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Bile acid analysis in human disorders of bile acid biosynthesis

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ABSTRACT

Bile acids facilitate the absorption of lipids in the gut, but are also needed to maintain cholesterol homeostasis, induce bile flow, excrete toxic substances and regulate energy metabolism by acting as signaling molecules. Bile acid biosynthesis is a complex process distributed across many cellular organelles and requires at least 17 enzymes in addition to different metabolite transport proteins to synthesize the two primary bile acids, cholic acid and chenodeoxycholic acid. Disorders of bile acid synthesis can present from the neonatal period to adulthood and have very diverse clinical symptoms ranging from cholestatic liver disease to neuropsychiatric symptoms and spastic paraplegias. This review describes the different bile acid synthesis pathways followed by a summary of the current knowledge on hereditary disorders of human bile acid biosynthesis with a special focus on diagnostic bile acid profiling using mass spectrometry.

1. Bile acid synthesis and metabolism

1.1. Introduction and scope

Bile acids are best known for their function in the absorption of fats and fat-soluble vitamins, however, they also are important for the maintenance of cholesterol homeostasis, excretion of endogenous and exogenous toxic substances, induction of bile flow and as signaling molecules that influence glucose homeostasis, lipid metabolism and energy expenditure (Halilbasic et al., 2013; Russell, 2009). This paper focuses on the mass spectrometry-based profiling of human disorders of bile acid biosynthesis. We will not discuss bile acid transport (Dawson et al., 2009; Halilbasic et al., 2013) and regulatory aspects of bile acids (Russell, 2003) and refer the reader to excellent reviews and references on these topics.

1.2. Enterohepatic circulation and secondary bile acids

The two primary bile acids, cholic acid (3α,7α,12α-trihydroxy-5β-cholanoic acid, CA) and chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanoic acid, CDCA), are synthesized from cholesterol in the liver. After synthesis, hepatocytes convert bile acids into their corresponding amine conjugates (glycine or taurine), the bile salts. Together with phospholipids and other bile components the bile salts are excreted into the canaliculi and the resulting bile is transported to and stored in the gallbladder. After a meal, cholecystokinin-mediated contraction of the gallbladder results in secretion of bile salts into the duodenum. There, and in the remainder of the small intestine, bile salts aid in dissolving and absorbing fats and fat-soluble vitamins. Part of the bile salts are modified and/or deconjugated by gut bacteria resulting in secondary bile acids, deoxycholic acid (3α,12α-dihydroxy-5β-cholanoic acid) from CA and lithocholic acid (3α-hydroxy-5β-cholanoic acid) from CDCA, and free primary bile acids (e.g. deconjugated bile salts). Other secondary bile acids are ursodeoxycholic acid (3α,7β-dihydroxycholanoic acid, UDCA), hyodeoxycholic acid (3α,6α-dihydroxycholanoic acid) and hyocholic acid (3α,6α,7α-trihydroxycholanoic acid). Both primary and secondary bile salts/acids are

Abbreviations: CA, Cholic acid = 3α,7α,12α-trihydroxy-5β-cholanoic acid; CDCA, chenodeoxycholic acid = 3α,7α-dihydroxy-5β-cholanoic acid; UDCA, ursodeoxycholic acid = 3α,7β-dihydroxycholanoic acid; DHCA, 3α,7α-dihydroxy-5β-cholestanoic acid; THCA, 3α,7α,12α-trihydroxy-5β-cholestanoic acid; BACS, bile acid-CoA ligase; AMACR, α-methylacyl-CoA racemase; ACOX2, acyl-CoA oxidase 2; DBP, D-bifunctional protein; SCPx, sterol carrier protein X; BAAT, bile acid CoA:amino acid *N*-acyltransferase; GC, gas chromatography; MS, mass spectrometry; FAB, fast atom bombardment; ESI, electrospray ionization; LC, liquid chromatography; MRM, multi-reaction monitoring; UPLC, ultra performance liquid chromatography; γ-GT, γ-glutamyltranspeptidase; CTX, cerebrotendinous xanthomatosis; 3β-HSOR, 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase; m/z, mass over charge ratio; OH-THCA, tetrahydroxy-5β-cholestanoic acid; diOH-THCA, pentahydroxy-5β-cholestanoic acid.

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reabsorbed in the terminal ileum and in the large intestine after which they are transported back to the liver via the portal circulation. Hepatocytes then take up the bile acids/salts thereby completing the so-called enterohepatic circulation. The enterohepatic circulation maintains a bile acid pool of about 2–4 g that goes through about 10–12 cycles per day (Heubi et al., 2007). Fecal loss (about 0.2–0.6 g per day, 5% of the total pool) is compensated by hepatic bile acid synthesis thereby maintaining bile acid homeostasis (Russell, 2003).

1.3. Bile acid synthesis

The biosynthetic pathway from cholesterol to the two primary bile acids is depicted in Figs. 1 and 2. This synthetic pathway, that is present in the liver, consists of at least 17 enzymes that are distributed across several subcellular locations/organelles including the cytosol, endoplasmic reticulum, mitochondria and peroxisomes (Figs. 1 and 2). Cholesterol is a C_{27}-sterol with one double bond at the 5-position (cholest-5-en-3β-ol). In short, for the conversion of cholesterol into the two primary bile acid CA (3α,7α,12α-trihydroxy-5β-cholanoic acid) and CDCA (3α,7α-dihydroxy-5β-cholanoic acid), one (7α) or two (7α, 12α) α-hydroxyl groups are added, the double-bond at the 5-position is reduced with the concomitant generation of a 5β-hydrogen, the 3β-hydroxyl group is converted into a 3α-hydroxyl group and the aliphatic side-chain is oxidized to a carboxyl group and shortened by three carbon atoms ($C_{27} \rightarrow C_{24}$, from cholestanoic to cholanoic acid). After synthesis of the primary bile acids CA and CDCA, conjugation to glycine or taurine generates the so-called bile salts.

Four different main pathways of bile acid biosynthesis have been delineated which are termed the classic or neutral pathway, the alternative or acidic pathway, the Yamasaki pathway and the 25-hydroxylation pathway (Fig. 1). The exact order of steps in the biosynthetic pathways of bile acids remains unclear because many intermediates are substrates for more than one biosynthetic enzyme (Russell, 2003). Little is also known about the transport of the bile acids and intermediates between the different compartments, which most likely is an important factor in the bile acid synthesis process.

In the classic pathway, which starts with the hydroxylation of cholesterol to 7α-hydroxycholesterol by sterol 7α-hydroxylase (CYP7A1), modifications of the steroid ring structure precede the oxidative chain-shortening of the aliphatic side-chain. 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase (HSD3B7) converts 7α-hydroxycholesterol into a 3-oxo-Δ^4-form after which the Δ^4-3-oxosteroid-5β-reductase (AKR1D1) reduces the Δ^4-double bond and generates a 5β-hydrogen configuration. The final step of the ring structure modification is the reduction of the 3-oxo group to a 3α-alcohol by 3α-hydroxysteroid dehydrogenase (AKR1C4). If the 12-position is hydroxylated by sterol 12-hydroxyase (CYP8B1), the final product will be CA, if this position is not hydroxylated CDCA is ultimately formed. After the ring structures have been modified, a carboxyl group is created at position C_{27} by sterol 27-hydroxylase (CYP27A1) producing the C_{27}-bile acid intermediates: 3α,7α-dihydroxy-5β-cholestanoic acid (DHCA) and 3α,7α,12α-trihydroxy-5β-cholestanoic acid (THCA). The hydroxylation of the pro-chiral aliphatic side-chain by CYP27A1 results in the exclusive formation of the 25R-stereoisomer. The C_{27}-bile acid intermediates are then activated to their corresponding CoA-ester. To date, two enzymes have been shown to be able to activate the C_{27}-bile acid intermediates, i.e. bile acid-CoA ligase (BACS) and very long-chain acyl-CoA synthetase, both located at the endoplasmic reticulum. The C_{27}-bile acyl-CoAs are transported into the peroxisome by ABCD3, also called peroxisomal membrane protein 70 (PMP70) where, prior to chain-shortening, the chiral carbon center at the 25-position needs to be racemized from the R- to the S-configuration by α-methylacyl-CoA racemase (AMACR). Only then can the side-chain be shortened by peroxisomal β-oxidation (Fig. 2). In the peroxisome, (D/T)HC-CoA is oxidized by acyl-CoA oxidase 2 (ACOX2) forming a double bond at the 24-position. This double bond is hydrated to 24-hydroxy-(D/T)HC-CoA followed by dehydrogenation to 24-keto-(D/T)HC-CoA, both catalyzed by D-bifunctional protein (DBP). Thiolytic cleavage of this ketone by sterol carrier protein X (SCPx) releases propionyl-CoA and CDCA-CoA or CA-CoA. These bile acyl-CoAs are substrates for the peroxisomal bile acid CoA:amino acid *N*-acyltransferase (BAAT) which generates the four primary bile salts (glycine or taurine conjugated CA/CDCA) (Fig. 2).

In the acidic pathway, a C_{27}-carboxylic acid is first generated, followed by modifications of the steroid nucleus and finally the peroxisomal chain-shortening to C_{24}-bile acids. After hydroxylation at the 27-position (which actually should be systematically named (25R)26-hydroxycholesterol (Fakheri and Javitt, 2012)), another enzyme than sterol 7α-hydroxylase is responsible for the 7α-hydroxylation, namely oxysterol-7α-hydroxylase (CYP7B1) after which the same enzymes as in the classic pathway complete the ring structure modifications. The acidic/alternative pathway preferentially produces CDCA and appears to be the major contributor to the bile acid pool in young infants (Setchell et al., 1988a). Later in life, the classic/neutral pathway gains in importance with respect to the contribution to the bile acid pool (Russell, 2003).

For the Yamasaki pathway, where the first steps coincide with the acidic pathway, side-chain oxidation and shortening result in the formation of the C_{24}-bile acid 3β-hydroxy-5-cholenoic acid followed by further modifications of the ring structure. In humans, 7α-hydroxylation prior or after peroxisomal β-oxidation is believed to generate 3β,7α-dihydroxy-5-cholenoic acid (Javitt et al., 1986), which ultimately results in the formation of CDCA (and not lithocholic acid). CDCA is believed to be the major product of the Yamasaki pathway in humans. The exact contribution of the Yamasaki pathway to the bile acid pool in humans is not clear, but because of the presence of monohydroxy bile acids in fetal bile and relatively high levels of these bile acids in meconium and amniotic fluid it was suggested that this pathway may be of quantitative importance, at least during development (Nakagawa and Setchell, 1990; Setchell et al., 1988a).

Another way to obtain a C_{24}-carboxylic acid after the ring structure modifications –without the need for 27-hydroxylation and subsequent peroxisomal β-oxidation– is by the 25-hydroxylation pathway. A microsomal sterol 25-hydroxylase (CH25H) generates 3α,7α,12α-trihydroxy-5β-cholestane-25-tetrol which is further hydroxylated at the 24-position to a 24S-pentol, then (likely) dehydrogenated to a 24-oxo-tetrol (Shefer et al., 1976), which is subsequently cleaved to yield CA and acetone (Salen et al., 1979).

1.4. Bile acid analysis

Bile acid analysis was previously done by ion exchange chromatography followed by gas chromatography (GC) and electron ionization mass spectrometry (MS). An important drawback with these methods was the need for cleavage of conjugated bile acids and subsequent derivatization to provide the necessary volatility. Later, fast atom bombardment (FAB)-MS made it possible to directly analyze native bile acids and salts with minimal sample preparation after which electrospray ionization (ESI) largely replaced FAB-ionization and is currently used by the majority of the laboratories. ESI-MS with prior liquid chromatography (LC) separation is now the method of choice for analyzing a cholanoid profile (Griffiths and Sjövall, 2010). Routinely, the MS operates in the multi-reaction monitoring (MRM) mode enabling detection and