysis indicates that the proximal TGR5 C-terminus contains an α-helix, which is disrupted by the 285-290 variants thereby causing ER retention.

Lysophosphatidylcholine induces apoptotic cell death in biliary epithelial cell: Implication to biliary oncogenesis associated with pancreaticobiliary maljunction

Akiko Sugiyama, Keishi Kanno, Miki Saito, Keiko Fujita, Nobusuke Kishikawa, Mayumi Okamoto, Yuka Kikuchi, Kennichi Yokobayashi, Shigeyuki Kushihata, Masahumi Mizooka, Toshinari Saeki, Mika Nakashima, Susumu Tazuma
Department of General Internal Medicine, Graduate School of Medical Science, Hiroshima University, Hiroshima, Japan

Introduction: Lysophosphatidylcholine (lysoPC), a derivative of phosphatidylcholine hydrolysis by phospholipase A2 (PLA2), is a highly abundant bioactive lipid mediator in bile. LysoPC has been reported to be significantly increased in bile of the patients with pancreaticobiliary maljunction, which is considered to be the major risk factor for biliary cancers. Biliary system is continuously exposed to bile containing lipids and bile acids, and therefore such increase of biliary lysoPC is possibly involved in carcinogenesis. In this study we aimed to investigate the influence of lysoPC on biliary systems in light of carcinogenesis.

Methods: HuCCT-1, a human cholangiocellular carcinoma cell line was treated with lysoPC in vitro. Cytotoxicity and apoptosis were studied by MTT assay, morphological analysis, and flow cytometric analysis. Key genes participate in apoptosis were quantified by real-time PCR. Caspase activities were assayed using a commercial assay kit. The expression of G protein-coupled receptor 132 (G2A), a lysoPC-related receptor was analyzed by real-time PCR and Western blot analysis.

Results: LysoPC (100 μM) exhibited significant time-dependent cytotoxicity with more than 50% decrease of cell viability at 10hr. Flow cytometric analysis revealed significant induction of apoptotic cells by lysoPC treatment. Fluorescent microscopic analysis of LPC-treated cells stained with DAPI also depicted the typical morphological changes observed in apoptotic cells such as the condensed chromatin gathering. In addition to up-regulation of Fas receptor mRNA, the activities of caspase 8 and 3, which are downstream of Fas, were significantly increased by the treatment of lysoPC. We also observed up-regulation of Bax mRNA and activation of caspase 9. Also, lysoPC markedly induced mRNA and protein levels of G2A.

Discussion/Conclusion: These data suggest that lysoPC-induced apoptosis in biliary epithelial cells is mediated through both the extrinsic (death receptor-dependent) and the intrinsic (mitochondria-dependent) signaling pathway. Thus, lisoPC derived by PLA2 may function in pancreaticobiliary maljunction-associated biliary carcinogenesis through G2A signaling.

88

Identification of functionally relevant lysine residues that modulate human farnesoid X receptor (hFXR) function

An-Qiang Sun[1], Yu-Huan Luo[1], Donald S. Backos[2], Shuhua Xu[1],
Natarajan Balasubramaniyan[1], Philip Reigan[2] and Frederick J. Suchy[1]
[1]Department of Pediatrics, University of Colorado Denver School of Medicine, Aurora, CO 80045, USA
[2]Skaggs School of Pharmacy and Pharmaceutical Sciences, Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA

Introduction: Lysine residues play an important role in the posttranslational modification of nuclear receptors (e.g. FXR) leading to enhanced or suppressed activity. Two highly conserved lysine residues (K210 and K339) of hFXR were identified following the rules for posttranslational modification (e.g. acetylation). Computational-based structural modeling suggested that K210 (at hinge region) could directly interact with DNA by hydrogen-bonding, and the K339 (at Ligand-Binding-Domain) of FXR is located at the entrance of ligand-binding pocket.

Methods: To further understand the role of the two lysine residues of FXR, K210 and K339 were replaced with arginine by site-directed mutagenesis, and the subsequent effects on transactivation, nuclear localization, protein-protein and protein-DNA interaction were investigated by Luciferase assay, co-immunoprecipitation, confocal microscopy, and electrophoretic mobility shift analysis (EMSA).

Results: The results demonstrated that, in a ligand-dependent manner, the mutants markedly reduced human Organic Solute Transporter-alpha and -beta expression in both liver and intestine cell lines, reduced promoter basal activity by ~11.3–25.2% (FXR-K210R) and 48.4–65.4% (FXR-K339R) in transfected HepG2 cells, and by ~21.9–32.7% (FXR-K210R) and 53.6–59.7% (FXR-K339R) in transfected CaCo2 cells, respectively, compared with cells transfected with wild-type FXR. In transfected HepG2 cells, FXR-K210R and -K339R mutants decreased hBSEP expression and basal promoter activity by ~25% and ~80%, respectively. Co-immunoprecipitation demonstrated that FXR-K210R significantly impaired the protein-protein interaction with RXR protein (a co-activator of FXR) by ~30% in a ligand-dependent manner. Confocal microscopy showed no difference in the nuclear localization of the FXR-K210R and -K339R mutants compared with wild type hFXR in transfected HepG2 cells. EMSA demonstrated that hFXR-K210R and -K339R reduced the protein-DNA (IR1 element at hBSEP promoter) binding affinity by ~75% and ~90%, respectively.

Discussion/Conclusion: In summary, the K210 and K339 of hFXR play a critical role in gene regulation and molecular interaction (protein-protein and protein-DNA), in a ligand dependent way.

Effect of 4-phenylbutyrate on the biliary transport maximum of taurocholate reduced by lipopolysaccharide in rats

H. Takikawa, N. Sano, M. Adachi, K. Miura, M. Mikami, M. Aiso, Y. Takamori, A. Tanaka
Department of Medicine, Teikyo University School of Medicine, Tokyo, Japan

Introduction: 4-Phenylbutyrate (4PB) has been reported to increase the amount of the bile salt export pump (Bsep, Abcb11) and the multidrug resistance protein 2 (Mpr2, Abcc2) at the canalicular membrane (CM). In the present study, the effect of 4PB on the biliary transport maximum (Tm) of taurocholate (TC) and BSP was studied in rats. The effect of 4PB on the reduced Tm of TC by lipopolysaccharide (LPS) was also examined.

Methods: 4BB (1,200 mg/kg) was orally given for 4 days to male Sprague-Dawley rats. After bile duct cannulation, 14C-TC (1.5 μmol/min/100 g) or BSP (0.2 μmol/min/100 g) was intravenous infused and bile samples were collected. In different experiments, rats were intravenously injected LPS (2.5 mg/kg) 18 h prior to the experiments. 14C-TC was infused at the rate of 1.0 μmol/min/100 g, and the effect of 4PB treatment was examined.

Results: The Tm of TC was increased by 4PB treatment from 0.86 ± 0.1 to1.26 ± 0.34 μmol/min/100 g, whereas the Tm of BSP was unchanged by 4PB treatment; 91 ± 10 vs. 87 ± 9 nmol/min/100 g in controls. The decreased Tm of TC (0.35 ± 0.21 μmol/min/100 g) by LPS treatment was relieved by 4PB (0.84 ± 0.23 μmol/min/100 g), and bile flow was also relieved from 0.35 ± 0.21 to 0.84 ± 0.23 μl/min/100 g.

Discussion/Conclusion: The increase of the Tm of TC suggests the further increase of Bsep amount at the CM. These data suggest that 4PB is effective in the treatment of intrahepatic cholestasis.

90

Hypertension enhances hepatic steatosis through oxidative stress associated with down-regulated expressions of LXR and FXR: Implication to the future strategy for non-alcoholic fatty liver diseases (NAFLD) by nuclear receptor ligands

Susumu Tazuma[1], Takuya Ikuta[1], Keishi Kanno[1], Mika Nakashima[1], Akiko Sugiyama[1], Nobusuke Kishikawa[1], Hisanobu Kodama[1], Mayumi Okamoto[1], Yuka Kikuchi[1], Shigeyuki Kushihata[1], Kennichi Yokobayashi[1], Masahumi Mizooka[1], Toshinari Saeki[1], and Koji Arihiro[2]
[1]Departments of General Internal Medicine, and [2]Anatomical Pathology, Hiroshima University Hospital, Hiroshima, Japan

Aim: Patients with NAFLD frequently have many co-morbidities including hypertension, which is known to increase vascular reactive oxygen species (ROS) production. Recently, we reported that hypertension enhances hepatic steatosis associated with oxidative stress using spontaneously hypertensive rats (SHR) fed with choline deficient diet (CD) as a NAFLD model (Hepatol Res 2012;42:310). Thus, hypertension might modulate the hepatic oxidative status and lipid metabolism to develop NAFLD. The aim of this study was to investigate the underlying mechanisms of potential effects of hypertension in the light of roles of nuclear factors and their regulating enzymes for cholesterol and bile acid metabolism.

Methods: SHR and Wistar-Kyoto (WKY) rats as normotensive controls were fed CD for 5 weeks, then hepatic histological changes, mRNA expressions of nuclear receptors, LXR and FXR, and their regulating molecules, were assessed.

Results: CD pronounced hepatic steatosis in SHR with an 8-fold increase of the hepatic triglyceride (TG), whereas unchanged in WKY. Changes in SHR were associated with reduced expression of mRNA for PPARalpha, ACOX, MTP, ApoB100 and both of LXR and FXR. Consistent with reduced expression of LXR and FXR, down-regulation of bile acid metabolism-related genes (cyp7a1, cyp8a1, cyp27a1) and canalicular membrane transporter genes (abcg5/g8, abcb11, abcb4) was evident in SHR fed CD.

Summary and Conclusions: *Hepatic steatosis found in CD-fed SHR* was associated with lipid accumulation caused by down-regulation of nuclear receptors, PPARalpha, LXR and FXR. Taken together, nuclear receptor ligands are promising agents for the future strategy of hypertension-related NAFLD.

Monitoring bile acid transport in single living cells using a genetically encoded FRET sensor

Stan F.J. van de Graaf[1], Maarten Merkx[2], Saskia W.C. van Mil[1], Leo W.J. Klomp[1], Ingrid T.G.W. Bijsmans[1], Misha V. Golynskiy[2*], Lieke M. van der Velden[1*]
[1]Department of Metabolic Diseases, University Medical Center Utrecht, The Netherlands
[2]Laboratory of Chemical Biology, Department of Biomedical Engineering, Eindhoven University of Technology, The Netherlands
*L.M.vdV and M.V.G contributed equally to this work

Introduction: Bile acids are pivotal for the absorption of dietary lipids and vitamins, and function as important regulators of glucose homeostasis, lipid metabolism and energy expenditure. Current methods to study cellular bile acid homeostasis and transport provide limited time and spatial resolution. Here, we describe a genetically encoded fluorescent sensor that allows for spatiotemporal monitoring of bile acid dynamics in single living cells.

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

Results: Changes in subcellular concentration of multiple bile acid species were detected as robust and reversible changes in Förster Resonance Energy Transfer (FRET) in multiple cell types. Influx of cell-impermeable bile acids was visualized in the presence of bile acid transport proteins. Uptake of cyprinol sulphate, the zebrafish bile alcohol, required expression of zebrafish ASBT. Similarly, Import of taurine- and glycine-conjugated bile acids was shown to depend on the expression of active NTCP. Combined cellular visualization of bile acid uptake with fluorescent labeling of cell surface resident NTCP showed that the low bile acid uptake activity of several NTCP variants was due to loss of transporter activity, as plasma membrane expression was unaffected. The reversible nature of the sensor also enabled measurements of bile acid efflux in living cells, and expression of the organic solute transporter αβ (OSTαβ) resulted in efflux of conjugated chenodeoxycholic acid.

Discussion/Conclusion: Genetically encoded fluorescent bile acid sensor (BAS) was developed that allows real time intracellular imaging of bile acid homeostasis in single living cells.

92

Genetic inactivation of the bile salt export pump in mice leads to massive cholesterol excretion

Mariëtte Y.M. van der Wulp[1,2], Theo H. van Dijk[2], Vincent W. Bloks[2], Albert K. Groen[1,2], and Henkjan J. Verkade[1,2]
[1]Top Institute Food and Nutrition, Wageningen, The Netherlands
[2]Pediatric Gastroenterology and Hepatology, Department of Pediatrics, Beatrix Children's Hospital, Groningen University Institute for Drug Exploration, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

Introduction: The bile salt export pump (Bsep) is the major hepatic bile salt (BS) transporter, facilitating BS transfer from liver to bile. Bsep$^{-/-}$ mice were previously shown to escape severe cholestasis by increasing biliary BS hydrophilicity, resulting in activation of alternative transporters. Bsep$^{-/-}$ mice surprisingly showed increased biliary cholesterol secretion. The consequence of strongly increased biliary cholesterol secretion for intestinal handling is not known. To determine these downstream effects of Bsep deficiency we determined biliary BS and whole body cholesterol fluxes.

Methods: We determined biliary BS composition and secretion, cholesterol input (intake, biliary secretion, absorption and synthesis), and fecal sterol output and origin (gas chromatography and stable isotope methodology) in Bsep$^{-/-}$ and Bsep$^{+/+}$ mice.

Results: Biliary BS secretion was decreased in Bsep$^{-/-}$ compared with Bsep$^{+/+}$ mice (-40%; $p < 0.01$). Biliary cholate secretion was decreased (-85%; $p < 0.01$), whereas beta-muricholate secretion was increased (+55%; $p < 0.01$) in Bsep$^{-/-}$ mice. In contrast to previous studies, we did not detect tetrahydroxylated BS in Bsep$^{-/-}$ mice. Cholesterol intake (+25%; $p < 0.01$), biliary secretion (by 3.5-fold; $p < 0.01$) and synthesis (by 10-fold; $p < 0.01$) were increased, whereas cholesterol absorption was decreased (-90%; $p < 0.01$) in Bsep$^{-/-}$ mice. Fecal cholesterol excretion increased 7-fold ($p<0.01$) in Bsep$^{-/-}$ mice. This was largely due to an increase of the trans-intestinal cholesterol excretion pathway.

Discussion/Conclusion: Bsep$^{-/-}$ mice show increased (transintestinal) cholesterol excretion, which appears to be induced by severely impaired cholesterol absorption in the presence of a hydrophilic BS pool. Potential health benefits of an increasingly hydrophilic BS pool in terms of cholesterol elimination form the body require further research.

Atp8B1 deficiency in mice, mimicking PFIC type 1, reduces the synthesis and pool size of the bile salt cholate

Henkjan J. Verkade[1], Karin E. Gooijert[1], Cindy Kunne[2], Renze Boverhof[1], Frans Stellaard[1], Coen C. Paulusma[2]
[1]Pediatric Gastroenterology/Hepatology, Beatrix Children's Hospital – University Medical Center Groningen, Groningen, The Netherlands
[2]Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, The Netherlands

Atp8b1 is expressed in many tissues, including the liver and the intestine. In humans, mutations in the ATP8B1 gene can cause Progressive Familial Intrahepatic Cholestasis type 1 (PFIC1). Atp8b1 deficient mice display mildly elevated serum bile salt levels without a defect in canalicular bile secretion. The canalicular membrane of Atp8b1-deficient hepatocytes has an increased sensitivity to extraction of cholesterol by taurocholate, resulting in reduced reduced activity of BSEP and MRP2 providing an explanation for the cholestasis. To further elucidate the mechanism, we now evaluated the physiological consequences of Atp8B1 deficiency on the kinetics of the enterohepatic circulation of cholate, the major bile salt specie in mice.

Methods: In Atp8b1$^{-/-}$ and control mice, cholate synthesis rate and pool size were determined by $^{2}H_{4}$-cholate stable isotope dilution technique. Plasma, bile and feces were collected for determination of cholate and total bile salts. Cycling time and intestinal absorption of cholate were calculated.

Results: Biliary bile flow, bile salt and phospholipid secretion and concentration were similar in Atp8b1$^{-/-}$ and control mice. Bile of Atp8b1 mice contained less cholic acid (-49), $P < 0.05$) and more beta- and omega muricholic acid (+231 and +131%, resp; each $P < 0.05$) compared with control mice. Cholate synthesis rate was decreased by 32% in Atp8b1 deficient mice ($P < 0.05$), which was associated with a reduction of the cholate pool size (46%; $P < 0.05$). Neither the calculated fractional turnover rate nor the cycling time of cholate were significantly altered. The fraction of cholic acid that was reabsorbed from the intestine per enterohepatic cycle was similar between the two groups (~7%; NS).

Conclusions: Atp8b1 deficiency in mice changes the bile composition and decreases the cholate synthesis rate and pool size. Despite expression of Atp8b1 in the intestine, its absence does not diminish the absorption efficacy of cholate. Present data support the hypothesis that the mechanism of PFIC1 cholestasis is primarily based in the liver rather than in the intestine.

94

Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-betamuricholic acid, a naturally occurring FXR antagonist

Annika Wahlström[1]*, Sama Islam[2]*, Jenny Felin[2], Hanns-Ulrich Marschall[1], Krister Bamberg[3], and Fredrik Bäckhed[2]
Sahlgrenska Academy, Institute of Medicine, Departments of [1]Internal Medicine and [2]Molecular and Clinical Medicine - Sahlgrenska Center for Cardiovascular and Metabolic Research/Wallenberg Laboratory, University of Gothenburg, S-413 45 Gothenburg, Sweden; [3]AstraZeneca R&D Mölndal, S-431 83 Mölndal, Sweden

Introduction: Farnesoid-X-receptor (FXR) is a nuclear receptor that is activated by bile acids and plays a key role in the regulation of bile acid synthesis and homeostasis. Bile acids are synthesized from cholesterol in the liver and further metabolized by the gut microbiota into secondary bile acids. In this study we aimed to investigate if the effects of the gut microbiota on bile acid homeostasis are mediated through FXR.

Methods: *Fxr*-deficient mice were rederived as germ-free (GF) in order to study the impact of the gut flora on bile acid metabolism through FXR. The expression of FXR target genes in the liver and distal ileum was analyzed. Taurine-conjugated beta-muricholic acid (TBMCA) was tested for direct FXR activity in a co-activator recruitment assay.

Results: Rederivation of *Fxr*-deficient mice as GF showed that the gut microbiota regulates expression of fibroblast growth factor-15 (FGF15) in the ileum and cholesterol-7α-hydroxylase (CYP7A1) in the liver by FXR-dependent mechanisms. Since GF mice have elevated bile acid levels, in particular TBMCA, but reduced FXR activation we hypothesized that GF bile contained a FXR antagonist. In agreement with this hypothesis we identified TBMCA as a potent endogenous FXR antagonist.

Discussion/Conclusion: We propose that the gut microbiota modulates bile acid synthesis by changing the bile acid profile and by alleviating FXR inhibition in the small intestine. In summary, we demonstrate that the microbial suppression of biosynthetic genes in the liver is consistent with increased FXR-dependent activation of FGF15 in the ileum due to reduced levels of TBMCA.

Reduced fibroblast growth factor 19 production in patients with primary bile acid diarrhoea

[1]Julian RF Walters, [1]Ian M Johnston, [1]Jonathan D Nolan, [1]Sanjeev S Pattni, [2]KD Bardhan, [1]Peter H Dixon, [1]Catherine Williamson
[1]Imperial College London, [2]Rotherham General Hospital, UK

Introduction: Primary bile acid diarrhea (PBAD), also known as idiopathic bile acid malabsorption, is a common cause of chronic watery diarrhoea which is under-diagnosed unless SeHCAT testing is available. We suggested that a possible cause was deficiency of the ileal hormone fibroblast growth factor 19 (FGF19), which regulates bile acid synthesis. We determined serum FGF19 in a prospective study of patients with unexplained chronic watery diarrhoea.

Methods: 211 patients were recruited prospectively and grouped into: normal SeHCAT (> 15%) 'diarrhoea controls'; reduced SeHCAT (< 15%) with no obvious disease 'PBAD'; those secondary to other causes were studied separately. Blood was assayed for FGF19. Some patients had repeated measurement throughout the day after meals. Polymorphisms in candidate genes were typed.

Results: Fasting FGF19 was significantly reduced in PBAD compared with diarrhoea controls (medians 147 vs. 225 pg/ml, $p < 0.001$). FGF19 and SeHCAT retention were significantly related, as were SeHCAT and BMI, and FGF19 with age. The response of patients with PBAD to sequestrants was significantly better in the group with reduced FGF19. Different patterns of FGF19 response to meals were seen; the integrated FGF19 response in PBAD correlated with SeHCAT retention. The subgroup of patients with all serum FGF19 levels < 300 pg/ml had SeHCAT values < 5% and appear more likely to have the common ASBT SNP A171S. This polymorphism however has been shown to not affect BA uptake.

Discussion/Conclusion: Reduced fasting and meal-stimulated FGF19 is a feature of PBAD There may be several causes of this. If stimulation of FGF19 can be achieved, there may be therapeutic benefits.

96

Bile acid precursors and oxysterol 7alpha-hydroxylase deficiency

Y. Wang[1], W.J. Griffiths[1], P. Crick[1], P.T. Clayton[2], A.A. Morris[3]
[1]College of Medicine, Swansea University, UK, [2]Institute of Child Heath, UCL, UK, [3]St Mary's Hospital, Manchester, UK

Abstract: Oxysterol 7α-hydroxylase deficiency presents in infants as neonatal cholestatic liver disease. Using an LC-MS^n strategy incorporating a novel derivatisation method we have profiled plasma from three patients and identified high levels of toxic C_{24} and C_{27} acids with a 3β-hydroxy-5-ene structure. Among other metabolites identified were high levels of monohydroxycholesterols, their sulphates and glucuronides.

Introduction: Oxysterol 7α-hydroxylase, CYP7B1, is the enzyme which adds a 7α-hydroxy group to oxysterols as part of the acidic and 25-hydroxylase pathways of bile acid biosynthesis. In infants enzyme deficiency presents as neonatal cholestasis, while in the adult as hereditary spastic paresis type 5. Here using a novel LC-MS^n methodology we have profiled the plasma sterolome of three infants with neonatal cholestasis in an effort to better understand the etiology of disease in infants.

Methods: Plasma samples from infants with oxysterol 7α-hydroxylase deficiency and controls were profiled using a novel LC-MS^n strategy utilising derivatisation. The derivatisation method greatly enhances sensitivity allowing the detection of cholesterol metabolites at the ng/mL level. Compounds were identified by reference to authentic standards or presumptively identified by accurate mass, retention time and fragmentation spectra.

Results: High levels of free 24-, 25- and (25R),26-hydroxycholesterols (> 50 ng/mL) were identified along with their sulphates and glucuronides in plasma from patients. Additionally, 3β-hydroxycholestenoic and 3β-hydroxycholenoic acids were particularly abundant (> 100 and > 1000 ng/mL respectively) in patient samples.

Discussion/Conclusion: Amongst the metabolites identified at high levels in patients with oxysterol 7α-hydroxylase deficiency are C_{24} and C_{27} acids with a 3β-hydroxy-5-ene function that are known to be hepatotoxic. Other metabolites of interest only found in patient samples are hydroxycholesterol sulphates and glucuronides. Monohydroxycholesterol sulphates have been suggested to be antagonists towards LXR.

The G protein-coupled bile acid receptor, TGR5, is expressed on colonic epithelial cells and regulates ion transport

Joseph Ward[1], Magdalena Mroz[1], Stephen Keely[1]
[1]Royal College of Surgeons in Ireland

Introduction: TGR5 has been implicated in the regulation of intestinal motility and has also been identified as a regulator of Cl^- secretion across gallbladder epithelium. However, there is a paucity of information regarding the expression and role of TGR5 on colonic epithelial cells (CECs). Here we sought to investigate a potential role for TGR5 in regulation of colonic epithelial ion transport.

Methods: 6alpha-ethyl-23(*S*)-methylcholic acid (INT-777) was used to activate TGR5. Ion transport was measured as changes in short-circuit current (I_{sc}) across voltage-clamped muscle-stripped segments of rat colon. mRNA expression was measured by RT-PCR. TGR5 expression/localisation was measured by confocal microscopy.

Results: TGR5 mRNA and protein were highly expressed in CECs. Confocal imaging revealed the protein to be localised bilaterally in rat colonic crypts. Acute bilateral addition of INT-777 (100 uM) to rat colonic mucosa caused a rapid and transient decrease in I_{sc} to 86.9 ± 5.2% (n = 8) of initial values, which returned to basal values by 15 minutes. INT-777 was effective from the basolateral, but not apical, side. The effects of TGR5 were not altered by pretreatment of the tissue with tetrodotoxin. Furthermore, treatment of rat CECs with INT-777 reduced subsequent Cl^- secretory responses to the cholinomimetic, carbachol (CCh), to 64.15 ± 1.73% of those in controls (n = 3).

Discussion/Conclusion: These studies reveal a novel role for TGR5 in regulating basal and secretagogue-induced colonic epithelial ion transport. TGR5 activation rapidly reduces basal I_{sc} and inhibits subsequent agonist-induced Cl^- secretory responses. Our data suggest that TGR5 may provide a new target for development of drugs to treat intestinal transport disorders.

The authors acknowledge Intercept Pharmaceuticals for supplying INT-777.

98

Bile acid binding resins affect diverse molecules to improve metabolic syndrome

Mitsuhiro Watanabe[1,2], Kohkichi Morimoto[1], Nao Kaneko-Iwasaki[1], Taichi Sugizaki[1], Yasushi Horai[1], Eri Arita[1], Karin Murahashi[1], Kristina Schoonjans[3], Johan Auwerx[3], and Hiroshi Itoh[1]
[1]Department of Internal Medicine, School of Medicine, Keio University, Tokyo 160-8582, Japan
[2]Graduate School of Media and Governance, Keio University, Kanagawa 252-0882, Japan
[3]Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland

Introduction: Bile acid binding resins (BABRs), anion exchange resins developed as medication for hypercholesterolemia, have been reported to improve glycemic control in patients and animal models with type 2 diabetes mellitus. Although the mechanism has not been clarified yet, we suspect that diverse factors including peripheral bile acid composition and incretins play significant roles in the effect. Besides BABRs, bile acids (BAs) administration has been recently reported to ameliorate metabolic status. In our study, we also administered BAs to metabolic syndrome model mice, to show similarities and differences of BABRs and BAs, to elucidate the mechanism of their anti-metabolic syndrome effects.

Methods: We evaluated the metabolic effects of BABR and BA by administering colestimide and cholic acid to male C57BL/6J and KK-Ay mice, at 6 weeks of age, fed normal or high-fat diet supplemented with/without colestimide or cholic acid. A dipeptide peptidase-4 (DPP-4) inhibitor had been also administered alone or combined with colestimide or cholic acid. We performed animal studies including body weight gain, food intake, OGTT, IPGTT, IPITT, serum GLP-1 and insulin concentrations. Morphological study of major tissues, bile acid composition analysis, and gene expression analysis were conducted with samples from the model mice.

Results: BABR administration increased energy expenditure to induce weight reduction and insulin sensitization. The effect was similar to that of BA administration on BA composition and thermogenesis. BABR and BA also stimulated GLP-1 secretion, and additional administration of DPP-4 inhibitor augmented antidiabetic effect of BABR and BA.

Discussion/Conclusion: Our data suggest that BABRs could be useful for the management of not only hypercholesterolemia but metabolic syndrome. Furthermore, Combination uses of DPP-4 inhibitor with BABRs or BAs could exhibit great efficacy, providing clues to elucidate the effect of BABRs, and proposing new combination of established medications.

Microscopy based method for the quantification of subcellular localization of hepatobiliary transporters

Katrin Weissenberger, Stefanie Kluge, Jan Stindt, Dieter Häussinger, Ralf Kubitz
Department of Gastroenterology, Hepatology and Infectious Diseases, University Hospital Düsseldorf, Germany

Introduction: Bile formation critically depends on ABC-transporters such as BSEP and MDR3. Mutations of these transporters often result in targeting defects or altered protein expression and turnover, eventually leading to liver damage due to retention of cholephils in hepatocytes.

Methods: In order to analyze the intracellular fate of these ABC transporters, they were fused to a yellow fluorescent protein and expressed in different human cell lines. Subcellular compartments were visualized by immunostaining with GM130 (Golgi), PDI (ER) and the Na^+K^+ATPase (plasma membrane). Pictures were acquired with fixed microscope settings (Zeiss, LSM510Meta) and analyzed with the AxioVision software to obtain pixel characteristics according to a preset threshold.

Results: With a customized workflow, total expression of transporter and marker proteins, the degree of co-localization and cell size were extracted from primary data. To validate the method, the BRIC-2 mutation p.G374S of BSEP was compared to wildtype BSEP. Both proteins were targeted to the plasma membrane. 60% of $BSEP^{wt}$ but only 50% of $BSEP^{G374S}$ positive pixels co-localized with Na^+K^+ATPase. Interestingly, the total expression of $BSEP^{G374S}$ was found to be 1.8-fold higher than that of the wildtype, indicating an altered turnover of $BSEP^{G374S}$. First results indicate a larger amount of $BSEP^{G374S}$ (36%) in co-localization with the ER marker PDI as compared to $BSEP^{wt}$ (20%).

Discussion/Conclusion: Our findings demonstrate the feasibility of image based quantification of subcellular transporter localization, which may serve to evaluate the molecular impact of BSEP mutations on protein expression and targeting.

100

A prospective national study of severe intrahepatic cholestasis of pregnancy

C. Williamson[1], V. Geenes[1], B. North[2], M. Knight[3]
[1]Maternal and Fetal Disease Group, Institute of Reproductive and Developmental Biology, Imperial College London, [2]Statistical Advisory Service, Imperial College London, [3]UK Obstetric Surveillance System, National Perinatal Epidemiology Unit, University of Oxford, UK

Introduction: Intrahepatic cholestasis of pregnancy (ICP) is characterised by maternal pruritus and raised serum bile acids (SBA). Previous studies have shown that high levels of maternal SBA are associated with increased rates of adverse pregnancy outcome. In the largest study to date, the risk of spontaneous preterm labour, asphyxial events and meconium-stained liquor, placenta or membranes did not occur until maternal SBA were ≥ 40 µmol/l (i.e. severe ICP). We aimed to establish whether the non-fasting maternal serum bile acid level was also associated with adverse pregnancy outcomes, including the risk of stillbirth, in a large prospectively collected national cohort of ICP cases known to have SBA ≥ 40 µmol/l.

Methods: Cases of severe ICP were identified from all UK maternity units between June 2010 and July 2011 by the UK Obstetric Surveillance System (UKOSS). Severe ICP was defined as a maternal SBA of > 40 µmol/l at any time during pregnancy. Information regarding maternal biochemistry and fetal outcomes was collected. Data were compared to matched controls and analysed using SPSS.

Results: 714 cases of severe ICP were identified from an estimated 510,325 deliveries giving an incidence of 0.14%. A combination of logistic regression and generalised additive models revealed a significant relationship between maternal SBA and meconium-stained amniotic fluid ($p = 0.0003$), spontaneous preterm labour ($p = 0.003$), Apgar score < 7 at 5 minutes post-delivery ($p = 0.019$) and stillbirth ($p = 0.009$).

Discussion/Conclusion: This prospective study is seven times larger than any previous study of adverse pregnancy outcome in severe ICP. This study suggests that stillbirth, spontaneous preterm labour, low Apgar score and meconium-stained liquor are all seen more frequently in pregnancies complicated by severe ICP and that the frequency of these complications rises with increasing levels of maternal serum bile acids.

FXR- and BDL-mediated transcriptional regulation of hepatic and extrahepatic glucuronidation in a humanized *tgUGT1A* mouse model

A. Winkler, S. Kalthoff, N. Freiberg, M.P. Manns, C.P. Strassburg
Department for Gastroenterology, Hepatology and Endocrinology, Hanover Medical School, Hanover, Germany

Introduction: Glucuronidation is an important detoxification process that is transcriptionally regulated by xenobiotics, oxidative stress and bile acids (farnesoid-X-receptor, FXR). Bile acids such as chenodeoxycholic acid (CDCA) are also substrates for glucuronidation, which leads to their detoxification and elimination. Aim of this study was to elucidate transcriptional *UGT1A* regulation *in vivo* during cholestasis and/or under treatment with the FXR agonist GW4064.

Methods: *TgUGT1A* WT mice were subjected to bile duct ligation (BDL) for 5 days; 4 days intraperitoneal (i.p.) injection with GW4064 or 4 days i.p. injection with GW4064 after BDL. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), as well as bilirubin were analyzed and *UGT1A* expression was determined by Taqman-PCR in liver, jejunum and colon.

Results: After BDL/BDL+GW4064, serum transaminase activities as well as bilirubin levels were significantly increased in *tgUGT1A WT* mice compared to controls, while GW4064 treatment only lead to a slight AST elevation.
In *tgUGT1A WT* mice (BDL), all hepatic *UGT1A* genes were significantly induced (1.5-4.2-fold), while induction of intestinal UGTs was only detected for *UGT1A3* and *UGT1A4*.
GW4064 treatment resulted in significant upregulation of all *UGT1A* genes in the liver (2-5-fold) and in colon (2.4-45-fold), but only 1.3-fold induction of *UGT1A1* in jejunum.
In BDL+GW4064 mice, only hepatic *UGT1A3* (1.5-fold) was significantly induced; no further significant induction was observed in jejunum.

Discussion/Conclusion: Our data demonstrate a differential and tissue specific upregulation of glucuronidation during obstructive cholestasis (BDL) and under treatment with the FXR agonist GW4064 *in vivo*. Interestingly, the additional treatment with GW4064 during cholestasis does not lead to further upregulation of glucuronidation, but rather an increased toxicity indicated by elevated ALT and AST levels. These data are relevant for the understanding of alterations in hepatic drug and xenobiotic metabolism in patients suffering from cholestatic diseases and the development of new therapeutic strategies.

102

A novel di-leucine motif at the N-terminus of human Organic Solute Transporter-beta (hOST-beta) is essential for protein interaction and membrane localization

Shuhua Xu[1], An-Qiang Sun[1], Donald S. Backos[2], YuHuan Luo[1], Philip Reigan[2] and Frederick J. Suchy[1]
[1]Department of Pediatrics, University of Colorado Denver School of Medicine, Aurora, CO 80045, [2]Skaggs School of Pharmacy and Pharmaceutical Sciences, Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA

Introduction: The heterodimer membrane protein [OST-alpha/OST-beta] appears to be the major bile acid efflux transporter in intestine. Physical association of hOST-alpha and -beta subunits is essential for their polarized basolateral membrane localization and function in the export of bile acids.

Methods: To identify the functionally important motifs of hOST-beta, the effects of mutagenesis, [$^{+}$H] estrone-3-sulfate transport assay, co-immunoprecipitation (co-IP), membrane biotinylation, and confocal microscopy analysis were investigated in hOST-alpha/beta co-transfected HEK-293 and MDCK cells.

Results: After deletion of 35 amino acids from the amino-tail of hOST-beta, the efflux transport activity and polarized membrane distribution of the truncated hOST-beta was abolished. Co-IP analysis verified that the amino-tail of hOST-beta was essential for the association with hOST-alpha. A highly conserved acidic di-leucine motif (-$EL_{20}L_{21}$EE) was identified at the amino-tail of OST-beta among various species. To further identify the protein interacting domain between hOST-beta and hOST-alphaand its effect on membrane localization, Lue20 and Lue21 on the amino-tail of hOST-beta were replaced with alanines by site directed mutagenesis. Co-IP study demonstrated that substitution of the leucine residues with alanines interrupted the interaction of the hOST-beta mutant with the hOST-alpha subunit. Membrane biotinylation demonstrated that the Leu20/21Ala mutant eliminated membrane expression of both subunits. Computational-based modelling of hOST-beta suggested that the Leu20/21Ala mutation substantially alters both the structure and lipophilic potential of the surface that has the potential to affect the interaction with the alpha-subunit. In addition, this change may also impact the capacity of hOST-beta to interact with the membrane as well as the migratory capability of the N-terminal domain.

Discussion/Conclusion: In summary, the di-leucine motif (Leu20/Leu21) on the N-terminal region of hOST-beta subunit plays a critical role on hOST-beta protein association with OST-alpha and polarized plasma membrane localization.

Bile acids and sphingosine-1 phosphate receptor signaling in cholangiocarcinoma progression

Huiping Zhou, Renping Zhao, Xiqiao Zhou, Deanna J.W. Campbell,
Alphonse E. Sirica, and Phillip B. Hylemon
Department of Microbiology and Immunology, Department of Pathology, Division of Cellular and Molecular Pathogenesis, School of Medicine, Virginia Commonwealth University, Richmond, VA 23298, USA

Introduction: The bile duct obstruction is a potent stimulus for intrahepatic cholangiocarcinoma (ICC) growth and progression. The sphingosine-1 phosphate (S1P)-mediated signaling pathways have been closely linked to the development and progression of various types of human cancers. Our recent studies demonstrated that conjugated bile acids, such as taurocholate (TCA), activate the ERK1/2 and AKT signaling pathways through the S1P receptor 2 ($S1P_2$) in hepatocytes. However, the role of S1P receptor signaling in bile acid-mediated ICC progression has not been identified and is the focus of this study.

Methods: Rat and human ICC cells and cancer-associated myofibroblasts (CAFs) cell lines were used. The mRNA and protein levels of target genes were determined by real-time RT-PCR and Western blot analysis, respectively. Cell proliferation was determined using CellTiter Aqueous One solution kit. The cell migration was assessed by scratch assay.

Results: The $S1P_2$ is the predominant S1P receptor expressed in both human and rat ICC cells and sphingosine kinase-1 (SphK1) is highly upregulated in both human and rat CAFs cells. TCA activated $S1P_2$, which further activated the downstream ERK1/2 and AKT signaling pathways and upregulated $S1P_1$ expression in ICC cells and CAFs. Both TCA- and S1P-induced cell migration and proliferation were inhibited by a specific $S1P_2$ chemical inhibitor (JTE-013) and shRNA.

Discussion/Conclusion: These results suggest that conjugated bile acids promote ICC progression by regulating the expression and activation of S1P receptors and SphKs in ICC cells and CAFs. The S1P receptors and SphKs may represent novel molecular targets for cholangiocarcinoma therapy.

104

Reduced FGF19 level in bile of patients with primary sclerosing cholangitis

S.J.L.B. Zweers[1], A. Shiryaev[2], T.H. Karlsen[2], P.L.M. Jansen[1,3], F.G. Schaap[1]
[1]Tytgat Institute for Intestinal and Liver Research, Academic Medical Center, Meibergdreef 69-71, 1105 BK Amsterdam, The Netherlands
[2]Norwegian PSC Research Center, Clinic for Specialized Medicine and Surgery, Oslo University Hospital, Rikshospitalet, Oslo, Norway
[3]Department of Hepatology and Gastroenterology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

Introduction: Human bile contains Fibroblast Growth Factor 19 (FGF19) at levels far exceeding those in the circulation (Zweers et al. Hepatology 55: 575–83). Apart from metabolic functions, binding of FGF19 to its receptor FGFR4 restrains TNFα-induced NFκB signaling (Drafahl et al. PLoS One 5: e14412). Considering this anti-inflammatory action of FGF19, we anticipated that there might be a deficiency of FGF19 in primary sclerosing cholangitis (PSC), a disease characterized by ongoing biliary inflammation. Therefore we studied FGF19 expression in bile and in gallbladders of patients with PSC.

Methods: Gallbladder bile (n = 13–14 per group) and gallbladder tissue (n = 12–13 per group) of PSC and non-PSC patients with various liver diseases were collected at the time of liver transplantation (LTx). FGF19 protein levels were measured by ELISA. Transcript levels of FGF19, the FXR target SHP, and the apical sodium dependent bile salt transporter (ASBT) were measured by RT-qPCR using mucosal epithelium-specific cytokeratin 7 and 19 as reference genes.

Results: In bile of PSC patients, the FGF19 level is significantly reduced when compared to non-PSC patients with liver disease and non-transplanted controls (7.0 ± 6.7 vs. 23.6 ± 25.9 ng/mL and 21.9 ± 13.3, respectively; $p < 0.005$). In gallbladder specimens of PSC patients, ASBT and SHP mRNA expression are significantly reduced (5.1 and 2.2 fold, respectively, both $p < 0.005$). In contrast, gallbladder FGF19 expression was not significantly different in PSC patients.

Discussion/Conclusion: In conclusion, FGF19 levels in bile of PSC patients are reduced. This is likely the result of decreased FGF19 production in the intrahepatic biliary tract. As FGF19 has anti-inflammatory properties, depletion may further enhance pre-exisiting biliary inflammation.

Author Index to Poster Abstracts

(Name – Poster Number)

Falk Foundation
Dr. Falk Pharma

Where medicine and pharmaceuticals meet – a tried and trusted link

Innovative Drugs
for bowel and liver diseases

Modern formulations and specially designed delivery systems ensure targeted release of the active drug

Scientific Dialogue
in the interest of therapeutic progress

Falk Symposia and Workshops
nearly 250, attended by more than 100,000 participants from over 100 countries since 1967

Continuing medical education seminars
over 14,000, attended by more than one million physicians and patients in Germany alone

Comprehensive literature service for healthcare professionals and patients
with more than 200 publications

www.falkfoundation.org **www.drfalkpharma.com**

Leinenweberstr. 5 79108 Freiburg Germany Tel +49 (0)761/1514-0 **Fax** +49 (0)761/1514-321 **Mail** zentrale@drfalkpharma.de