Turning over or turning around: hepatic phosphatidylcholine in the mouse model for progressive familial intrahepatic cholestasis type 3

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Abstract

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Turning over or turning around: Hepatic phosphatidylcholine in the mouse model for progressive familial intrahepatic cholestasis type 3 (PFIC3)

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Modern life style in Western societies has lead to an increase of artherosclerosis, the consequences of which are presenting a major health care problem. Artherosclerosis positively correlates with plasma cholesterol levels, which is determined by dietary cholesterol intake, cholesterol biosynthesis and cholesterol excretion. The latter occurs either by biosynthesis of bile salts starting from cholesterol in hepatocytes or by cholesterol excretion into bile. Biliary cholesterol excretion critically depends on hepatic bile salt and phosholipid secretion (1). Biliary phospholipids consist mostly of phosphatidylcholine (PC). PC secretion into the canalicular lumen requires the coordinate action of ABCB4/Abcb4 (human/rodents) (1) and BSEP/Bsep (or ABCB11/Abcb11) (2). The mechanism of biliary PC secretion has been worked out with the help of knockout mice defective in functional Abcb4 and by elucidating the pathophysiologic alterations in patients with inherited human diseases progressive familial intrahepatic cholestais (PFIC) type 3 and PFIC2 (or BSEP and MDR3 deficiency syndrome, respectively) (3). Both, mice with a disrupted *Abcb4* gene and humans with mutations in the *ABCB4* gene are not capable of biliary PC secretion, but display normal bile salt secretion. Moreover, the amount of PC secreted into bile is positively correlated with the amount of canalicular bile salt secretion, which releases PC from the canalicular membrane and subsequently promotes the formation of mixed PC/bile salt micelles. If either canalicular bile salt secretion or PC secretion is defective, biliary cholesterol secretion is also impaired, due to the lack of a suitable cholesterol acceptor in bile. Hence, ABCB4 may indirectly contribute to serum cholesterol homeostasis.

Abcb4 knockout mice (and patients with PFIC3) have no PC in their bile, but secrete high amount of bile salts. These mice develop liver disease with portal inflammation and ductular proliferation leading to dysplasia, dysplastic nodules and with increasing age (beginning at an age of 4 to 6 months) hepatocellular carcinoma (4). These pathologies are attributed to the toxic action of bile salts on the bile duct epithelium as well as to retention of bile salts in hepatocytes (5). Along these lines, it was recently reported that patients with PFIC2 having

bile salt export pump deficiency display an increased risk of hepatobiliary malignancy (6, 7), hence supporting the concept that bile salts can act as tumor promoting agents. Taken together, studies in Abcb4 knockout mice (and interpretation of pathophysiologic findings from patients with PFIC2 and PFIC3) have focused on pathological consequences resulting from canalicular secretory defects, while little or no attention has been paid to hepatocellular PC metabolism as a cause for liver pathology in situations of absent PC secretion.

The biosynthesis of phosphatidylcholine occurs either from choline via the CDP-choline pathway (or the so-called "Kennedy pathway") or by methylation of phosphatidylethanolamine (PE) through phosphatidylethanolamine *N*-methyl transferase (PEMT) (8, 9). Besides being an important lipid component of intracellular and plasma membranes in liver 8and all other) cells, PC is secreted via lipoproteins into serum or mediated by Abcb4 into bile. In Abcb4 knockout mice, this latter pathway is non-existing, so these animals might have an altered PC homeostasis. Interestingly, the total phospholipid content of such livers is not different from wild type animals, suggesting an unaltered PC content (10, 11). Furthermore, serum lipoprotein content in these mice is also reduced in normal and cholestatic conditions, indicating lower phospholipid content associated with lipoproteins, which could be due to reduced lipoprotein secretion from the liver (10, 11). Finally, the biosynthesis and turnover of PC, e.g. by phosholipase A_2 to toxic lysoPC (12) may be altered as a consequence of nonfunctional Abcb4.

In this issue, Baghdasaryan et al. (13) focused on PC metabolism in Abcb4 knockout mice to test, whether impaired biliary PC secretion would lead to formation and/or retention of abnormal and potentially toxic PC metabolites in liver. To this end, they fed knockout mice with a diet enriched in soybean lecithin or with a choline deficient diet and compared these animals with knockout mice fed on a control (standard) diet. The key finding of their study is that the different feeding regimens did neither alter hepatic, serum nor biliary phospholipids nor that toxic PC metabolites were increased. The authors also observed that the soybean lecithin diet (additional supply of PC to the liver) decreased serum levels of alkaline phosphatase and bilirubin in comparison to knockout mice fed a standard chow. However, degree of portal fibrosis as well as hydroxyproline content did not differ among the different treated animal groups, indicating no improvement bile ductular damage for the soybean lecithin diet.

Of note, the authors investigated the enzymes involved in PC biosynthesis and found no changes in mRNA levels of choline kinase α and β, choline-phosphate cytidyl transferase (or phosphocholine transferase) (CT) and of PEMT. Among these enzymes, CT catalyzes the rate

limiting step of PC biosynthesis. Transcriptional regulation of this enzyme is complex and depends on Sp1, but other factors are most likely important, as data from the regulation of this enzyme during the cycle suggest (9). In addition to transcriptional regulation, CT exists in a soluble or cytosolic, inactive and a membrane bound, active form (8, 9). The translocation of CT to membranes is fast and allows a quick response of a cell to the need for PC. Hence, while mRNA level of CT in this study was not altered by the different feeding regimens, the possibility remains that a shift from the membrane bound to the cytosolic pool of CT occurs as a consequence of non-existent canalicular PC secretion, which in turn would down-regulate PC biosynthesis. Last but not least, CT is undergoing phosphorylation and may hence be subject to extensive posttranscriptional regulation.

The observation of the soybean lecithin induced reduction in serum liver markers alkaline phosphatase and bilirubin is difficult to explain at this moment. Both markers represent the the state of health of hepatocytes. Hence the data suggest that in these animals that the state of health of hepatocytes may be improved by the soybean lecithin diet. This is supported by the reduction of the proliferative activity of hepatocytes in this diet group. Dietary lecithin also protects against the negative effects of cholate feeding on livers of Abcb4 knockout mice, again supporting a protective role of lecithin in the diet for the liver of these animals (14). The direct link between the diet and the health state of hepatocytes in these situations is however not evident. While additional components of the diets used in such experiments may exert the beneficial effect, soybean lecithin diets could also lead to subtle changes in the relative cellular amounts of PC and PE in liver, even though the study of Baghdasaryan et al. (13) found no obvious differences in the content of PC and PE in the different diet groups. It can however not be ruled out at the moment that the diet regimen could lead to subtle alterations in PC and PE content of hepatocellular membranes. These two lipids are asymmetrically distributed in membranes, whereby PE is enriched in the cytosolic leaflet of membranes, while PC is enriched in the non-cytosolic or luminal leaflet of the membrane. The relative transmembrane distribution of these two lipids is essential for a "normal" membrane behavior (15). In conclusion, this study has opened a new potential avenue to treat patients with cholestatic liver diseases not only with ursodeoxycholic acid, but also by dietary means.

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