Understanding mouse bile acid formation: 
Is it time to unwind why mice and rats make unique bile acids?

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Current knowledge on bile acid metabolism is largely based on extrapolations from animal experiments where the mouse has taken a front position, much due to the development of techniques making it feasible to construct mouse models where specific functions are deficient or overexpressed. However, there are several major differences between mice and humans as regards bile acid metabolism that are important to recognize when interpreting data obtained from experiments on mice and extrapolating that data to humans. One such difference is that in mice as well as in rats, bile acids are hydroxylated at the 6-β position to form MuriCholic Acids (MCAs), a set of bile acids that is unique to these species. Although the long pathway for the synthesis of bile acids in humans has progressively been uncovered (1) the structure(s) responsible for the formation of MCAs has remained a dark area although it has previously been concluded that chenodeoxycholic acid (CDCA) should be at least one substrate for the formation of MCAs (2). In the present issue of Journal of Lipid Research, Takahashi et al (3) report that the Cyp2c70 gene is key for the synthesis of MCAs. By screening liver extracts from different knock out mouse strains for the absence of MCAs, they found that Cyp2c gene cluster knock out (Cyp2c-null) mice are completely deficient in MCAs. Of the 15 functional candidate genes within that gene cluster, the authors came to the conclusion that the Cyp2c70 gene is necessary for the formation of α-MCA from CDCA as well as for the formation of β-MCA from ursodeoxycholic acid (UDCA). In liver extracts from WT mice the formation of α-MCA from CDCA occurred with an approximately 40-fold higher affinity than the formation of β-MCA from UDCA. However, in line with previous findings on gallbladder bile (4) or liver extracts (5, 6), they found that in liver extracts from WT mice, β-MCA was 5-fold more abundant than α-MCA, and that the level of conjugated tauro (T) β-MCA was 20-fold higher than that of Tα-MCA. It was proposed that presumably, epimerization of 7α-MCA into 7β-MCA, possibly mediated by intestinal microbiota, might explain the higher abundancy of β-MCA. The ultimate model to prove these results, knock out mice for Cyp2c70 solely, is now highly warranted.
Are MCAs of interest in human medicine?

The long and complex pathways for the synthesis of bile acids is a classic example where it has been recognized that the level of synthesis is suppressed by the end products made, the bile acids. How this occurred was not well understood until the discovery that the farnesoid X receptor (FXR) serves as a specific receptor for bile acids in this negative feedback regulation (7, 8). This knowledge has in turn led to an increased awareness that the FXR agonistic activity varies greatly between different bile acids. Thus, CDCA and deoxycholic acid (DCA) are clearly more potent than cholic acid (CA) and UDCA, the latter two being poor FXR activators when studied alone in vitro (8, 9). However, when UDCA is used in the presence of potent FXR activating agonists such as DCA or CDCA it can dampen the FXR stimulation from the agonists (9). In this respect the MCAs are of particular interest.

Although never described, it could already in the report by Makishima et al (7) be seen that the CYP7A1 protein in HepG2 cells was dose-dependently induced when cells were exposed to MCAs. Later studies on germ-free mice have highlighted that such animals have an enlarged pool of bile acids rich in MCAs and that $\text{T}_{\beta}-\text{MCA}$ and $\text{T}_{\alpha}-\text{MCA}$ can serve as antagonists to CDCA as determined with a coactivator recruitment assay with recombinant human FXR (10). Germ-free mice have been shown to have an improved resistance against high fat feeding (11). Interestingly, cholic acid free $\text{CYP8B1}^{-/-}$ mice and mice treated with antibiotics share several features with germ-free mice. They have an induced bile acid synthesis and increased expression of the intestinal ASBT protein, an enlarged pool of bile acids enriched in MCAs, and their intestinal absorption of dietary fat including cholesterol is reduced (12-14). $\text{CYP8B1}^{-/-}$ mice are also less prone to develop steatosis (12). Further, feeding WT mice with $\alpha$- or $\beta$-MCAs reduces the intestinal absorption of cholesterol from 38 to 11% (15).

The present identification of $\text{Cyp2c70}$ as key for the synthesis of MCAs from CDCA or UDCA should now make it feasible to synthesize MCAs in vivo in species other than rats and mice. One may thus speculate whether the production of significant quantities of MCAs by human intestinal microbiota


or in liver may result in beneficial metabolic effects such as reduced body weight, improved insulin sensitivity and reduced liver lipids? One important substrate for this, CDCA is certainly highly available in humans.

Other conditions where it may be of interest to generate MCAs in humans are different situations with cholestasis associated with high systemic levels of the potentially toxic CDCA. In bile duct ligated rats the urinary excretion of the water soluble MCAs increases about 400-fold (16) to become the major excretory route for bile acids. In addition, bile acid synthesis is induced.

Although such approaches may at first appear attractive, the fact that administered bile acids are often rapidly converted in vivo may result in unexpected responses. Thus, the administration of CDCA to mice induces the formation of MCAs from CDCA that dampens the expected suppression of bile acid synthesis by this treatment, while on the other hand administration of the weak FXR agonist CA suppresses CYP7A1 strongly, presumably due to the pronounced reduction of FXR antagonistic MCAs that is seen during such treatment (5, 6).

Further understanding of the physiologic function of MCAs in rats and mice

It is well known that mice and rats are relatively resistant to high fat feeding and that mice with boosted levels of MCAs, such as germ-free, Cyp8b1−/−, and antibiotic-treated mice show even stronger such resistance. The question of whether MCAs are important for this resistance may now be investigated by high fat feeding of MCA deficient mice. Will these mice respond more like humans? It is also known that basal bile acid synthesis in mice is about double that seen in humans. Are the FXR antagonistic MCAs important for that species difference? In the present study there was a clear trend for higher CYP7A1 mRNA in the WT animals but due to small animal numbers this did not reach statistical significance. Another issue that also will be of interest is to learn how MCA deficient mice will respond to bile duct ligation. Will bile acid synthesis be suppressed as in humans or will it be
induced as in WT mice? These and many other questions to clarify the physiologic functions of MCAs should now be possible to answer.

References


