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Biliary lipid secretion

Safra lipid sekresyonu

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The liver has many biochemical functions, of which one of the most important is bile formation. Bile is both a secretory and an excretory fluid and two of its most important functions are the delivery to the intestinal tract of: (i) bile acids to assist in fat digestion and absorption; and (ii) liver-derived metabolites of potentially toxic materials prior to their elimination from the body in the feces. Bile contains numerous solutes, including bile acids, phospholipids and cholesterol. Biliary lipids mainly consist of cholesterol and phospholipids and their secretion into bile is affected by the secretion of bile acids. Phospholipids and cholesterol are synthesized in the hepatocytes and are thought to be transferred via vesicle- and non-vesicle-mediated mechanisms into the bile canaliculus. Hepatocytes acquire biliary lipid by three pathways, which are biosynthesis, lipoproteins and existing molecules drawn from intracellular membranes, with the newly synthesized biliary lipid accounting for less than 20% of the total lipids. The hepatic determinants of biliary cholesterol elimination are not limited to total cholesterol homeostasis, but also concern biliary disease conditions, since excess biliary cholesterol secretion is involved in cholesterol gallstone formation, as well as being a major risk factor for gallbladder cancer. The purpose of this review was to highlight some of the major mechanisms involved in biliary lipid secretion.

Key words: Bile, cholesterol, phospholipid, lipid, liver

Birçok biyokimyasal fonksiyonu olan karaciğerin, en önemli işlevlerinden biri de safra oluşturmasıdır. Safra, aralarında safra asitleri, fosfolipidler ve kolesterolün de olduğu çok sayıda çözünmüş bileşik içerir. Fosfolipidlerin ve kolesterolün hepatositlerde sentezlenerek veziküler ve veziküler olmayan mekanizmalarla hepatositlerin safra kanalikusuna transfer edildiği düşünülmektedir. Safra lipidleri, çoğunlukla kolesterol ve fosfolipidlerden oluşur ve safraya sekresyonları da safra asitleri sekresyonundan etkilenir. Hem salınan hem de ekskrete edilen bir sıvıdır. En önemli iki fonksiyonu, muhtemelen (i) yağ sindirimi ve absorbsiyonuna yardım eden safra asitlerinin ve (ii) potansiyel toksik materyallerin karaciğer-orijinli metabolitlerinin, vücuttan dışkıyla eliminasyonlarından önce intestinal sisteme taşınmalarıdır. Hepatositler; üç ayrı yolakla, biyosentez, lipoproteinler ve intrasellüler membranlardan çekilen mevcut moleküllerle safra lipidi yapabilirler. Yeni, sentezlenen safra lipidleri, toplam lipidlerin %20'sinden biraz azını oluşturur. Safrada bulunan kolesterolün eliminasyonunun hepatik ölçütleri, sadece tüm kolesterol homeostazisi ile değil, safra ile ilgili hastalık koşullarıyla da ilişkilidir. Aşırı safra kolesterol sekresyonu, kolesterol safra taşı oluşumuyla ilişkili olup, safra kesesi kanseri için de esas risk faktörüdür. Bu derlemenin amacı, safra lipid sekresyonuyla ilgili, bazı ana mekanizmaların öneminin vurgulanmasıdır.

Anahtar kelimeler: Safra, kolesterol, fosfolipid, lipid, karaciğer

INTRODUCTION

Bile formation occurs by secretion of bile acids, cholesterol, phospholipids, and inorganic anions, and many transport systems for these substances have been identified in both the sinusoidal plasma membrane and canalicular membrane of hepatocytes. Some ATP-binding cassette (ABC) transporters such as ABCG5, ABCG8, multidrug resistance protein 2 (MRP2), and bile salt export pump (BSEP) have also been found in the canalicular membrane, with ABCG5 and ABCG8 transporters being responsible for the majority of sterol secretion into bile (1, 2).

Bile salts are secreted by the human liver in huge amounts, up to 36 g per day (3). These detergent molecules are derived from cholesterol and have enough capability to damage cellular membranes at 5 to 30 mM concentrations in bile. The main part of biliary phospholipids consists of phosphatidylcholine (PC) with a distinct fatty acid pattern such as C16:0 C18:2 fatty acids (4). Cholesterol is

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available in its free form in bile, and excessive cholesterol as a result of dietary intake and/or synthesis of cholesterol in the liver can be removed via biliary secretion (5). It is important that excessive cholesterol should be eliminated, otherwise it will gather in the bile system, which can result in the constitution of gallstones, since cholesterol is almost insoluble in water. Solubilization of cholesterol is accomplished by biliary secretion of PC along with bile salts, to this extent, increasing cholesterol's water solubility over a million-fold. This is also the mechanism by which lipid soluble compounds (including bilirubin and xenobiotics) are removed from the body.

PHOSPHOLIPIDS

Phospholipid secretion into bile is dependent upon bile salt secretion. The more hydrophobic the bile salts, the higher the level of phospholipid secretion that occurs. This means that detergent effect of the bile salts is important in the phospholipid secretion, which indicates that bile salts may extract phospholipids from the canalicular membrane of the hepatocytes.

However, phospholipid compositions of the bile and of the canalicular membrane are different. The phospholipids of the canalicular membrane are PC, phosphatidylethanolamine, and sphingomyelin; however, PC comprises more than 95% of biliary phospholipid. There are also differences between canalicular membrane phospholipid and biliary phospholipid in terms of the fatty acyl species. The phospholipid of the canalicular membrane contains a large range of fatty acyl species, whereas biliary phospholipids are predominantly esterified palmitic (80%) or stearic (20%) acids, and linoleic, oleic and arachidonic acids. The biliary phospholipid species are more hydrophilic than the phospholipids of the canalicular membrane. They are extracted from the membrane by bile salts (10).

Phosphatidylcholine and sphingomyelin are located more in the exoplasmic hemi-leaflet of the canalicular membrane, whereas phosphatidylethanolamine is evenly distributed, and phosphatidylserine and phosphatidylinositol are mostly in the endoplasmic hemi-leaflet of the canalicular membrane of the hepatocytes (11). Any mechanism which is suggested for the transport and secretion of biliary phospholipids must take into account differences in the phospholipid classes and their distribution in the canalicular membrane and bile (16). Phosphatidylcholine is the principal biliary lipid, accounting for more than 95% of total biliary phospholipids in dogs, rats, oxen, pigs, guinea-pigs and humans. It has a fatty acid pattern which is distinct from membrane PC and is particularly rich in two molecular species 1- palmitoyl 2- linoleyl (16:0-18:2) and 1- palmitoyl 2- oleol (16:0-18:1) PC, whereas membrane PC is much richer in stearoyl- (18:0) and arachidonyl (20:4) species (12-15).

In the absence of mdr2 transporter, phospholipids can not be secreted into the bile, even though the bile salts exist in the canaliculi. This means that, in the absence of mdr2, phospholipids in the outer leaflet of the canalicular membrane are detergent-resistant. mdr2 may transfer the phospholipids from the inner leaflet to the outer leaflet and destabilize the outer leaflet, which may expose it to the effects of bile salts (26, 27).

Attempts to isolate vesicles containing biliary-type PC have been unsuccessful (9, 23), even though a vesicle population has been isolated from the human liver (24). Biliary PCs are derived from the endoplasmic reticulum (19) and transported rapidly to the plasma membrane; this transport is ATP-independent and unaffected by agents inhibiting protein transport (25).

Canalicular Membrane Secretion

Phosphatidylcholine transport protein (PC-TP) moves PC (or other phospholipids) from the endoplasmic reticulum to the canalicular membrane (6), and the simple diffusion of PC across the canalicular membrane to the bile is an exceedingly slow process. A phospholipid translocase protein in the canalicular membrane facilitates rapid PC transport across the canalicular membrane to the bile. In humans, this protein is called mDR3 p-glycoprotein, while in rodents it is called mdr2 p-glycoprotein. Smit et al. (26) observed that mice lacking the mdr2 p-glycoprotein gene fragment, which is responsible for coding the production of the phospholipid translocase protein, were incapable of secretion of PC into bile. Smit et al. (26) produced evidence to support this, suggesting that the mdr2 p-glycoprotein gene fraction codes for phospholipid translocase, and in support of this, Ruetz and Gros (27) have noted enhanced PC translocation in the presence of bile salts. Mice with the mdr2 gene that was disrupted by homologous recombination [mdr2 (-/-) mice] displayed a complete absence of phospholipids in bile and a strong depression of

cholesterol secretion. Heterozygous (+/-) mice, which are expected to have 50% of the normal expression of this gene, had a significantly reduced phospholipid secretion (60% of normal) and this was the only abnormality observed in these animals. It was therefore proposed that the loss of phospholipid secretion was the primary consequence of the mdr2 gene disruption.

The mdr2 knockout mouse is incapable of secreting phospholipid into bile (26). It has been thought that the mdr2 p-glycoprotein functions as a phospholipid translocase (flippase), moving biliary type PCs from the internal to the external hemi-leaflet of the canalicular membrane (27). The PC is thus exposed to bile salts which extract it into bile by their detergent action.

It was suggested that it was the formation of vesicle either by exovesiculation at the canalicular-bile interface or selective extraction of biliary lipids from the canalicular membrane that enable PC secretion into the bile. Ultrarapid cryofixation techniques have identified phospholipids on the luminal face (i.e. on the interface between the canalicular membrane a bile) of the canalicular membrane (8) and their presence was dependent upon bile salt co-secretion. Since the canalicular membrane is nearly always maintained, hepatocyte exocytosis into the bile is not a feasible mechanism for delivering PC into the bile. Crawford et al. (8) have quantitatively estimated that the vesicular mechanism can account for all phospholipids secretion into bile (16). (Figure 1).

The Origin of Biliary Phospholipid

The origin of biliary phospholipid may be derived from several sources: 1) synthesis via acylation of glycerol-3-phosphate to phosphatidic acid, dephosphorylation to form diglyceride, and reaction with CDP-choline to form PC, and 2) uptake of phospholipids from circulation lipoproteins. Newly synthesized phospholipid contributes only about 3% to phospholipid output (18).

Zilversmith and Van Handel (7) suggested that biliary phospholipid was derived from a small highly active pool of PC. The withdrawal of biliary PC from the performed pool is under the control of bile acids. Bile acids also stimulated lipoprotein uptake and phospholipid synthesis in the liver, in order to help replenish the pool. Small (17) suggested that bile acids may solubilize PC from the external hemi-leaflet of the canalicular membrane during their secretion into bile (20).

CHOLESTEROL

The liver is a key organ in the regulation of cholesterol metabolism. The liver acquires cholesterol from plasma lipoproteins and endogenous synthesis (3).

Physical Form of Cholesterol

Cholesterol is almost insoluble in water; therefore, solubilization with a combination of PC and bile salts, forming bile-salt-phospholipid-cholesterol micelles, dramatically improves the aqueous solubility of cholesterol. Micelle formation in the solu-

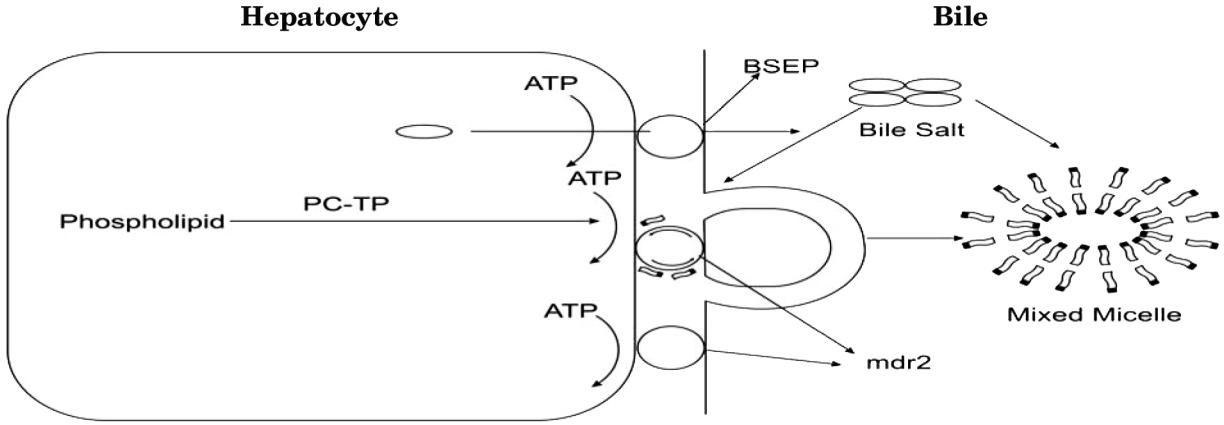

Figure 1. Suggested mechanism for phospholipid transport into bile: Bile acids are transported into bile by ATP-dependent bile salt export pump (BSEP). Phospholipids are delivered to the canalicular membrane by phosphatidylcholine transport protein (PC-TP) and across the canalicular membrane by the PC "flippase" (mdr2). When the PC is transported to the external hemi-leaflet of the canalicular membrane, the PC is thus exposed to bile salts, which extract it into bile by their detergent action. Adapted from (16, 43)

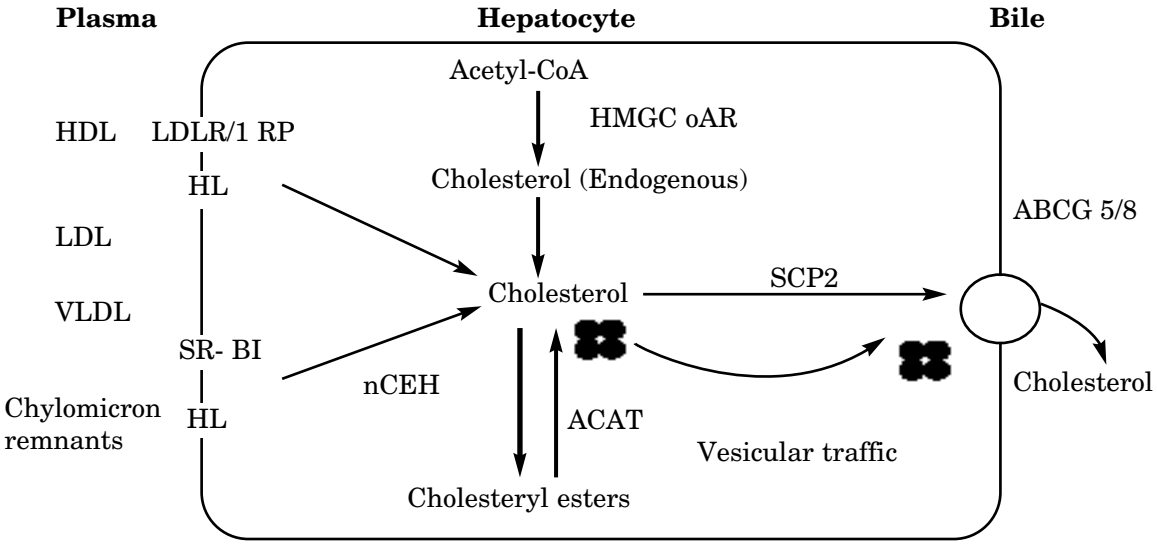

Figure 2. Suggested mechanisms for cholesterol transport into bile: The liver uptakes cholesterol from plasma lipoproteins, principally low-density lipoprotein (LDL), high-density lipoprotein (HDL) and chylomicron remnants and endogenous synthesis. Selective cholesterol uptake from plasma to the liver is supplied by endocytosis or interaction of apolipoproteins and various cell surface molecules, including the LDL receptor (LDLR) and LDLR-related protein (LRP), hepatic lipase, and scavenger receptor class B, type I (SR-BI). Intrahepatic cholesterol may be transported by sterol carrier protein-2 (SCP-2) and into vesicles. Hepatic cholesterol destined for biliary secretion is also controlled by acyl-coenzyme A cholesterol acyltransferase (ACAT) and neutral cholesterol ester hydrolases. Biliary cholesterol secretion is finally determined by the canalicular ATP-binding cassette transporters ABCG5 and ABCG8. Modified from (40)

bilization of cholesterol is suggested to be a secondary event (28) where vesicles were suggested to be the primary mechanism for cholesterol transport in bile (29). Cholesterol is also extracted from the canalicular membrane by luminal bile salts in the absence of PC co-secretion (30).

Intracellular Distribution of Cholesterol

Cholesterol is found almost exclusively in cell membranes. Despite the intracellular hepatocyte membrane having a surface area 20 times greater than the hepatocyte plasma membrane (31), 80% of cholesterol is present in the plasma membrane. The canalicular membrane has the highest cholesterol to lipid ratio of 0.76:1 compared to any other hepatocyte membrane fraction (32). Liscum and Underwood (33) have suggested that cholesterol has a high affinity for membrane rich in sphingomyelin, and the ratio of sphingomyelin in the canalicular membrane is three times greater than that in any other hepatocellular membrane. Another binding protein (i.e. caveolin) found in the plasma membrane also binds cholesterol and thus may play a role in maintaining the plasma membranes high cholesterol concentration (34, 16).

Centers of Cholesterol and Hepatic Uptake

Most of the cholesterol secreted into bile is derived from circulating plasma lipoproteins (35), principally low-density lipoprotein (LDL), high-density lipoprotein (HDL) and chylomicron remnants, respectively (10). Newly synthesized cholesterol does not contribute to a major extent to biliary cholesterol secretion. Lipoprotein cholesterol exists either as free cholesterol (in the outer phospholipid monolayer) or as cholesterol esters within the triglycerides core (36). The free cholesterol in the outer monolayer diffuses to the basolateral membrane of the hepatocyte, partitioning into the outer plasma membrane. This process is particularly true for HDL cholesterol, and is mediated by transient binding of HDL to its receptor on the hepatocyte basolateral membrane (37). Cholesterol diffusion in the plasma membrane enables the rapid distribution among intracellular membranes. In addition, receptor-mediated endocytosis of lipoprotein leads to lysosomal break down of the cholesteryl esters and release of free cholesterol for distribution throughout the cell (38). However, receptor-mediated endocytosis seems to be the primary mechanism for lipid and apoprotein from LDL and

other lipoprotein (39), which is in contrast to HDL secretion.

Cholesterol Secretion

It is thought that ABCG5 and ABCG8 heterodimer transporters may translocate cholesterol from the inner to the outer canalicular membrane in an ATP hydrolysis-dependent manner. Therefore, ABCG5/8 may make cholesterol available for phospholipid vesicles and bile salt micelles into bile. On the other hand, Small (21) suggested that ABCG5/8 transporters may increase biliary cholesterol secretion due to a cholesterol flippase activity and form a putative channel that pushes cholesterol into the canalicular lumen where it can be removed easily by vesicular or micellar acceptors. (Figure 2).

CONCLUSION

The liver is a key organ for body cholesterol metabolism. In addition to endogenous synthesis, the liver obtains cholesterol from plasma lipoproteins via endocytosis or selective cholesterol uptake mediated by the interaction of apolipoproteins with various cell surface molecules, including the LDL receptor (LDLR) and LDLR-related protein (LRP), hepatic lipase, and scavenger receptor class B, type I (SR-BI). Intrahepatic cholesterol may be transferred into vesicles and by different sterol binding/transfer proteins, such as sterol carrier protein-2 (SCP-2); both of these transport mechanisms may transport cholesterol to the canalicular region of hepatocytes for secretion into the bile. Hepatic cholesterol destined for biliary secretion is also controlled by acyl-coenzyme A cholesterol acyltransferase (ACAT) and neutral cholesterol ester hydrolases. Biliary cholesterol secretion is finally determined by the canalicular ATP-binding cassette transporters ABCG5 and ABCG8 (40, 36).

If biliary cholesterol output could be increased with no risk of increase in gallstone formation or decreased without altering overall cholesterol homeostasis, drugs for modulating ABCG5/ABCG8 function might open new therapeutic horizons for atherosclerosis and gallstone disease (22, 41, 42).

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