Will the real bile acid sulfotransferase please stand up? Identification of Sult2a8 as a major hepatic bile acid sulfonating enzyme in mice

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Will the real bile acid sulfotransferase please stand up? Identification of *Sult2a8* as a major hepatic bile acid sulfonating enzyme in mice[1]

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This year marks the 50th anniversary of the publication by Robert Palmer (1, 2) recognizing the formation of bile acid sulfates as a mechanism for bile acid elimination in humans. Like steroids, bile acids undergo sulfonation in liver and other tissues [reviewed by Alnouti in (3)]. This important phase II detoxification reaction transfers a sulfonate group (SO_3^-) from the universal donor, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), to a hydroxyl, amine, or carboxylic acid group of a substrate. In humans, almost half of the lithocholic acid (LCA) secreted into bile is present in divalent, sulfated, and amidated form (4), and bile acid sulfates comprise a large proportion of the bile acids excreted in urine (5). Sulfonation is not specific to LCA because sulfated forms of cholic acid, deoxycholic acid, chenodeoxycholic acid, and ursodeoxycholic acid are also found in serum and urine, usually as double-conjugates with glycine and taurine in the side-chain (6). Notably, the levels of bile acid sulfates are elevated in physiological and pathophysiological cholestasis (7, 8). Sulfonation significantly alters the physicochemical, pharmacokinetic, and signaling properties of bile acids, reducing their cytotoxicity. For example, polar bile acid sulfates are choleretic, and sulfation promotes rapid elimination of bile acids by limiting their intestinal reabsorption and by efflux of divalent bile acid sulfates from liver into blood to increase their urinary excretion. In general, there is an inverse relationship between hydroxylation and sulfation whereby addition of hydroxyl groups prevents sulfation. In contrast to humans, hydroxylation rather than sulfation is thought to be the more dominant pathway for bile acid detoxification in mice (3, 9). However, sulfonation may still have a role under certain pathophysiological conditions in mice (10, 11). Another species difference between humans and mice is the position of the sulfate moiety on the steroid nucleus. Bile acids are primarily mono-sulfated in humans (although traces of disulfates are found in urine, possibly through renal sulfation) and in mice. However, the location of the sulfate group on the bile acid steroid nucleus differs, preferentially occurring at the C-3 position in humans and at the C-7 position in mice (with the exception of LCA, which is a minor bile acid in mice) (3, 6, 12). This is important because the sulfate moiety's position has a profound effect on the physiological behavior of bile acids. Unlike bile acids sulfated at the C-3 position, C-7-sulfates are resistant to hydrolysis and metabolism by the intestinal microbiota (13), thereby blocking their intestinal reabsorption and potentially providing cytoprotection to the intestinal mucosa (14).

Sulfonation is carried out by members of the superfamily of cytosolic sulfotransferases (SULTs). In humans, there are 13 SULT genes, which are distributed between 4 families (15). SULT2A1, the lone SULT2A gene in humans, appears to be the major human SULT enzyme capable of sulfonating bile acids at the 3α-OH position (15–17). In mice, there are 21 SULT genes, and these are subdivided between 6 families. Whereas there is only one human SULT2A gene, seven (and now eight) Sult2a genes are present in mice (15, 18). Most mouse studies have focused on Sult2a1 or Sult2a1/Sult2a2 as the putative hepatic bile acid sulfotransferase. However, the significant female predominance of hepatic Sult2a1 expression and dehydroepiandrosterone (DHEA) sulfotransferase activity in mice (19, 20) is not associated with a greater abundance of bile acid sulfates in urine or tissue (12). These observations as well as the plethora of mouse Sult2a genes raise questions as to which mouse ortholog(s) of human SULT2A1 are responsible for this important reaction and whether expression of Sult2a1 mRNA/protein can be equated with hepatic synthesis of bile acid sulfates. In the present issue of the *Journal of Lipid Research*, Feng et al. (21) identify a novel Sult2a isoform that begins to answer these questions.

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In a true 'tour-de-force', the authors identify a novel PPARα-regulated gene using a differential display approach comparing the expression profile of wild-type and PPARα knockout mice under fed or fasted conditions. Cloning of the full-length transcript and sequence analysis revealed that the differentially expressed cDNA (initially called ml-STL) shared highest amino acid sequence identity with the Sult2a family of sulfotransferases. The gene (now designated *Sult2a8*) was then localized to mouse chromosome 7, embedded in a 0.9 Mb region harboring the cluster of Sult2a genes (*Sult2a5 – Sult2a2 – Sult2a1 – Sult2a4 – Sult2a3 – Sult2a6 – Sult2a8 – Sult2a7*), as well as several Sult2a pseudogenes. Extensive characterization of the activity of recombinant Sult2a8 confirmed that the enzyme is a bile acid sulfotransferase with high activity toward 7α-hydroxylated bile acids such as taurocholic acid, cholic acid, taurochenodeoxycholic acid, chenodeoxycholic acid, tauro-α-muricholic acid, and α-muricholic acid, but little activity toward bile acids lacking a 7α-hydroxy group, such as deoxycholic acid or lithocholic acid. Sult2a8 was highly specific for 7α-hydroxylated bile acids/salts and exhibited little or no ability to sulfonate other prototypic SULT substrates, including steroids (androsterone, DHEA, prenenolone, and cholesterol), phenols, catecholamines, or thyroid hormones. As such, studies using the substrate DHEA as a surrogate for bile acid sulfonation missed this activity. Analysis of the tissue sites and ontogeny of expression (mRNA and protein) in mice revealed that Sult2a8 is liver-specific and first detected approximately seven days after birth, rising sharply to a maximal level at one month of age. In striking contrast to Sult2a1, Sult2a8 is expressed at higher levels in males, with expression declining in female mice over the time period analyzed (from one month to 12 months of age). However, even female mice express a considerable amount of Sult2a8 mRNA. The authors also demonstrate that the synthetic PPARα agonist Wy-14,643 downregulates Sult2a8 mRNA and protein expression in a PPARα-dependent fashion, in agreement with the original cloning strategy.

In the course of studying novel PPARα-regulated pathways, the authors have identified a major hepatic bile acid sulfonating enzyme and provided insight to the pathways responsible for bile acid sulfonation in mice (**Fig. 1A**). Although Sult2a1 may be important for LCA sulfonation in mice, the results suggest that Sult2a8 plays a prominent role, particularly in males. Indeed, interrogation of publically available RNA-SEQ data (Gene Expression Omnibus GSE93380) (22) for livers of chow-fed adult male and female CD-1 mice reveals a striking difference in the hepatic mRNA expression of Sult2a8 versus other Sult2a family members (Fig. 1B). Because much of the previous work has focused on hepatic Sult2a1 or Sult2a2 expression as an indicator of hepatic sulfonation in mice (3), the regulation of hepatic bile acid sulfonation and its role in bile acid metabolism in the mouse should be revisited in light of these new findings for Sult2a8. Clearly, future studies will be required to understand the contribution of Sult2a1 and Sult2a8 to formation of bile acid sulfates and the protective role of this detoxification mechanism under pathophysiological conditions. However, the present identification of mouse Sult2a8 as a major hepatic bile acid sulfotransferase revealed an important and unrecognized gap in our understanding of the regulation of bile acid metabolism in mouse models and demonstrates that there is more to be learned many years after Palmer's original description of bile acid sulfates.
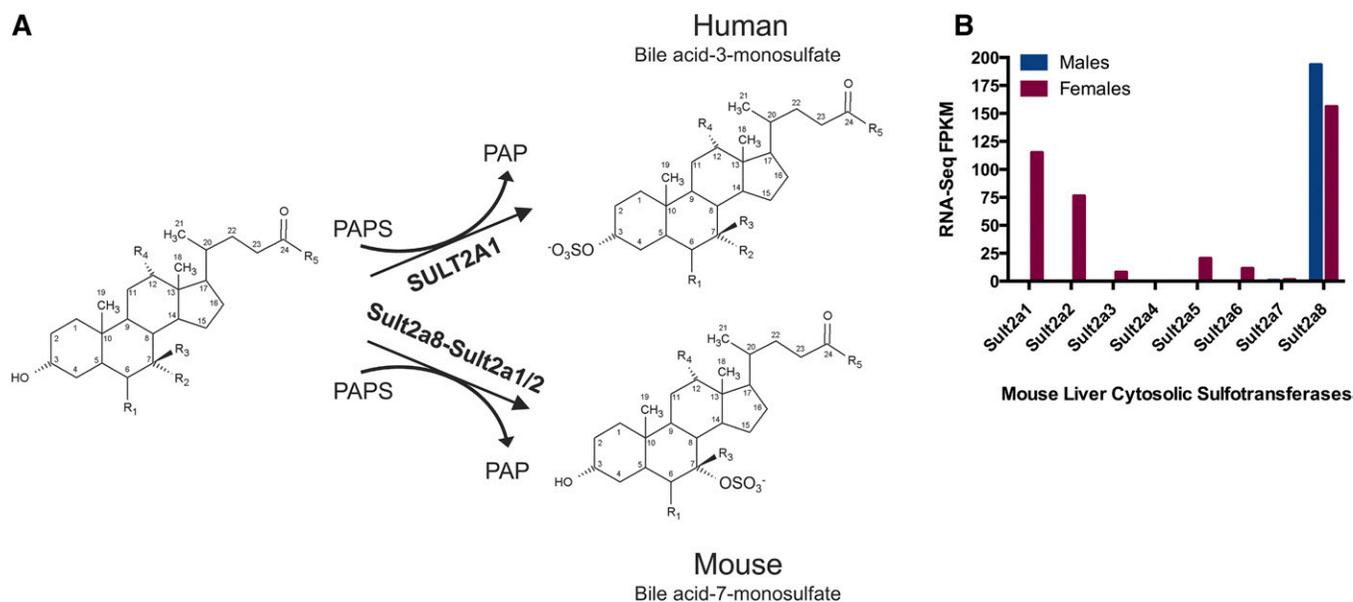

Fig. 1. Bile acid sulfonation reaction and mRNA expression of the cytosolic sulfotransferases in liver of adult wild-type mice. A: Schematic of the major mechanisms responsible for the formation of bile acid sulfates in humans and mice. B: Expression of the Sult2a mRNA in adult male and female mice. FPKM (fragments per kilobase of transcript per million mapped reads) expression values obtained from RNASeq analysis (22).

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