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Metformin impacts cecal bile acid profiles in mice

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ABSTRACT

Bile acids (BAs) are major components of bile synthesized from cholesterol and take part in the digestion of dietary lipids, as well as having signaling functions. They undergo extensive microbial metabolism inside the gastrointestinal tract. Here, we present a method of ultra-high pressure liquid chromatography coupled to ion trap mass spectrometry for quantification of 45 BAs in mouse cecum. The system was validated in regard to sensitivity with limits of detection and quantification (0.6–24.9 nM), interday accuracy (102.4%), interday precision (15.2%), recovery rate (74.7%), matrix effect (98.2%) and carry-over effect (< 1.1%). Afterwards, we applied our method to investigate the effect of metformin on BA profiles. Diabetic mice were treated with metformin for 1 day or 14 days. One day of treatment resulted in a significant increase of total BA concentration (2.7-fold increase; *db/db* metformin 5.32 µmol/g, *db/db* control mice 1.95 µmol/g), most notable in levels of 7-oxodeoxycholic, 3-dehydrocholic and cholic acid. We observed only minor impact on BA metabolism after 14 days of metformin treatment, compared to the single treatment. Furthermore, healthy wild type mice had elevated concentrations of allocholic and ω-muricholic acid compared to diabetic mice. Our method proved the applicability of profiling BAs in cecum to investigate intestinal BA metabolism in diabetes and pharmacological applications.

1. Introduction

Bile acids (BAs) are key compounds in determining co-microbial metabolism in the intestinal tract of mammals. BAs are classified into primary, secondary and tertiary BAs. Cholesterol is converted by classical, acidic or alternative pathways into the primary BAs chenodeoxycholic (CDCA) and cholic acid (CA) [1]. CA and CDCA are common primary BAs detected in human bile, whereas CA and muricholic acid (MCA) are major BAs in mice [2]. In rodents, CDCA is converted into more hydrophilic MCAs, resulting in six unique BAs including alpha (α), beta (β), omega (ω) MCA. Furthermore, BAs undergo taurine (rodents) or glycine (humans) conjugation in hepatocytes before entering the biliary system and excreted into the gut. Here, conjugated BAs are hydrolyzed into their free forms by bile salt hydrolases which are found in several bacteria including Enterococci, Bacteroides, Lactobacillus and Clostridia [3]. Released CA and CDCA are converted by several bacteria (7α-dehydroxylation and 12α-dehydroxylation) into the secondary BAs deoxycholic acid (DCA) and lithocholic acid (LCA), respectively [4]. DCA and LCA can be further transformed into their isoforms, for example by *Ruminococcus gnavus* [5]. βMCA is converted into the secondary BA ωMCA which is a product of bacterial conversion performed through Clostridium group III or cooperative work of three strains [6].

Recently, BA separation and quantification is preferably performed by liquid chromatography (LC) coupled to mass spectrometry (MS). Here, extensive and tedious preparation steps such as hydrolysis of conjugated BAs and derivatization are not needed compared to gas chromatography (GC) methods [7]. Most of the published methods used octadecyl (C18) reversed-phase (RP) chemistry mainly coupled to triple quadrupole MS. BAs were profiled in humans, mice and rats in many different matrices such as serum, plasma, urine, bile, liver and adipose tissue. There is a large variation in the number of validated and quantified BAs ranging from 9 to 57 BAs with total analysis times between 5 and 32 min. Mouse plasma, urine, bile and liver contain mainly taurine conjugated BAs (TBAs), especially TMCAs (α, β and ω) and TCA [8–10]. Reported concentrations vary strongly, for example TCA ranges between 74.1 and 3090 ng/mL in murine serum or plasma samples [8–20].

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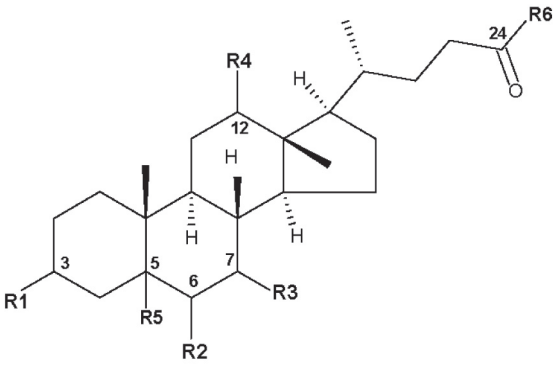

Conjugations	R6
unconjugated (uBA)	OH
glycine conjugated (GBA)	$NHCH_2COOH$
taurine conjugated (TBA)	$NH(CH_2)_2SO_3H$

BA	R1	R2	R3	R4	R5	Classification
CA-7S	α-OH	H	α-SO_3OH*	α-OH	β-H	primary
7-epiCA	α-OH	H	β-OH	α-OH	β-H	secondary
7,12-dioxoLCA	α-OH	H	=O	=O	β-H	secondary
ωMCA	α-OH	α-OH	β-OH	H	β-H	secondary
αMCA	α-OH	β-OH	α-OH	H	β-H	primary
7-oxoDCA	α-OH	H	=O	α-OH	β-H	secondary
βMCA	α-OH	β-OH	β-OH	H	β-H	primary
MDCA	α-OH	β-OH	H	H	β-H	secondary
HCA	α-OH	α-OH	α-OH	H	β-H	primary
3-dehydroCA	=O	H	α-OH	α-OH	β-H	secondary
alloCA	α-OH	H	α-OH	α-OH	α-H	primary
CA	α-OH	H	α-OH	α-OH	β-H	primary
UDCA	α-OH	H	β-OH	H	β-H	primary
HDCA	α-OH	α-OH	H	H	β-H	secondary
6-oxo-alloLCA	α-OH	=O	H	H	α-H	secondary
6,7-dioxoLCA	α-OH	=O	=O	H	β-H	secondary
7-oxoLCA	α-OH	H	=O	H	β-H	secondary
12-oxoLCA	α-OH	H	H	=O	β-H	secondary
apoCA	α-OH	H	α-OH	β-OH	β-H	secondary
3-dehydroCDCA	=O	H	α-OH	H	β-H	secondary
CDCA	α-OH	H	α-OH	H	β-H	primary
DCA	α-OH	H	H	α-OH	β-H	secondary
alloLCA	α-OH	H	H	H	α-H	secondary
isoLCA	β-OH	H	H	H	β-H	secondary
7,12α-dihydroxy-5β-cholanic acid	β-OH	H	H	α-OH	β-H	secondary
Lithochol-11-enoic acid	α-OH	H	H	= **	β-H	secondary
LCA	α-OH	H	H	H	β-H	secondary
dehydroLCA	=O	H	H	H	β-H	secondary

*sulfated BA (SBA)
**double bond at position 11-12

Fig. 1. Chemical structure of bile acids and conjugates.

Few studies quantified BAs in the gastrointestinal tract of mice although BAs are highly abundant and diverse in this organ [13,21]. They are released from the gall bladder into the small intestine, a process triggered by nutrients, promoting absorption of lipophilic dietary compounds [22]. Most BAs are reabsorbed by enterohepatic circulation but a small percentage of 5% escapes into cecum and further into the colon of mice. Here, BAs face extensive bacterial metabolism including deconjugation, dehydroxylation or epimerization. So far, studies analyzing murine cecal content for BA quantities and composition examined their role in the context of germ-free and specific pathogen free mice [23], the impact of diet [24], interplay between FXR receptor and gut microbiota [25], protection from *Clostridium difficile* [26], alcohol consumption [27], and prebiotics [28,29]. Total cecal BA content in mice was reported to range between 0.11 and 20 µmol/g, depending on the number of BAs taken into account [23,24,26–28,30–36].

In this study, a wide range of BAs, 45 in total, were quantified in the luminal content of cecum in mice, performed by UHPLC-MS, with an octyl reversed-phase chemistry column and an ion trap mass spectrometer. Our method was validated according to intra- and interday accuracy and precision, recovery rate, matrix effect and carry-over. Finally, we applied our method to investigate the effects of metformin on bile acid metabolism in a diabetic mouse model.

2. Material and methods

2.1. Reagents and materials

Forty-five BAs and 4 deuterated BAs were used for validation and quantification purposes. Their specifications are summarized in supplementary information (Table S1). Methanol (MeOH) and acetonitrile were purchased from FLUKA, Sigma-Aldrich (LC-MS grade, CHROMASOLV®, St Louis, MO, USA). Ultrapure water was taken from Milli-Q Integral Water Purification System (Millipore, Billerica, MA, USA), ammonium acetate was purchased from Sigma-Aldrich, and acetic acid