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Quantitative profiling of 19 bile acids in rat plasma, liver, bile and different intestinal section contents to investigate bile acid homeostasis and the application of temporal variation of endogenous bile acids

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ARTICLE INFO

Abbreviations:
LC–MS/MS
high-performance liquid chromatography-tandem mass spectrometry
BAs
bile acids
G-BAs
glycine-conjugated bile acids
T-BAs
taurine-conjugated bile acids
beta-MCA
beta-muricholic acid
CA
cholic acid
UDCA
ursodeoxycholic acid
HDCA
hyodeoxycholic acid
DCA
deoxycholic acid
CDCA
chenodeoxycholic acid
LCA
lithocholic acid
IS
internal standard
MeOH
methanol
ACN
acetonitrile
QC
quality control

Keywords:
Bile acid
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Profile

ABSTRACT

Bile acid homeostasis is maintained by liver synthesis, bile duct secretion, microbial metabolism and intestinal reabsorption into the blood. When drug insults result in liver damage, the variances of bile acids (BAs) are related to the physiological status of the liver. Here, we established a method to simultaneously quantify 19 BAs in rat plasma, liver, bile and different intestinal section contents (duodenum, jejunum, ileum, cecum and colon) using high-performance liquid chromatography-tandem mass spectrometry (LC–MS/MS) to reveal the pattern of bile acid homeostasis in the enterohepatic circulation of bile acids in physiological situations. Dynamic changes in bile acid composition appeared throughout the enterohepatic circulation of the BAs; taurine- and glycine-conjugated BAs and free BAs had different dynamic homeostasis levels in the circulatory system. cholic acid (CA), beta-muricholic acid (beta-MCA), lithocholic acid (LCA), glycocholic acid (GCA) and taurocholic acid (TCA) greatly fluctuated in the bile acid pool under physiological conditions. Taurine- and glycine-conjugated bile acids constituted more than 90% in the bile and liver, whereas GCA and TCA accounted for more than half of the total bile acids and the secretion of bile mainly via conjugating with taurine. While over 80% of BAs in plasma were unconjugated bile acids, CA and HDCA were the most abundant elements. Unconjugated bile acids constituted more than 90% in the intestine, and CA, beta-MCA and HDCA were the top three bile acids in the duodenum, jejunum and ileum content, but LCA and HDCA were highest in the cecum and colon content. As the main secondary bile acid converted by microflora in the intestine, LCA was enriched in the cecum and DCA mostly in the colon. As endogenous substances, the concentrations of plasma BAs were closely related to time rhythm and diet. In conclusion, analyzing detailed BA profiles in the enterohepatic circulation of bile acids in a single run is possible using LC–MS/MS. Based on the physiological characteristics of the metabolic profiling of 19 BAs in the total bile acid pool and the time rhythm variation of the endogenous bile acids, this study provided a new valuable method and theoretical basis for the clinical research of bile acid homeostasis.

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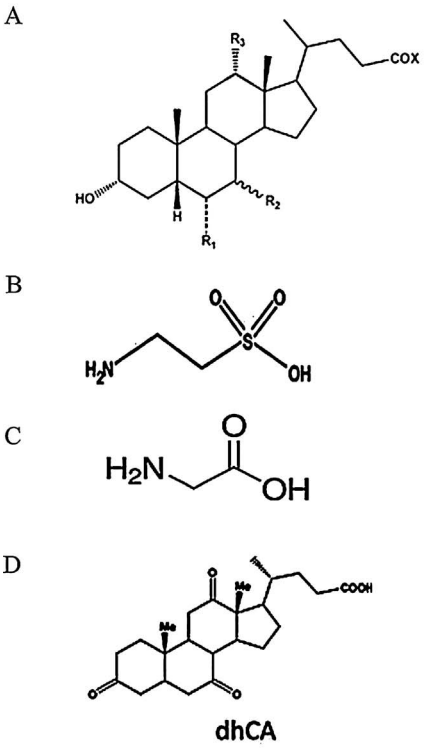
LC–MS/MS
Chronopharmacokinetics
Rat

1. Introduction

Bile acids (BAs) play an important role in physiological and pathological processes. BAs are mostly involved in the enterohepatic system not only to facilitate the intestinal absorption of lipids and fat-soluble vitamins and the elimination of cholesterol and to protect against bacterial overgrowth [1,2] but also to regulate energy expenditure, glucose and lipid metabolism, thyroid hormone signaling, and cellular immunity [1,3]. BAs are synthesized in hepatocytes from cholesterol by pathways involving at least 17 different enzymes, and the immediate products of these synthesis pathways are referred to as primary bile acids [4]. Upon conjugation, primary BAs combine with amino acids (mainly the taurine and glycine) before they are excreted into the small intestine via the bile duct. In the gut, primary BAs are deconjugated and converted by microflora to secondary bile acids, which are mainly deoxycholic acid (DCA) and lithocholic acid (LCA). Most BAs in the intestine are reabsorbed into the portal circulation [5]. Following reuptake by the liver, BAs conjugate with taurine and glycine and are excreted into bile again, a process called enterohepatic circulation. The interruption of bile acid homeostasis can cause changes in the bile acid profile and the accumulation of toxic bile acids. A variety of pathologic changes induced by BAs, including cholestasis, bile duct infarction, liver fibrosis, liver cirrhosis, liver cancer and irritable bowel syndrome, were demonstrated in previous studies [6–8]. Meanwhile, the changes in the bile acid profile or some particular BAs may serve as potential pathogenesis-related biomarkers of liver diseases. Thus, investigating the metabolic profiling of bile acids in the total bile acid pool under physiological conditions may provide a new valuable method for the study of liver diseases.

As shown in Fig. 1, varying with the number and orientation of hydroxyl groups, BAs are complex compounds because of their numerous polarity structural isomers [9]. Therefore, dehydrocholic acid (dhCA) was used as an internal standard (IS) for quantification of the samples as it has similar functional groups as BAs and is not a natural component of rat samples. Individual bile acid has differential effects on bile acid signaling in mice, and the activities of individual bile acids vary markedly under physiological and pathophysiological conditions [10,11]. For example, secondary bile acid (LCA) is the most toxic BA and a potent ligand for pregnenolone X receptor (PXR) [12], whereas chenodeoxycholic acid (CDCA) is less toxic and is a potent farnesoid x receptor (FXR) ligand [13]. Studies have shown that T-beta-MCA and T-alpha-MCA are FXR antagonists [14]. BAs are activators of several mammalian nuclear receptors, and the affinities for FXR are as follows: CDCA > LCA > DCA > CA [15]. TGR5 is principally activated by secondary BAs, including DCA and LCA. Bile acid affinities for TGR5 are as follows: LCA > DCA > CDCA > CA. Therefore, bile acid homeostasis and the composition of the bile acid pool must be strictly controlled to maintain physiological levels of BAs in the liver and extrahepatic tissues.

BAs also affect the pharmacokinetics of drugs that are mainly excreted through bile. The regulation of hepatic and ileal relevant enzymes and transporters result from the alternations of individual bile acid after cholecystectomy. Of note, the downregulation of hepatic CYP3A11 suggested that undesirable pharmacokinetic alternations of drugs, especially CYP3A11 substrates such as rifampicin, might occur under cholecystectomy conditions [16]. As endogenous substances, serum levels of BAs vary during the day following a rhythm dictated by the ingestion of meals [3]. Studies have shown that the



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Bile acids

abbreviation	R1	R2	R3	X
CA	H	OH(α)	OH	OH
beta-MCA	OH(β)	OH(β)	H	OH
UDCA	H	OH(β)	H	OH
HDCA	OH(α)	H	H	OH
CDCA	H	OH(α)	H	OH
DCA	H	H	OH	OH
LCA	H	H	H	OH
TCA	H	OH(α)	OH	$NHCH_2CH_2SO_3H$
TUDCA	H	OH(β)	H	$NHCH_2CH_2SO_3H$
THDCA	OH(α)	H	H	$NHCH_2CH_2SO_3H$
TCDCA	H	OH(α)	H	$NHCH_2CH_2SO_3H$
TDCA	H	H	OH	$NHCH_2CH_2SO_3H$
TLCA	H	H	H	$NHCH_2CH_2SO_3H$
GCA	H	OH(α)	OH	$NHCH_2CO_2H$
GUDCA	H	OH(β)	H	$NHCH_2CO_2H$
GHDCA	OH(α)	H	H	$NHCH_2CO_2H$
GCDCA	H	OH(α)	H	$NHCH_2CO_2H$
GDCA	H	H	OH	$NHCH_2CO_2H$
GLCA	H	H	H	$NHCH_2CO_2H$

Fig. 1. Chemical structure of the bile acids analyzed in this study. 19 bile acids are complex compounds for their varied number and orientation of hydroxyl groups and binding groups. (A) Backbone and side chain structures of the BAs and their taurine and glycine conjugates. (B) Molecular structure of taurine. (C) Molecular structure of glycine. (D) Structure of the internal standard (dhCA). (E) Structure of major unconjugated, glycine-and taurine-conjugated bile acids.