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HEPATOLITHIASIS—EPIDEMIOLOGY AND PATHOGENESIS UPDATE

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TABLE OF CONTENTS

1. ABSTRACT

Hepatolithiasis or intrahepatic calculi are prevalent in East Asia, including Japan, but occurs much less frequently in Western countries. Hepatolithiasis appears mostly as brown pigment stones (calcium bilirubinate stones) but contain more cholesterol in composition. The disease is characterized by its intractable nature and frequent recurrence, requiring multiple operative interventions, in distinct contrast to gallbladder cholesterol or black pigment stones. Moreover, the most unfavorable complication of the disease is an intrahepatic cholangiocarcinoma. In view of the lack of information on the pathogenesis, a multidisciplinary approach has been carried out through the Hepatolithiasis Research Group organized by the Ministry of Health and Welfare of Japan. In this review, the up-to-date data on the epidemiology and pathogenesis of hepatolithiasis are introduced and discussed. Furthermore, potential medical treatments targeting pathogenetic molecules, which may be important for the etiological process of gallstone formation, are introduced as future therapeutic options for the disease.

2. DEFINITION, STONE COMPOSITION, AND CLINICAL FEATURE OF HEPATOLITHIASIS

Hepatolithiasis is defined as gallstones present in all bile ducts peripheral to the confluence of the right and left hepatic ducts, irrespective of the coexistence of gallstones in other parts of the biliary tract, such as the extrahepatic bile duct and/or the gallbladder (1). The gallstones found above the confluence are considered to be intrahepatic gallstones, regardless whether the confluence is located intrahepatically or extrahepatically.

Gallstones in hepatolithiasis are brown, soft, and friable. They consist of two groups, i.e., brown pigment stones (calcium bilirubinate stones) and cholesterol stones—the former predominating (2-4). It should be stressed that intrahepatic brown pigment stones contain less bilirubin and bile acid and more cholesterol than those either in the common bile duct or in the gallbladder (3,5,6). The presence of brown pigment with a wider range of cholesterol contents as well as cholesterol stones among hepatolithiasis suggests that the complex nature of the pathogenesis should be considered, e.g., not only the formation and precipitation of calcium bilirubinate but also the solubility of cholesterol in hepatic bile.

Typically, the clinical course of hepatolithiasis is intractable, requiring multiple operative interventions due to frequent stone recurrences. The disease has a high association with cholangiocarcinoma (7,8). It is distinct contrast to cholesterol and black pigment stones in the gallbladder.

3. EPIDEMIOLOGY OF HEPATOLITHIASIS

Hepatolithiasis is prevalent in East Asia, including Japan (9,10). Although the disease is extremely rare in Western countries, it has been increasingly encountered in those countries because of increased

Table 1. Relative Prevalence of Hepatolithiasis in the World

China– Shenyang	21.2%	Nakayama	1986
– Beijing	9.2%	Nakayama	1986
Taiwan	20.3%	Su	1992
Korea	10.8%	Han	1992
Hong Kong	3.1%	Nakayama	1986
Singapore	1.7%	Nakayama	1986
Chile	1.5%	Yarmuch	1989
Italy	1.0%	Gandini	1990
Japan	2.2%	Tanimura	1992

* Proportion of hepatolithiasis to all cholelithiasis cases

immigration from Asia. The highest incidence of hepatolithiasis occurs in the fifth to sixth decades and is usually distributed between the 30- and 70-year age group (11). Intrahepatic type is more frequent in the younger age group, while intra- and extrahepatic type occurs in the elder groups. The first sign of hepatolithiasis appears between 30 and 50 years of age but sometimes even earlier.

Based on the recent data of the surveys of hepatolithiasis worldwide (Table 1) (10,12-16), a large geographical difference in the relative proportion of hepatolithiasis to all cholelithiasis is observed between the East and the West. Furthermore, in spite of similar ethnic backgrounds, the proportion differs significantly in East Asia. In Taiwan, hepatolithiasis comprises more than 50% of all cholelithiasis (12), in Hong Kong 3.1% (10) and in Singapore 1.7% (10). Regional differences also exist within China itself. Shenyang in northeastern China had a higher relative proportion of hepatolithiasis to all cholelithiasis, i.e., 21.1% (10), while in Beijing it was only 9.2% (10). In the Western world, the prevalence of hepatolithiasis seems to be much less, less than 1% (17,18), as revealed by only occasional reports emerging from the West. However, the overall relative proportion of hepatolithiasis is increasing, since the number of immigrants from endemic areas entering the West is considerably increasing. In fact, most reports on hepatolithiasis appearing from the West concern such immigrant populations. In Latin America, the relative proportion of hepatolithiasis seems to be relatively high and reported to be 2-7% (11). In Japan, the relative prevalence is recently reported to be 2.2% (16).

Focusing on the data of the five surveys conducted by the Hepatolithiasis Research Group with the support of the Ministry of Health and Welfare of Japan, a chronological shift in the prevalence of hepatolithiasis has been noted (16,19-22). A survey conducted on Japan from 1970-1977 showed that the relative proportion of hepatolithiasis cases was in the years from 1970-1977, 4.1% (1,590/38,606) (19), in the years from 1975-1984, 3.0% (4,381/148,017) (19), in the years from 1985-1988, 2.3% (1,813/79,052) (21), in the years from 1989-1992, 2.2% (2,353/105,062) (16), and, in the years from 1993-1995, 1.7% (21). The increase of ordinary cholelithiasis in postwar Japan may have partly contributed to the apparent decrease in the relative proportion of hepatolithiasis. The relative proportion is also decreasing in Taiwan (23) as well as in Korea (24). Regarding the chronological shift in stone category of hepatolithiasis in Japan, hepatolithiasis appears mostly as brown pigment stones and the stone category has not changed so much (10). This is in much contrast to the increased number of gallbladder cholesterol stones, probably due to the Westernization of diet in postwar Japan. However, an increased number of primary cholesterol hepatolithiasis has been reported recently (4,25).

The Research Group has proposed a grade classification in order to scale the severity of hepatolithiasis (26). Grade 1 is defined as having no symptom, grade 2 as having abdominal pain, grade 3 as having either transient jaundice or cholangitis, and grade 4 as having either continuous jaundice, sepsis, or cholangiocarcinoma. In a recent survey of 473 hepatolithiasis patients (in 1998), the number of cases falling under grade 1 was at 20%, grade 2, 25%, and grades 3 and 4, 55% (26). It should be noted that more than half of the hepatolithiasis cases were classified as grade 3 or 4, the more severity of the disease. When viewing the results of postsurgical prognosis of 303 patients with hepatolithiasis over a ten year period, 12% of hepatolithiasis patients were still symptomatic, with fever or abdominal pain, and surprisingly 30% had died (27). Regarding the causes of death, it should be noted that 25% of dead cases were because of cholangiocarcinoma. In the survey, the association of biliary tract carcinomas with hepatolithiasis was 5.2% (27), and among the carcinomas, intrahepatic cholangiocarcinoma was reported most frequently (27). The frequency of associated hepatolithiasis among all cholangiocarcinoma cases in Japan varies from 5.7% to 17.5% (28-30). More attention should be paid on this association in the daily practice of hepatolithiasis patients.

The surveys reveal (a) a decreased proportion of hepatolithiasis to gallstone diseases, (b) an increased number of primary cholesterol hepatolithiasis, (c) no decreased number of intracterable cases, and (d) an increased number of death cases because of cholangiocarcinoma.

4. PATHOGENESIS OF HEPATOLITHIASIS

The majority of gallstones of hepatolithiasis are brown pigment stones (calcium bilirubinate stones) (31), which differ from cholesterol and black pigment stones in their composition (32) and etiology (33,34). Previous studies have suggested that bacterial infection (35) and bile stasis caused by bile duct strictures (36) are thought to be of pathogenetic importance of hepatolithiasis. However, the etiological process would be more complicated and may be governed by multiple factors, e.g., ethnics and environments, bacterial and parasitic infection, cholangitis and bile stasis, and biochemical/metabolic defects in the liver, all of which may affect the rheology of bile flow within the intrahepatic biliary tree as well as the formation and secretion of lithogenic hepatic bile.

4.1. Environmental and ethnic factors

The geographical differences in the relative proportion of hepatolithiasis to all cholelithiasis cases suggest ethnic and environmental factors in the

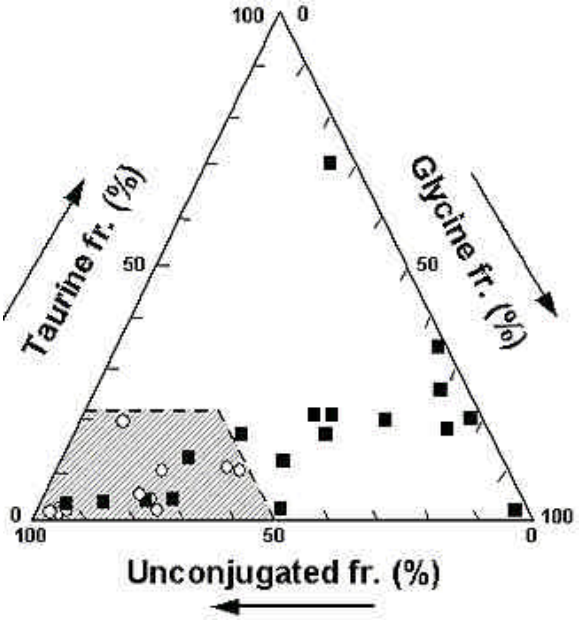

Figure 1. Bile acid composition of brown pigment stones in the intrahepatic bile ducts (hepatolithiasis) and in the extrahepatic bile ducts. Triangular coordinate diagram viewed with the C-24 unconjugated, glycine-conjugated, and taurine-conjugated bile acid fractions. Each fraction is expressed as weight percentage of total bile acids. Gallstones were classified based on visual inspection and infrared analysis: ■ (solid square), brown pigment stones in the intrahepatic bile ducts; ○ (open circle), brown pigment stones in the extrahepatic bile ducts. The dashed lines indicate the lower limit of unconjugated bile acid content or the upper limit of taurine-conjugated bile acid content in the extrahepatic brown pigment stones. The shaded area represents their distribution. Thirteen of 18 brown pigment stones were outside the area.

pathogenesis. In the previous surveys conducted in East Asia and some other countries (16,19-22), the relative proportion of hepatolithiasis to all cholelithiasis cases in China, Taiwan, Korea, Hong Kong, Singapore, Chile, and Italy was compared to that in Japan (Table 1). The relative population differs considerably from country to country in spite of a similar ethnic background. Moreover, focusing on the chronological change in the relative proportion of hepatolithiasis in Japan, the epidemiology shows that the proportion of hepatolithiasis had decreased from 4.1% in 1970-1977 (19) to 1.7% in 1993-1995 (22), in contrast to the increased number of gallbladder cholesterol stones, probably due to the Westernization of diet in postwar Japan. The surveys also showed an increased number of cases of primary cholesterol hepatolithiasis. All of these data on the epidemiology imply the importance of environmental factors in the pathogenesis.

The case-control studies in Japan and Taiwan, with special reference to the food habits and environmental factors associated with the high incidence of hepatolithiasis (11,37,38), were conducted by the Hepatolithiasis Research Group. The surveys reveal possible risk factors associated with the incidence of hepatolithiasis: (a) being born in the countryside, (b) using well water, (c) having a past history of parasitic infestation, (d) low hygienic conditions of the present domicile, and (e) consuming a traditional Japanese diet (11). Thus, the Group showed a close association between the high incidence of hepatolithiasis and the lower socioeconomic status, that is, poor nutrition (e.g., more fish and less butter) and low hygienic conditions, or traditional living habits (e.g., non-flush toilet).

The dietary habits may be causatively related to the etiological process of hepatolithiasis: the diet low in saturated fat and protein may lead to a relative increase in bile stasis and an increased chance for bacterial infection and its related precipitation of calcium bilirubinate in the intrahepatic bile ducts, since the dietary components, i.e., saturated fat and protein, cause cholecystokinin release and sphincter of Oddi relaxation (39,40). In addition, the low-calorie diet may increase the amount of unconjugated bilirubin by decreasing biliary glucuronolactone, a major inhibitor of bacterial beta-glucuronidase (41), to which is referred later in “bacterial infection.”

Ethnic or genetic factors should also be considered in hepatolithiasis, since there are definite differences in the incidences between the East and the West, and the age-related incidence between intrahepatic and gallbladder brown pigment stones, that is, the high incidence of hepatolithiasis in younger generations. Genetic factors in patients with hepatolithiasis, e.g., the specified types of HLA, were also studied in detail (42). However, the results as yet are not sufficient as to indicate a significant role.

4.2. Bacterial and parasitic infection

The high incidence of bacteria in bile (43) and gallstones (44) suggests a close association between bacterial infection and the formation of brown pigment stones. In addition, bacterial 16S rRNA is detected at a high frequency in specimens of intrahepatic brown pigment stones, whereas it is detected at a low frequency in intrahepatic cholesterol stones (44). The infectious route of bacteria into the biliary tract would be due to either ascending infection through sphincter of Oddi, bacteriobilia through the portal venous system, or transient infection because of some degree of stasis. Bacterial beta-glucuronidase is believed to be responsible for the formation of calcium bilirubinate (45): the unconjugated bilirubin formed by bacterial beta-glucuronidase combines with ionized calcium present in the bile to form calcium bilirubinate, leading to the formation of brown pigment stones. Among the bacterial species isolated from the bile of patients with hepatolithiasis, E. Coli, Clostridium and Bacteroides show beta-glucuronidase activity (35).

However, there has been some evidence reported which stands in opposition to the central role of bacterial infection in the pathogenesis. Nonbacterial transformation is also known to take place in the bile (46,47), where tissue-associated beta-glucuronidase may be responsible for the hydrolysis of bilirubin glucuronides in the bile. In an analysis of chemical composition of intrahepatic brown pigment stones, stones with a large percentage of

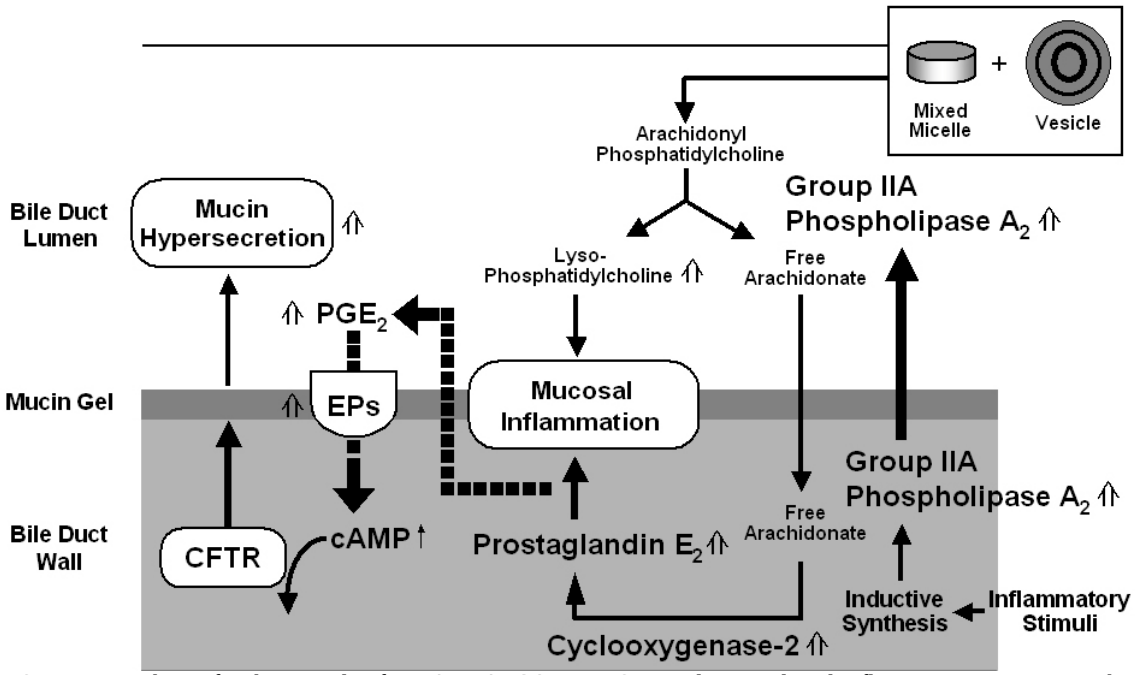

Figure 2. Putative scheme for the cascade of $sPLA_2$-IIA, COX-2, PGE_2, and EP-mediated inflammatory responses in both bile duct wall and hepatic bile in hepatolithiasis. Induction of $sPLA_2$-IIA and COX-2 synthesis in response to pro-inflammatory stimuli may occur in the bile duct epithelia. The synthesized $sPLA_2$-IIA is secreted into the lumen and then hydrolyzes arachidonyl-phosphatidylcholine in the biliary mixed micelles and vesicles or membranous arachidonyl-phosphatidylcholine on the mucosa, yielding lysophosphatidylcholine and free arachidonate. The bile becomes enriched with free arachidonate, which in turn enriches the epithelia with arachidonate. Enrichment of the mucosa with arachidonate provides a substrate of COX-2 for PGE_2. The generated PGE_2 mediate not only propagation of mucosal inflammation but also epithelial proliferation and mucin hypersecretion in the bile ducts, both of which are important for the pathobiology of chronic proliferative cholangitis. The growth promotion and mucin secretagogue action of PGE_2 in the bile duct epithelia might be mediated through the activation of the EP_4-receptor. CFTR, cystic fibrosis transmembrane conductance regulator; EPs, prostaglandin E-receptor subtypes; PGE_2, prostaglandin E_2

cholesterol and less bilirubin were encountered in about 20% of the cases, although most stones were identical to the usual brown pigment stones found in common bile ducts (3,5). The composition of bile acids present in gallstones may reflect the etiological process of gallstone composition (48). A detailed analysis of the bile acid composition of gallstones present in the intrahepatic duct shows that intrahepatic brown pigment stones have a lower total bile acid content, a smaller proportion of bacterial metabolites of bile acids, i.e., unconjugated bile acid, secondary bile acids (e.g., deoxycholate) and ketonic bile acids compared with those in the extrahepatic bile ducts (Figure 1) (5,6). Although bacterial infection plays a pivotal role in the formation of brown pigment stones, when considering the bile acid composition (5,6), it is suggested that bacterial infection does not solely play a major role in the pathogenesis and that by implication additional factors must be considered.

Parasitic infestation has often been cited as a major cause of hepatolithiasis (49). However, infestation seems incidental rather than causative since the endemic areas of infestation are not correlated with the areas with a high prevalence of hepatolithiasis in Taiwan (10), parasites and ova (e.g., Clonorchis) are found infrequently in cases with hepatolithiasis, and the high incidence of infestation was largely eliminated in postwar Japan but hepatolithiasis still persists (10).

4.3. Cholangitis and bile stasis

The main morphologic feature of stone-containing bile ducts in hepatolithiasis is chronic proliferative cholangitis (50,51), a condition in which intramural and extramural peribiliary glands proliferate to a marked degree and in which the lining epithelia are hyperplastic. Chronic inflammation precedes bile duct deformations, i.e., strictures and/or dilatation, both of which in turn may cause bile static alterations of biliary flow owing to an uneven inner surface of the affected bile ducts. Moreover, the majority of proliferating glands have mucin-producing activity (50). Excessive amounts of mucin secreted into the inner surfaces of the ducts may provide a microenvironment that initiates a nidus for stones by trapping calcium salts (52) and lipids (53), and also cause stones to expand by altering biliary flow in bile ducts. In terms of the biliary mucin molecules, an increased amount of sulfomucins and sialomucins were found in the bile of hepatolithiasis patients (54). Importantly, these acid mucins reduce pH in the bile and this in turn leads to diminished solubility of unconjugated bilirubin or calcium

bilirubinate in the bile. The expression levels of mucin core polypeptide (MUC) genes in the bile ducts were found to be heterogeneous. The abundance of secretory-type mucins (MUC2, MUC3, MUC5AC, MUC5B, MUC6) was significantly higher in the bile ducts of hepatolithiasis patients than in the ducts of controls (54). Gel-forming mucins of MUC2 and 5AC may be more important for the pathogenesis. The events initiating chronic proliferative cholangitis are not fully addressed but thought to be the acute inflammation of bile ducts caused by infective organisms via the portal vein or alterations of bile components.

Seeking biochemical factors involving chronic proliferative cholangitis in hepatolithiasis, interest has recently been focused on the role of enhanced arachidonate metabolism in the bile ducts. Inflammatory cytokine-induced phospholipases A_2 (PLA_2s) and cyclooxygenase-2 (COX-2) are key enzymes in the activation of the cascade, and the PLA_2 and COX-2-derived PGE_2 plays an important role in the initiation and propagation of inflammatory changes of cholangitis. In the in vivo experiment, a long-term administration of PGE_2 may contribute to the pathological changes analogous to the changes found in human hepatolithiasis (55). The mRNA levels of secretory-type group IIA PLA_2 ($sPLA_2$-IIA) and COX-2 were significantly increased in the bile ducts with a change of chronic proliferative cholangitis compared with the levels in the bile ducts of control subjects (54). Immunostaining revealed an increased level of COX-2 protein in the proliferating glandular elements and the hyperplastic lining epithelia. These changes were associated with concomitant increases in PGE_2 in both the bile ducts and the bile. The mRNAs of prostaglandin E-receptor (EP) subtypes, EP_2, EP_3 and EP_4, were amplified in the affected bile ducts and expressed mostly in the glands and epithelia expressing COX-2 by *in situ* hybridization. These observations suggest an enhanced arachidonate metabolism and $sPLA_2$-/COX-2-derived PGE_2 synthesis and its actions mediated via the EP subtypes in the bile ducts may be of pathobiological significance for chronic proliferative cholangitis in patients with hepatolithiasis (Figure 2).

In the biliary composition, the $sPLA_2$-IIA as well as PLA_2 enzyme activity was significantly increased in the bile of hepatolithiasis patients, compared to their levels in gallbladder stone or common bile duct stone patients. There was a concomitant increase in the concentration of lysophosphatidylcholine (54), which could potentiate mucosal inflammation of bile ducts. In regard to the mucin secretagogue action of the increased PGE_2 levels, the total mucin concentrations were significantly increased in the bile (54). The mucin secretagogue action of PGE_2 might be mediated through the activation of EP_4 (56).

Bile stasis caused by bile duct stenosis and stricture formation in primary hepatolithiasis, usually associated with areas of ductal dilatation, has been considered as the cause of the disease. However, in some cases with primary hepatolithiasis, no stenosis nor stricture can be observed in spite of the presence of stones. Left hepatic duct involvement predominates for still unknown reasons (10). A comparable study on the anatomical variation of bile ducts between biliary tract diseases, including cholecystolithiasis, choledocholithiasis, and control subjects, clearly indicated that the incidence of the mode of confluence of main intrahepatic bile ducts are not closely associated with the presence or absence of intrahepatic stones (57). Regarding the nature of bile duct strictures in cases of primary hepatolithiasis, there has been no report of absolute strictures of intrahepatic large bile ducts found before the development of hepatolithiasis (50). The histology of the stricture is quite similar to that of the adjacent parts of the stone-containing ducts (50). It has been suggested that chronic inflammation (e.g., chronic proliferative cholangitis) precedes ductal stricture in hepatolithiasis and that the stricture is secondary to the development of intrahepatic stones. The abnormal biliary flow due to an uneven inner surface may promote further growth of preexisting initial stones.

Importantly, the issue of chronic proliferative cholangitis not only causes strictures and/or dilatations of the bile ducts but also is involved in biliary carcinogenesis. In some cases with recurrent cholangitis, atypical epithelial hyperplasia was recognized (58). Chronic inflammation causes epithelial cell death, which in turn increases epithelial cell proliferation. This is followed by an increased rate of cellular DNA synthesis and production of endogenous mutagens coupled with a compromised cellular repair function (59). If these processes are sustained for a long period of time, they would cause the multiple chromosomal changes necessary to trigger the development of cholangiocarcinoma.

4.4. Biochemical/metabolic defects

It is of particular interest that intrahepatic stones with higher cholesterol and lower bilirubin contents are encountered in about 20% of the cases with hepatolithiasis (3). A series of our analytical works (5,6) has shown that, in chemical composition, intrahepatic brown pigment stones contain more cholesterol than do extrahepatic brown pigment stones (43% vs. 20%). Moreover, primary pure cholesterol stones within the intrahepatic bile ducts have been reported more frequently in Japan (25). In searching for any metabolic defects underlying the formation of cholesterol-rich brown pigment stones, a comparison of hepatic cholesterol and bile acid metabolism was made between hepatolithiasis and gallbladder cholesterol stones.

In the liver of patients with hepatolithiasis, (a) de novo synthesis of cholesterol (determined by the enzyme activity of HMG-CoA reductase) is up-regulated significantly in both affected and unaffected hepatic segments, irrespective of whether the stone category is brown pigment or pure cholesterol, and (b) bile acid synthesis (determined by the activity of cholesterol 7alpha-hydroxylase), which is involved in the catabolism of cholesterol, is down-regulated significantly in both hepatic segments (44,60). Up-regulated hepatic cholesterogenesis and decreased bile acid synthesis in the liver may underlie the formation of cholesterol-supersaturated hepatic bile. The cholesterol saturation indices in hepatic bile are significantly higher in patients with hepatolithiasis, for the

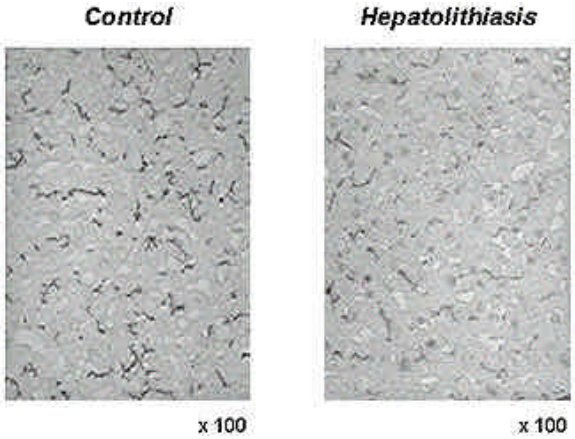

Figure 3. Immunohistochemical localization of MDR3 P-glycoprotein in liver sections. Immunostaining of MDR3 Pgp shows a diffuse and linear pattern outlining the canalicular membrane domain of the liver section from a control subject (original magnification x 100) In a section of the liver from a patient with hepatolithiasis (brown pigment stone case), the staining pattern of MDR3 Pgp outlines the canalicular membrane domain, but is less defined and focally absent (original magnification x 100) In a hepatolithiasis patient, sections of the hepatic segments affected by stones were used for immunohistochemistry.

specimens from both affected and unaffected ducts, than in control subjects and patients with gallbladder cholesterol stones (44,60). In turn, the alterations of hepatic bile decreased bile acid but unchanged cholesterol concentrations appear to reflect the cholesterol and bile acid contents of intrahepatic brown pigment stones (44,50).

Furthermore, we have recently suggested hepatic transport and secretion defects of phospholipid (phosphatidylcholine) in the setting of increased cholesterogenesis and decreased bile acid synthesis (44). Phospholipid concentrations are significantly decreased in the hepatic bile issuing from both affected and unaffected bile ducts of intrahepatic stone patients in this study, irrespective of stone category (44). This possibility is supported by the decrease in both affected and unaffected hepatic segments of mRNA and protein levels of multidrug resistance 3 P-glycoprotein (MDR3 Pgp), which is rate-limiting for phospholipid secretion into bile (62). Phosphatidylcholine transfer protein (PCTP) may play a role in resupplying the canalicular membrane with biliary phosphatidylcholines during bile formation (63). The decreased biliary phospholipid secretion rates in patients with hepatolithiasis are found to be associated with a significant down-regulation of both PCTP and MDR3 Pgp mRNA and protein levels compared with those in patients with cholesterol gallstones and in control subjects. The immunostaining of MDR3 Pgp outlined the canalicular membrane domain but was less defined and focally absent in the liver of patients with hepatolithiasis, especially in affected segments (Figure 3). An alteration of the distribution of MDR3 Pgp from the canalicular membrane to the subapical domain or missorting to the canalicular membrane domain would correlate with impaired biliary phospholipid secretion. To elucidate whether the decreased MDR3 Pgp mRNA and protein expression in hepatolithiasis patients is associated with the gene mutations, a mutation analysis on the cDNA of the MDR3 Pgp gene with special reference to the coding region was performed using the liver specimens of hepatolithiasis patients. The results revealed that the heterozygous mutations were detected in only two of the 14 patients (unpublished observation), which in turn suggested either unknown mutations in the promoter region of the MDR3 Pgp gene or a possible involvement of unknown genes possibly regulating MDR3 Pgp transcription and/or trafficking in the liver.

Phosphatidylcholine and bile acids form mixed micelles in bile and protect the biliary epithelium from detergent action of unconjugated bile acids (64). The loss of phospholipid secretion appears to be the main cause of the nonsuppurative destructive cholangitis in FVB mice with a disrupted mdr2 Pgp gene (65) and in patients with progressive familial intrahepatic cholestasis-3 (PFIC-3) (66). Human bile acids are more hydrophobic than those of rodents and might therefore contribute to more aggressive ductular lesions. Under these conditions, the presence of hydrophobic bile acids in the bile ducts could solubilize the plasma membrane of the biliary epithelia. Hyposecretion of phospholipids, coupled with hydrophobic bile acid (e.g., chenodeoxycholate) secretion, may thus contribute to chronic proliferative cholangitis.

In hepatolithiasis, the observed biochemical /metabolic defects in the liver may be interpreted as the consequence of primary events, since these changes are specific to patients with hepatolithiasis and observed in both affected and unaffected segments of the liver, irrespective of whether the stones are brown pigment or cholesterol. However, specified factors initiating the defects in the liver, e.g., food and environmental issues stated before, or even gene mutations, have not been well elucidated yet.

5. SIMILARITY AND DIFFERENCE BETWEEN HEPATOLITHIASIS AND GALLBLADDER STONES OR COMMON BILE DUCT STONES

We hypothesize that in hepatolithiasis primary events in the liver underlying biliary cholesterol supersaturation may be secondarily modified by local events in the bile ducts, e.g., cholangitis, mucin hypersecretion, or bacterial infection, all of which interplay to form cholesterol-rich brown pigment stones in the intrahepatic bile ducts. Here, the major similarities and differences between hepatolithiasis and gallbladder stones (cholesterol stones) or common bile duct stones (brown pigment stones) are summarized in Figure 4. A high degree of biliary cholesterol-supersaturation is similar between hepatolithiasis and gallbladder stones, but the formation mechanism may be different. Phospholipid concentration in hepatic bile and MDR3 Pgp expression in liver are decreased in patients with hepatolithiasis but preserved in those with gallbladder stones and those with common bile duct stones. Bacterial infection, cholangitis, and a high

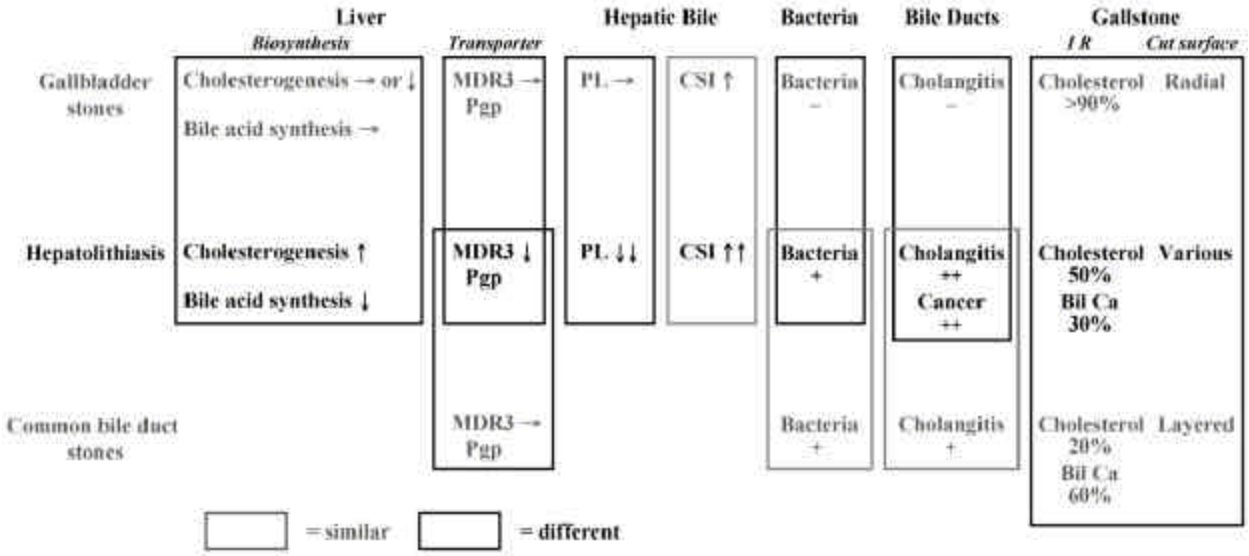

Figure 4. Similarities and differences in terms of factors important for the pathogenesis of hepatolithiasis between hepatolithiasis and gallbladder stones or common bile stones. A high degree of biliary cholesterol-supersaturation is similar between hepatolithiasis and gallbladder stones, but the formation mechanism may be different. Phospholipid concentration in hepatic bile and MDR3 Pgp expression in liver are decreased in hepatolithiasis but preserved in gallbladder stones and common bile duct stones. Biliary bacterial infection and cholangitis differentiates hepatolithiasis from gallbladder stones. Visual inspection and chemical composition show great differences between hepatolithiasis and gallbladder stones, making hepatolithiasis rather similar to common bile duct stones. CSI, cholesterol saturation index; PL, phospholipid; Bil Ca, calcium bilirubinate.

association with cholangiocarcinoma differentiates hepatolithiasis from gallbladder stones. Visual inspection and chemical composition show great differences between hepatolithiasis and gallbladder stones, making hepatolithiasis rather similar to common bile duct stones. Hepatolithiasis, in terms of etiology and chemical composition, lies intermediately between gallbladder stones (cholesterol stones) and common bile duct stones (brown pigment stones). The disease is considered to be a unique hybrid of these two types of gallstones.

6. POTENTIAL THERAPIES FOR HEPATOLITHIASIS

The surveys reveal that the percentage of cases undergoing hepatectomy remains largely unchanged. Although we notice a significant increase in the percentage of cases undergoing endoscopic treatment, therapeutic options are still limited. Briefly, we introduce future medical treatments with some drugs and agents which target the molecules important for the pathogenesis of the disease.

6.1. Urosodeoxycholate

Ursodeoxycholate (UDC) has been widely used for dissolution of cholesterol gallstones (67).?With a long-term administration, it also seems to reduce the cumulative occurrence rate of biliary colics in symptomatic gallstone patients (68). Another effect of UDC might be on decreased levels of various proteins and nucleation-promoting activity in bile during the treatment (69). The reduction in the cumulative occurrence rate of biliary colics (68) as well as the decreased levels of various pronucleating proteins (69) may be related to its membrane-protective effects on the inflamed gallbladder mucosa during the treatment. Long-term UDC administration lowers the increased biliary level of $sPLA_2$-IIA protein in gallbladder cholesterol stone patients (70). These beneficial effects of UDC should be applicable to treatment of chronic proliferative cholangitis in hepatolithiasis. In another regard, UDC exerts a potent choleretic activity by stimulating hepatobiliary secretory function due in part to stimulation of vesicular exocytosis and insertion of Mrp2 and Bsep into the canalicular membrane in the liver (71). This choleretic effect may contribute to lowering the risk of recurrence after the surgical or endoscopic removal of intrahepatic stones. A substantial benefit of UDC treatment has been already reported in a few cases of primary hepatolithiasis associated with Caroli's disease (72).

6.2. Selective COX-2 inhibitors and Prostaglandin E-receptor antagonists

An enhanced synthesis of Cox-2-derived PGE_2 and its actions mediated via the EP subtypes in the bile ducts may be of pathobiological significance for chronic proliferative cholangitis in patients with hepatolithiasis. Selective COX-2 inhibitors or EP-selective antagonists may be potent candidates as therapeutic agents for the disease. In the in vitro study, treatment with an EP_4 selective antagonist abolished the biological effects of PGE_2 on DNA synthesis, colony number, and mucin secretion of biliary epithelial cells (submitted elsewhere for publication).

6.3. Fibrates

Fibrates have been used as antilipidemic drugs. Recently the drugs have been reported to up-regulate the

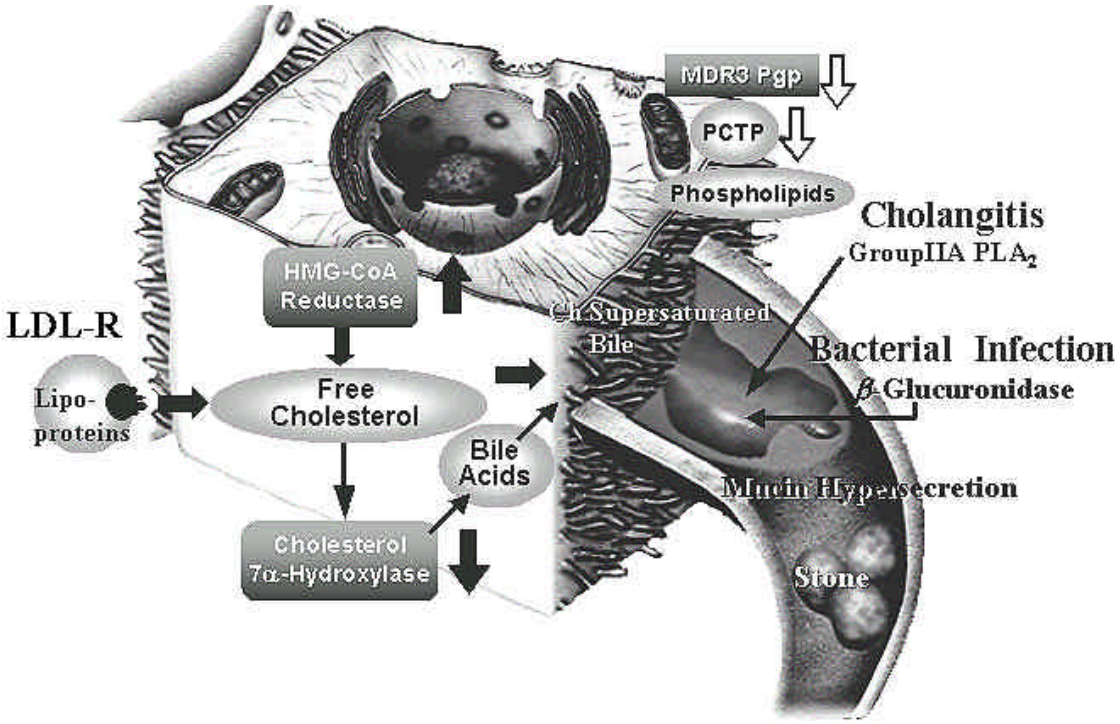

Figure 5. Putative scheme for the pathogenesis of hepatolithiasis. The decreased phospholipid secretion, probably due to the low level expression of MDR3 Pgp and PCTP, in the setting of the increased cholesterogenesis and decreased bile acid synthesis, may be a basic biochemical/metabolic defect in the liver and underlie the biliary cholesterol supersaturation. And then, these primary events may be secondarily modified by local events in the bile ducts, e.g., cholangitis, mucin hypersecretion, and bacterial infection, all of which underlie the bile static conditions and then interplay to form cholesterol-rich brown pigment stones. The liver supplies the calcium bilirubinate nidus with cholesterol-supersaturated bile for coating.

expression of mdr2 Pgp (murine homologue of human MDR3 Pgp) through the gene transcription (73). MDR3 Pgp is rate-limiting for phospholipid secretion into bile (62). There is an accompanying increase in biliary phospholipid secretion. The impaired function of mdr2 Pgp was confirmed to be associated with the development of cholangitis in the experiments using mdr2 knockout mice (65). Treatment with bezafibrate, a second-generation of fibrate analog, improves the elevated serum levels of biliary enzymes (ALT, ALP, and gamma-GT) in patients with primary biliary cirrhosis (74), a chronic cholestatic liver disease characterized by progressive inflammatory destruction of the intrahepatic bile ducts. These findings provide a rationale for the therapeutic role of fibrates in hepatolithiasis.

6.4. Herbal medicines

A Kampo (Chinese/Japanese herbal) medicine, Inchin-ko-to (ICKT), has long been recognized in Japan and China as a choleretic (75-77) and hepatoprotective (78) agent for various types of liver diseases. Considerable evidence (77,79,80) suggests that IKCT may exert its choleretic activity in a “bile acid-independent” mechanism through a selective stimulation of Mrp2-mediated bile formation and secretion (submitted elsewhere for publication). An impairment of Mrp2 expression and function in cholestasis may be an important target for specific pharmacotherapeutic interventions. IKCT should be used as a potent drug aimed at stimulation and restoration of defective expression and function of Mrp2 in hepatobiliary diseases, including hepatolithiasis.

7. SUMMARY

Hepatolithiasis is prevalent in East Asia, though extremely rare in the West. Gallstones of hepatolithiasis appear mostly as brown pigment stones (calcium bilirubinate stones) but contain more cholesterol in composition. Hepatolithiasis has a high rate of gallstone recurrence as well as a high association with cholangiocarcinoma. Therefore, the clinical course of the disease is often intractable and the postsurgical morbidity and mortality are still substantial.

In the epidemiology, the chronological surveys conducted by the Research Group in Japan have revealed a decreased proportion of hepatolithiasis to all cholelithiasis, an increased number of cholesterol hepatolithiasis, no decreased number of intracterable cases, and an increased number of death cases because of cholangiocarcinoma. In the pathogenesis, the results of a retrospective study in East Asia suggest that environmental rather than ethnic factors are implicated in their cause (10). Among the environmental factors to be considered are dietary habit, bacterial infection, and parasites. However, parasites such as Clonorchis sinensis are not very common in Japan. The improvements of sanitary and/or socioeconomic conditions may be involved in the decreased proportion of

hepatolithiasis. The genetic or ethnic role in the pathogenesis remains unknown, although it may play a certain role.

A definite tendency for stones to form within the left lobe is observed (10), and the majority of primary intrahepatic stones consists of brown pigment stones, except for a few pure cholesterol stones. Also, the type of intrahepatic stones is dependent on the patient's age and the presence of a concomitant biliary infection. The stones that form during the first or second decades of life in the absence of biliary infection are usually cholesterol or mixed stones. Gallstones formed in the presence of biliary infection are brown pigment stones. The observations have led us to hypothesize that primary events in the liver may be secondarily modified by local events in the bile ducts as described in Figure 5. The decreased phospholipid secretion, probably due to the low-level expression of MDR3 Pgp and PCTP, in the setting of the increased cholesterogenesis and decreased bile acid synthesis, may be a basic defect underlying the biliary cholesterol supersaturation. And then, this basic defect may be secondarily modified by local events, e.g., cholangitis, mucin hypersecretion or bacterial infection, all of which underlie the bile static conditions and then interplay to form cholesterol-rich brown pigment stones in the bile ducts. After all, the liver supplies the calcium bilirubinate nidus with cholesterol-supersaturated bile for coating. However, factors initiating the primary events in the liver as well as those triggering the local events in the bile ducts have not yet been well understood. All the data on the epidemiology and pathogenesis suggest that hepatolithiasis is a unique hybrid having combined features of cholesterol stones in the gallbladder and brown pigment stones in the common bile duct.

Considering the intractable course and the substantial postsurgical morbidity and mortality, efforts should be made to gain an understanding of the pathogenesis of the disease with special reference to an identification of environmental and/or ethnic factors involved in the primary events in the liver or those triggering the local events in the bile ducts, and furthermore an identification of hepatolithiasis susceptibility genetic loci in the human genome which correspond to the Lith genes identified in experimental cholesterol cholelithiasis in inbred mice (81) and involved in the defects, as well as an understanding of the molecular mechanism of inflammation-associated biliary carcinogenesis. In therapeutic aspects, some potent adjuvant therapies after surgical or endoscopic treatment, which target the pathobiological factors, or chemopreventions against biliary carcinogenesis should be introduced in the future practice, especially for the intractable cases.

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