Research Article

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Ursodeoxycholic acid exerts farnesoid X receptor-antagonistic effects on bile acid and lipid metabolism in morbid obesity

Michaela Mueller[1,2], Anders Thorell[3,4], Thierry Claudel[1], Pooja Jha[1,2], Harald Koefeler[5], Carolin Lackner[6], Bastian Hoesel[7], Guenter Fauler[8], Tatjana Stojakovic[8], Curt Einarsson[9], Hanns-Ulrich Marschall[10,*,†], Michael Trauner[1,*,†]

[1]*Hans Popper Laboratory of Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria;* [2]*Laboratory of Experimental and Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Medicine, Medical University of Graz, Graz, Austria;* [3]*Karolinska Institutet, Department of Clinical Science at Danderyds Hospital, Stockholm, Sweden;* [4]*Department of Surgery, Ersta Hospital, Stockholm, Sweden;* [5]*Core Facility for Mass Spectrometry, Medical University of Graz, Graz, Austria;* [6]*Institute of Pathology, Medical University of Graz, Graz, Austria;* [7]*Department of Vascular Biology and Thrombosis Research, Center for Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria;* [8]*Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria;* [9]*Karolinska Institutet, Department of Medicine, Karolinska University Hospital Huddinge, Stockholm, Sweden;* [10]*Institute of Medicine, Department of Molecular and Clinical Medicine, Sahlgrenska Academy, University of Gothenburg, Sweden*

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Background & Aims: Bile acids (BAs) are major regulators of hepatic BA and lipid metabolism but their mechanisms of action in non-alcoholic fatty liver disease (NAFLD) are still poorly understood. Here we aimed to explore the molecular and biochemical mechanisms of ursodeoxycholic acid (UDCA) in modulating the cross-talk between liver and visceral white adipose tissue (vWAT) regarding BA and cholesterol metabolism and fatty acid/lipid partitioning in morbidly obese NAFLD patients.

Methods: In this randomized controlled pharmacodynamic study, we analyzed serum, liver and vWAT samples from 40 well-matched morbidly obese patients receiving UDCA (20 mg/kg/day) or no treatment three weeks prior to bariatric surgery.

Results: Short term UDCA administration stimulated BA synthesis by reducing circulating fibroblast growth factor 19 and farnesoid X receptor (FXR) activation, resulting in cholesterol 7α-hydroxylase induction mirrored by elevated C4 and 7α-hydroxycholesterol. Enhanced BA formation depleted hepatic and LDL-cholesterol with subsequent activation of the key enzyme of cholesterol synthesis 3-hydroxy-3-methylglutaryl-CoA reductase. Blunted FXR anti-lipogenic effects induced lipogenic stearoyl-CoA desaturase (SCD) in the liver, thereby increasing hepatic triglyceride content. In addition, induced SCD activity in vWAT shifted vWAT lipid metabolism towards generation of less toxic and more lipogenic monounsaturated fatty acids such as oleic acid.

Conclusion: These data demonstrate that by exerting FXR-antagonistic effects, UDCA treatment in NAFLD patients strongly impacts on cholesterol and BA synthesis and induces neutral lipid accumulation in both liver and vWAT.

Keywords: Non-alcoholic fatty liver disease; FGF19; 3-hydroxy-3-methylglutaryl-CoA reductase; Lipogenesis; Stearoyl-CoA desaturase.

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* Corresponding authors. Addresses: Professor of Clinical Hepatology, Sahlgrenska Academy, Institute of Medicine, Department of Molecular and Clinical Medicine, S-41345 Gothenburg, Sweden. Tel.: +46 70 8774073; fax: +46 31 827458 (H.-U. Marschall) or Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria. Tel.: +43 1 40 40047410; fax: +43 1 40 400 47350 (M. Trauner).

E-mail addresses: hanns-ulrich.marschall@gu.se (H.-U. Marschall), michael.trauner@meduniwien.ac.at (M. Trauner).

† Shared senior authorship and correspondence.

Abbreviations: BAs, bile acids; NAFLD, non-alcoholic fatty liver disease; UDCA, ursodeoxycholic acid; vWAT, visceral white adipose tissue; FXR, farnesoid X receptor; SCD, stearoyl-Coa desaturase; NASH, non-alcoholic steatohepatitis; TGs, triglycerides; FAs, fatty acides; CYP7A1, cholesterol 7α-hydroxylase; SHP, small heterodimer partner; FGF19, fibroblast growth factor 19; SREBP1c, sterol regulatory element-binding protein-1c; FASN, fatty acid synthase; VLDL, very low density lipoproteins; CDCA, chenodeoxycholic acid; CA, cholic acid; C4, 7α-hydroxy-4-cholesten-3-one; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; ABC, ATP-binding cassette; LDLR, low density lipoprotein receptor; MA, myristic acid; PA, palmitic acid; SA, stearic acid; OA, oleic acid; MTTP, microsomal triglyceride transfer protein; ApoB, apolipoprotein B; FATP1, fatty acid transport protein 1; nCEH, neutral cholesterol ester hydrolase.

Introduction

Obesity is a major risk factor for the development of type 2 diabetes, hypertension and dyslipidemia exerting adverse effects on the liver. The prevalence of non-alcoholic fatty liver disease (NAFLD), as hepatic manifestation and major complication of obesity and the metabolic syndrome, is dramatically rising and comprises a spectrum ranging from steatosis over non-alcoholic

steatohepatitis (NASH) to advanced fibrosis/cirrhosis, ultimately leading to liver cancer. NAFLD is found in over two thirds of the obese population, regardless of diabetic status, and in more than 90% of morbidly obese individuals (>40 kg/m^2 body mass index (BMI)); NASH is diagnosed in 19% and almost 50% of these individuals, respectively [1]. A key feature of NAFLD is the hepatic accumulation of triglycerides (TGs) and free cholesterol. Expansion of white adipose tissue (WAT) with increased lipolysis and flux of fatty acids (FAs) to the liver due to insulin resistance (IR) critically links WAT dysfunction to NAFLD/NASH development [2].

In addition to their detergent properties in lipid digestion, bile acids (BAs) serve as signaling molecules by activating dedicated receptors such as the nuclear farnesoid X receptor (FXR) in the liver and intestine, which impacts on BA and lipid metabolism [3,4]. Upon FXR activation, BA homeostasis is maintained via a negative feedback loop decreasing expression of cholesterol 7α-hydroxylase (CYP7A1), the key enzyme in BA *de novo* synthesis, which mediates the conversion of cholesterol into BAs. Hepatic CYP7A1 is repressed by FXR-induced small heterodimer partner (SHP) and by fibroblast growth factor 19 (FGF19). In addition, FXR stimulation lowers triglyceridemia and hepatic TG deposition by reducing the expression of lipogenic genes and their regulators including sterol regulatory element-binding protein-1c (SREBP1c), fatty acid synthase (FASN) and stearoyl-CoA desaturase (SCD) [3]. Conversely, FXR deficiency in mice results in marked hypertriglyceridemia as a result of low apolipoprotein C-II and high apolipoprotein C-III, which reduces the interactions of chylomicrons and very low density lipoproteins (VLDL) with the lipoprotein lipase and their breakdown [5].

Ursodeoxycholic acid (UDCA) is currently used as 'panacea' for pharmacological treatment for a wide range of hepatobiliary disorders and has been shown to improve steatosis and inflammation in mice [6]. Taurine-conjugated UDCA reduced hepatic steatosis and enhanced insulin action in mouse liver, muscle and WAT [7] and enhanced hepatic and muscle insulin sensitivity in obese humans [8].

Clinical studies with UDCA in NAFLD have generated results raising questions about therapeutic mechanisms of BAs: While two randomized placebo-controlled trials did not show overall histological improvement including ballooning and inflammation [9,10], a recent high-dose UDCA study attenuated hepatic IR [11]. Importantly, understanding the mechanism(s) of action of UDCA may be instrumental for the development of more effective BA-based therapies for NAFLD/NASH.

In the present study, we aimed to investigate potential effects and underlying mechanisms of short term UDCA exposure on the interplay between hepatic and visceral WAT (vWAT) metabolism by analyzing; (i) BA and cholesterol homeostasis; (ii) biliary transporter expression; and (iii) FA/lipid partitioning in morbidly obese patients with NAFLD/NASH. We herein uncover numerous mechanistically interrelated changes in serum parameters and mRNA expression patterns of genes involved in BA, cholesterol and lipid metabolism. Moreover, we provide a detailed lipidomic profile of liver and vWAT uncovering altered storing properties upon UDCA administration.

Patients and methods

Study population

Patients with morbid obesity (BMI >35 kg/m^2) scheduled for laparoscopic Roux-en-Y gastric bypass surgery at Ersta Hospital, Stockholm, were asked to participate in a clinical pharmacodynamic study of the metabolic and molecular effects of UDCA. All candidates completed a detailed questionnaire about the patient's history and life-style. A total of 40 well-matched patients were equally randomized by drawing lots to treatment with UDCA, 20 mg/kg/day, for three weeks (Ursofalk®, Dr. Falk, Freiburg, Germany; kind gift of MEDA, Stockholm, Sweden) or no medication (controls) before surgery. UDCA was administered open-label in two daily doses until the day before surgery, i.e. the first dose was given after drawing blood on day 1, the last dose in the evening before surgery on day 21. Liver, kidney, intestinal or metabolic diseases other than NAFLD/NASH (alanine aminotransferase (ALT)/aspartate aminotransferase (AST)/gamma-glutamyl transferase (γGT) <3 × ULN) were exclusion criteria, as well as the use of medications known to affect liver function and metabolism. Blood samples were taken in the fasting state at 8:00 AM, and tissue samples by ultrasound dissector during surgery. No day 21 serum samples were taken in the control group. Based on a widely accepted histological scoring system [12], the lesions of NAFLD were classified as fatty liver or steatohepatitis on liver biopsies after surgery by a board certified pathologist (C.L.).

All participants provided written informed consent. The study protocol (ClinicalTrials.gov NCT01548079) was approved the by Ethics Committee at Karolinska Institutet (Dnr 2008/2:3) and the Swedish Medical Products agency (EudraCT 2007-005531-28).

For more details, see Supplementary Materials and Methods.

Results

Patient characteristics

Out of 40 randomized patients, 19 finished per protocol in the UDCA and 18 in the control groups. Drop-outs were due to diarrhea in the UDCA, and pregnancy and minor intraoperative bleeding in the control groups. Gender, age, BMI, liver function tests and IR (estimated by HOMA-IR) did not differ between groups. Compliance to UDCA (>95% in each patient randomized to treatment) was confirmed by pill counts and UDCA measurements in serum. All participants had been instructed not to change their dietary habits, thus, BMI increased during the study period, both in UDCA (42.4 ± 5.1 kg/m^2 to 43.2 ± 5.2 kg/m^2, p <0.05) and control (40.6 ± 3.9 kg/m^2 to 41.1 ± 3.7 kg/m^2, p <0.05) groups. Interestingly, histological analysis revealed a higher steatosis grade (1.2 to 1.9, p <0.05) and thereby NAFLD activity score (NAS) (1.9 to 2.5, p <0.05) in the UDCA treated patients compared to untreated controls at the day of surgery. Baseline HOMA-IR classified 18/19 UDCA and 15/18 control patients as insulin resistant. Fasting glucose and HbA1c levels were normal in all patients.

UDCA increased BA synthesis and cholesterol turnover

UDCA treatment resulted in reductions of serum AST, γGT, as well as free FA, total and LDL-cholesterol (LDL-C), whereas TGs increased (Table 1).

Upon UDCA, BAs increased 10-fold with UDCA enrichments in the range of recently reported peak concentrations in non-cholestatic subjects [13]. UDCA constituted 87.7 ± 3.7% of total BAs, in equal amounts unconjugated or glycine-conjugated (Supplementary Table 1). Of note, also the amounts of primary BAs chenodeoxycholic acid (CDCA) and cholic acid (CA) increased as their glycine-conjugates (Supplementary Table 1). Whereas CDCA elevations may have resulted from intestinal and/or hepatic epimerization of UDCA [14], the simultaneous increase of CA indicated enhanced *de novo* synthesis.

We first focused on changes in BA metabolism. Serum BA precursors, 7α-hydroxy-cholesterol and 7α-hydroxy-4-cholesten-3-one (C4), were increased (Table 1) and mRNA and protein expression levels of CYP7A1 were higher in liver samples of