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Review Article

New insights into the role of *Lith* genes in the formation of cholesterol-supersaturated bile

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ABSTRACT

Cholesterol gallstone formation represents a failure of biliary cholesterol homeostasis in which the physical-chemical balance of cholesterol solubility in bile is disturbed. Lithogenic bile is mainly caused by persistent hepatic hypersecretion of biliary cholesterol and sustained cholesterol-supersaturated bile is an essential prerequisite for the precipitation of solid cholesterol monohydrate crystals and the formation of cholesterol gallstones. The metabolic determinants of the supply of hepatic cholesterol molecules that are recruited for biliary secretion are dependent upon the input-output balance of cholesterol and its catabolism in the liver. The sources of cholesterol for hepatic secretion into bile have been extensively investigated; however, to what extent each cholesterol source contributes to hepatic secretion is still unclear both under normal physiological conditions and in the lithogenic state. Although it has been long known that biliary lithogenicity is initiated by hepatic cholesterol hypersecretion, the genetic mechanisms that cause supersaturated bile have not been defined yet. Identification of the *Lith* genes that determine hepatic cholesterol hypersecretion should provide novel insights into the primary genetic and pathophysiological defects for gallstone formation. In this review article, we focus mainly on the pathogenesis of the formation of supersaturated bile and gallstones from the viewpoint of genetics and pathophysiology. A better understanding of the molecular genetics and pathophysiology of the formation of cholesterol-supersaturated bile will undoubtedly facilitate the development of novel, effective, and noninvasive therapies for patients with gallstones, which would reduce the morbidity, mortality, and costs of health care associated with gallstones, a very prevalent liver disease worldwide.

1. Introduction

Gallstone disease is not only a very prevalent liver disease worldwide, but also a very old human disorder, going back thousands of years, as it has been found in ancient mummies in Egypt and China. Although gallstone disease was not recognized by ancient Chinese, abdominal pain as a result of hepatobiliary diseases and gastric malfunction, jaundice caused by liver diseases, and epigastric colic owing most likely to gallstones or biliary ascariasis were often treated with bear's bile.[1] The earliest medical record for these therapeutic interventions was found in Treatise on Properties of Drugs (c. 643 CE or earlier) written by an ancient Chinese doctor Zhen Quan (c. 540 to 643 CE).[1] Modern chemical analysis of the bile of Asian black bears (*Ursus thibetanus* or *Selenarctos thibetanus*) and brown bears (*Ursus arctos*) found that ursodeoxycholic acid (UDCA) is the major composition of the bile acid pool in these animals. Notably, UDCA, a hydrophilic bile acid, is now first-line pharmacological therapy in a subgroup of symptomatic patients with small, radiolucent cholesterol-enriched gallstones.[2] Long-term administration of UDCA promotes the dissolution of cholesterol gallstones, especially in patients with small (≤5 mm in diameter), cholesterol-rich and uncalcified stones

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(radiolucent on plain X-ray film) in a functioning gallbladder with preserved kinetics and a patent cystic duct.[3–5] However, the therapeutic effect of UDCA is not always achieved in clinical practice because of a high recurrence rate of gallstones.[5] Although laparoscopic cholecystectomy is nowadays the first choice of treatment options for gallstone disease, it is invasive and can cause surgical complications regarding morbidity and mortality, and not all patients with symptomatic gallstones are candidates for surgery.[6]

To reduce the morbidity, mortality and costs of health care associated with gallstones, it is imperative to elucidate the pathogenesis of gallstone disease. This will promote the development of a novel, effective, and noninvasive therapy for patients with gallstones. Since the first gallstone gene, *Lith1* was identified by quantitative locus trait (QTL) mapping methods in inbred strains of mice in 1995,[7] a mouse gallstone gene map that contains 25 *Lith* genes has been established through genetic analysis of cholesterol gallstone formation in different strains of inbred mice fed a lithogenic diet for 8 weeks.[8] This greatly promotes the discovery of human *Lith* genes because of homologues between human and mouse chromosomes. Such a successful study is the confirmation of *ABCG5/G8* as a human *Lith* gene based on mouse studies. The *Abcg5/g8* was first identified as the mouse *Lith9* by the QTL mapping methods,[9–11] and subsequently, two major gallstone-associated variants in *ABCG5/G8* (*ABCG5*-R50C and *ABCG8*-D19H) were found not only in German and Chilean populations, but also in Chinese and Indian populations.[12–19] Therefore, based on the mouse gallstone (*Lith*) gene map, more human *Lith* genes will be identified and their pathogenic mechanisms will be elucidated in the near future.

2. History of cholesterol and bile acid research

Bile is a yellow, brownish, or olive-green liquid that is composed primarily of water, organic solutes (such as lipids), inorganic salts, and some proteins. In bile, cholesterol, phospholipids, and bile acids are three major lipids, and bile pigments are minor lipids. Chemical studies of bile and gallstones for more than 200 years led to the discovery of cholesterol and bile acids, two major organic molecules in bile. The “cholesterol” was first identified in gallstones in the mid-18th century, and subsequently, this material was isolated from gallstones by some researchers. Accumulated evidence showed during the second half of the 18th century that the major component of gallstones was a white crystalline substance that is soluble in alcohol and ether, but not in water. It was not until 1816 that the compound “cholesterine” was named by chemist Michel Chevreul.[20] After cholesterine was found to be an alcohol by Berthelot in 1859,[21] a new name “cholesterol” was largely used in French and English scientific literature. The term cholesterol originated from the ancient Greek chole- (bile) and stereos (solid) followed by the chemical suffix -ol for an alcohol. Although cholesterol was recognized as a distinct chemical compound in the early 19th century, its chemical structure has not been known for many decades. In 1888, Reinitzer[22] identified that the empirical formula of cholesterol was $C_{27}H_{46}O$, indicating that cholesterol was not a straight-chain compound with a double bond, since it did not have enough hydrogen atoms to bind to all the carbon valency of four. However, he saw it was consistent with a structure containing four rings with two shared carbon atoms at each ring junction (four fused rings). Subsequently, some substances isolated from fungi and green plants were found to be cholesterol-like crystalline compounds. In 1889, Tanret[23] isolated a substance from rye seeds infected with ergot, which closely resembled cholesterol. This compound was named ergostérine (now called ergosterol). Furthermore, the empirical formulae of cholic acid ($C_{24}H_{40}O_5$), which was found by Strecker in 1848,[24] and of deoxycholic acid ($C_{24}H_{40}O_4$), which was found by Mylius in 1886,[25] displayed a highly similar ratio (1.67) of hydrogen to carbon atoms compared with that (1.70) in cholesterol. Because both bile acids and cholesterol are present in bile, it was reasonable to hypothesize that the structural features of these two compounds could be similar. In 1919, Windaus and his colleagues[26] found that the carbon skeleton of bile acids was the same as that of the cholesterol molecule, for the most part. This discovery greatly promoted the study of the chemical structure of cholesterol because the presence of the hydroxyl group in ring C of cholic and deoxycholic acids enabled Windaus and other researchers to further investigate the steroid ring system through the bile acid approach.

In 1928, the Nobel Committee for Chemistry announced that the Nobel Prize in Chemistry 1927 was awarded to Heinrich Wieland “for his investigations of the constitution of the bile acids and related substances,” as well as that the Nobel Prize in Chemistry 1928 was given to Adolf Windaus “for the services rendered through his research into the constitution of the sterols and their connection with the vitamins.” Thus, on December 10, 1928, two Nobel Prizes in Chemistry were awarded to Wieland and Windaus, respectively. In his Nobel lecture,[27] Wieland first described a brief history of how three bile acids (including cholic, deoxycholic, and lithocholic acids) were discovered and then, summarized his chemical experiments of bile acids. Based on his experimental results, he proposed a possible chemical structure of bile acid. In his Nobel lecture,[28] Windaus presented his discovery that the chemical precursor of vitamin D was a member of the sterol group and also showed how sunlight broke one of the chemical bonds in the parent molecule, converting it into the active vitamin. This finding clearly explained why exposure to sunlight could prevent rickets, a disease caused by vitamin D deficiency in humans. In addition, Windaus proposed a possible chemical structure of cholesterol. He spent some 30 years studying the chemical structure of cholesterol, which was part of his study of the complex alcohols, known as sterols. He found that sterols were closely related to bile acids by transforming cholesterol into cholanic acid. Unfortunately, the steroid nucleus of bile acid and cholesterol shown in their Nobel lectures was incorrect.[29] However, this did not significantly influence their excellent findings and conclusions for which their prizes were awarded.

It must be noted that modern physical techniques for structural analysis of steroids were not available to these early talented scientists that time. It was a challenging task for these early scientists to precisely identify the chemical structures of cholesterol and bile acids. However, the development of new physical techniques led to the discoveries of the correct chemical structures of these steroids. Desmond Bernal used X-ray diffraction methods to study vitamin D, cholesterol, and ergosterol, and reported the chemical structures of these compounds in *Nature* in 1932.[30] Subsequently, two research groups, led by Rosenheim and King in the UK and Wieland and Dane in Germany, further investigated the chemical structure of bile acids.[31] Each group independently proposed the structure of cyclopentanoperhydrophenanthrene for the steroid nucleus of bile acids. These structures were confirmation by both X-ray diffraction and chenodeoxycholic acid synthesis.[32] Obviously, the X-ray diffraction methods played a critical role in the determination of the correct chemical structures of these lipids in bile, which was proposed in 1932 and has been used ever since. The determination of the sterol ring structure promoted identification of the chemical structures of many other biologically important sterols. For example, Adolf Butenandt identified the structures of the male and female sex hormones even from 25 mg of the male hormone sample. Fig. 1 shows, from left to right, the molecular structures, the standard chemical formulae, the perspective formulae, and the space-filling models of cholesterol and cholic acid, respectively.

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