

Overexpression of sterol carrier protein-2 mRNA in patients with cholesterol gallstones

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BACKGROUND: Hypersecretion of biliary cholesterol is believed to be one of the important causes of lithogenic bile. Sterol carrier protein-2 (SCP₂) participates in cholesterol trafficking and metabolism and may play a key role in cholesterol gallstone formation. This study was undertaken to investigate the expression of liver SCP₂ mRNA in patients with cholesterol gallstone and those patients with non-cholesterol gallstone.

METHODS: The expression of liver SCP₂ mRNA was studied in 36 patients with cholesterol gallstone and 30 patients with non-cholesterol gallstone by reverse transcription-polymerase chain reaction (RT-PCR).

RESULT: The expression of SCP₂ mRNA was increased more significantly in patients with cholesterol gallstone than in patients with non-cholesterol gallstone.

CONCLUSION: The SCP₂ gene was overexpressed in patients with cholesterol gallstone, indicating that SCP₂ may be one of the important causes of cholesterol gallstone.

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KEY WORDS: SCP₂ mRNA expression;
lithogenic biles;
cholesterol gallstone

Introduction

Hypersecretion of cholesterol in bile, which results in the formation of lithogenic bile, is believed to be one of the major causes of cholesterol gallstone.^[1,2] Sterol carrier protein-2 (SCP₂) or nonspecific lipid transfer protein, is a 13.2 kDa base

protein and exists in peroxisome, mitochondria, endoplasmic reticulum and cytoplasm.^[3-5] As a moderator factor of cholesterol metabolism, the protein is involved in the biosynthesis of cholesterol^[6-8] and the trafficking of cholesterol to bile acid,^[9,10] cholesteryl ester^[11] and sterol.^[12] As a transporting tool, on the other hand, it participates in the transportation of cholesterol inside the cell and through the cytoplasm membrane^[13,14] as well as the rapid transportation of the newly synthesized cholesterol from the endoplasmic reticulum into the bile without the intervention of cytomicrotubule system and Golgi bodies.^[15] Hence hypersecretion of biliary cholesterol with the formation of lithogenic bile might explain the mechanism of the formation of cholesterol stones in the gallbladder.

In this study, we investigated the expression of SCP₂ mRNA in the liver tissue of patients with cholesterol stones and non-cholesterol stones, using reverse transcription-polymerase chain reaction (RT-PCR) technique.

Methods

Patients

Patients were divided into the cholesterol stone group and control group. The cholesterol stone group consisted of 36 patients with cholesterol stones in the gallbladder, with a cholesterol level of >50% and the control group comprised 30 patients with primary intrahepatic cholangiolithiasis, with a cholesterol level of <20%, and those patients with peptic ulcer, and cancer of the stomach or the colon, in whom no gallstones were verified by ultrasonography. The following patients were excluded: diabetic patients and patients with other endocrine metabolism disorders, obese individuals, and patients with other diseases of the liver and the gallbladder. Informed consent was obtained from the patients and their family members.

All the patients were fasted for 12 hours before operation. Under general anesthesia, a liver specimen of 50 mg was obtained. In the cholesterol stone group, 26 women and 10 men, aged 25-80 years, the stones were yellow or light yellow, with smooth or nodular surfaces.

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In the control group, 16 women and 14 men, aged 15-78 years, 11 patients had primary hepatocholedocholithiasis with brown or dark-brown stones, friable, similar to bile pigment stones, 5 gastric or duodenal peptic ulcer, 4 gastric cancer, 4 colon cancer, 4 non-cholesterol polyp in the gallbladder, and 2 portal hypertension. No significant differences were observed in age and body mass index between the two groups ($P > 0.05$).

Reagents

Total RNA kit was purchased from Promega Co., USA, RT-PCR kit from Dalianbao Bioengineering Co., Ltd., China, and the primer was synthesized and supplied by Dalianbao Bioengineering Co., Ltd., China. The sequence of primers were as follows; upper reach, 5' ATGGGGTTTTCCGGAAGCCGCCAGTT, and lower reach, 5' TCAGAGCTTAGCGTTGCCTGGCTGA, and the expansion product: 432bp. Biochemical kit purchased from Centric Co., USA was used for the determination of cholesterol level in gallstones, and other kits were purchased from Zhongsheng Bioengineering High-Tech Co., Beijing, China for the determination of serum total cholesterol (CHO), triglyce-ride (TG) and high density lipoprotein-C (HDL-c) levels with an automatic biochemical analyzer (Merck-Mega).

Methods

The extraction of total RNA in liver tissue and RT-PCR were performed following the instructions of the kits. Reverse transcription (RT) expansion parameters were 50 °C RT 30 minutes, 94 °C 2-minute reaction pause, 94 °C degeneration 30 seconds, 60 °C annealing 30 seconds, 72 °C extension 1 minute, after 30 cycles, 72 °C extension 8 minutes, and 5 μ l PCR product 1% agarose electrophoresis. The electrophoresis photodensity value was determined by the gel imaging analysis system. The cholesterol level in gallstones was determined. The stones obtained during the operation were rinsed with water and put in a dryer, until a constant weight was obtained. Subsequently, the stones were grounded, and dried naturally for 12 hours. A 10 mg sample of gallstone powder was weighed and dissolved in 5 ml anhydrous alcohol, stirred for 3 minutes, and centrifuged at 3000 rpm for 5 minutes. The supernatant was collected for further analysis. Another 10 mg sample was accurately weighed and dissolved in 5 ml chloroform, stirred for 3 minutes and centrifuged at 3000 rpm for 5 minutes, and the supernatant was collected for analysis. The wt/wt ratio of cholesterol in stones was determined by the enzyme method; and the bile pigment content determined by the azo method. The data were expressed as mean \pm standard deviation. Student's *t* test with software SPSS10.0 was used in the analysis of independent-samples and bivariate correlation.

Table. The levels of serum lipid in the cholesterol gallstone group and non-cholesterol gallstone group (mmol/L)

Group	CHO	TG	HDL-c
Control	4.7990 \pm 1.2967	1.5203 \pm 1.0572	1.1423 \pm 0.2572
Cholesterol gallstone	4.8868 \pm 1.7188 *	1.6695 \pm 1.8553 *	1.0455 \pm 0.2446 *

* : compared with control group, $P > 0.05$.

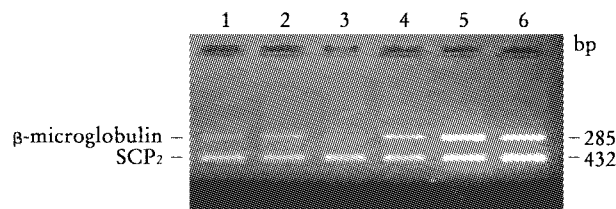


Fig. 1. The expression of SCP₂ mRNA in the cholesterol gallstone group and non-cholesterol gallstone group. Lanes 1-3: the cholesterol gallstone group; Lanes 4-6: the non-cholesterol gallstone group.

Results

The relative photodensity value could be calculated from the ratio of the photodensity value expressed by SCP₂ mRNA to the photodensity value expressed by internal referral value. In the cholesterol gallstone group, the photodensity value (0.98 ± 0.45) expressed by SCP₂ mRNA was higher than that (0.60 ± 0.30) in the control group ($t = 2.786$, $P < 0.01$, Fig. 1). The levels of serum CHO and TG in the cholesterol gallstone group were slightly higher than those of the control (4.85 ± 1.82 vs 4.72 ± 1.32 mmol/L, 1.70 ± 1.10 vs 1.45 ± 1.00 mmol/L). The level of serum HDL-c was lower than that of the control group (1.03 ± 0.24 vs 1.11 ± 0.25 mmol/L) ($P > 0.05$). The expression of SCP₂ mRNA in the cholesterol gallstone group did not show a linear relationship among the levels of serum CHO, TG, and HDL-c ($P > 0.05$, Table).

Discussion

The formation of cholesterol stones in the gallbladder is a multi-factor mediated process, and its pathogenesis is not well elucidated. The primary condition is the supersaturation of cholesterol in bile as a consequence of abnormal cholesterol metabolism in the liver, which is dependent on several factors, including transportation of cholesterol to the liver through low density lipoproteins (LDL); 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), cholesterol biosynthetic rate-limiting enzyme; 7 α hydroxylase (C7H), rate-limiting enzyme for transformation of cholesterol to bile acid; ACAT, rate-limiting enzyme for transformation of cholesterol to cholesteryl ester and sterols; and high-density lipoprotein

(HDL), through which cholesterol in hepatocytes is transported to the whole body. Investigations on abnormal cholesterol metabolism in the past half a century have concentrated on the above-mentioned proteins, attempting to explore the causes of supersaturation of cholesterol in bile, but with no success.^[16-20]

The SCP₂ gene is known to be involved in cholesterol transportation and metabolism, as shown in extensive studies. Ito et al.^[19] reported that 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), 7 α hydroxylase (C7H), acyl-CoA: cholesterol acyltransferase, ACAT activities in gallstones of 24 patients with cholesterol stones were not significantly different from those in the control group, while SCP₂ level in the gallstone group increased significantly. Therefore, the correlation of SCP₂ cholesterol transportation/metabolism with the formation of cholesterol stones in the gallbladder was suggested. As a result, the role of SCP₂ and molecular biological level were investigated to understand the cause of lithogenic bile formation, suggesting that hypersecretion of biliary cholesterol, forming lithogenic bile, might be one of the major causes of cholesterol stone formation. Animal experiments also proved that the change of SCP₂ gene expression plays an important role in the formation of cholesterol stones.

In this study, the expression of SCP₂ mRNA was found to increase significantly in patients with cholesterol stones, compared with the control group. Gene expression is regulated mainly at the level of transcription, so increased level of SCP₂ mRNA expression will lead to the marked increase of synthesis and secretion of corresponding proteins.^[21] SCP₂ mRNA expression is tissue-specific,^[22,23] i. e., the highest expression in liver tissues, moderate expression in muscle tissues, and little or least expression in fat, lung, brain and spleen tissues. Simonet^[24] reported a hepatic control region in the promoter area of the liver, which directed specifically the high expression of apo E and apo C genes; hence the expression of both genes is considered tissue-specific. Subsequently, Ohba^[25] found that the SCP₂ gene promoter in XI introne has a TATA deficient box structure with cell host specific patterns of promoter activity. It is hypothesized that there exists a specific hepatic control region, through which the increased level of hepatocyte cholesterol and the increase of quantity and speed of cholesterol transportation by SCP₂ might enhance the expression of SCP₂ mRNA. SCP₂ may increase the cholesterol level in hepatocytes through the following processes: promoting the formation of cholesterol from 7-dehydrocholesterol; inhibiting C7H activities and in turn decreasing the transformation of cholesterol to bile acids;^[26] inhibiting ACAT activities to decrease the transformation of cholesterol to cholesterylester; inhibi-

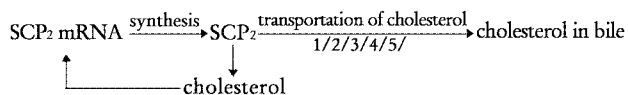


Fig. 2. The process of stone formation.

ting HDL-cholesterol secretion^[27] and HDL receptor expression, and increasing HDL-cholesterol concentration. The high concentration of cholesterol may have a positive feedback on the expression of SCP₂ mRNA and protein, resulting in transportation of a large amount of cholesterol into the bile, supersaturation of biliary cholesterol, and precipitation for stone formation. The process is illustrated in Fig. 2.

In this study no abnormal blood lipid levels were noted in patients with cholesterol stones. Though the level of serum total cholesterol and triglyceride were slightly higher than those in the control group (4.85 ± 1.82 vs 4.72 ± 1.32 , 1.70 ± 1.10 vs 1.45 ± 1.00), and the level of high-density lipoprotein cholesterol was slightly lower than that in the control group (1.03 ± 0.24 vs 1.11 ± 0.25) no significant statistical differences were seen between them ($P > 0.05$). This finding was consistent with those reported by Ito and Gu.^[19,28] Others reported a lower level of serum cholesterol in patients with cholesterol stones than that in normal individuals,^[29] and the higher levels of total cholesterol and triglyceride and HDL-c in cholesterol stone patients than those in controls.^[30] Hence it is uncertain whether abnormal blood lipid levels exist in patients with cholesterol stones. In this study no linear relations were observed between the expression level of SCP₂ mRNA and the levels of serum total cholesterol, triglyceride and HDL-c ($P > 0.05$), indicating that SCP₂ mRNA expression is not affected by blood lipid level, but is positively linear-related to cholesterol level in gallstones ($P < 0.05$).

In conclusion, overexpression of SCP₂ mRNA might be one of the important causes of cholesterol stones in the gallbladder and SCP₂ might be one of the major pathogenic genes of cholesterol cholecystolithiasis. The expression level of SCP₂ mRNA in patients with cholesterol stones is not linearly related to the levels of serum total cholesterol, triglyceride and high-density lipoprotein cholesterol. Patients with cholesterol gallstones may not show abnormal level of blood-lipid.

Competing interest

The author or authors do not choose to response to the statements listed in Instructions for Authors.

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