Changes of lipid metabolism in plasma, liver and bile during cholesterol gallstone formation in rabbit model

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Abstract

AIM To find out the relationship between the disturbances of lipid metabolism and the formation of cholesterol gallstones by studying the changes of lipid metabolism in plasma, liver tissue and the bile.

METHODS Male and female white Japanese rabbits were divided randomly into a control group (Con) and four experimental groups of 10 rabbits each fed with a diet containing 1.2% cholesterol for one, two, three and four weeks (1 wk, 2 wk, 3 wk and 4 wk group). The measurement of plasma triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and its subfractions (HDL2-C, HDL3-C), very low and low density lipoprotein cholesterol (VLDL-C, LDL-C) was taken with standard enzymatic techniques. Apolipoprotein (apo) concentrations in plasma were measured by radial immunodiffusion assay for apoA1, apoB100, apoC¢ò and apoC II. Total cholesterol of liver was measured by the enzymatic procedure for each animal. Bile acids, mainly glycocholate (GCA) and glycodeoxycholate (GDCA) were detected by dual wavelength thin layer scanner.

RESULTS In all the experimental groups fed with dietary cholesterol, cholesterol crystal was found in the gallbladder in 2/10 cases of the 1 wk group, 4/10 of the 2 wk group, 6/10 of the 3 wk group and 7/10 of the 4 wk group respectively. The concentration of plasma total cholesterol (TC), triglyceride (TG), phospholipid (pl), VLDL-C, LDL-C, apoB100, apoC II, apoC III gradually increased (P<0.05) with the prolonged feeding time of dietary cholesterol. High density lipoprotein cholesterol and its subfractions (HDL-C, HDL2-C, HDL3-C) showed a tendency to decrease, but without statistical significance (P>0.05). ApoA1 was reduced with increased feeding time of dietary cholesterol (P<0.05). The hepatic and biliary cholesterol increased 1-1.5 times as compared with the control group (t = 5.221 and 3.445, P<0.05). The GCA gradually decreased beginning from the control group to the 4 wk group (P<0.05).

CONCLUSION Owing to the high cholesterol diet, the increased concentrations of plasma TC, TG, VLDL-C, LDL-C, hepatic TC and TG, apoB100, apoC II and apoC III possibly enhanced the secretion of biliary cholesterol into bile; the decreased plasma apoA1 level might reduce the secretion of antinucleating factor into bile. All those factors mentioned above probably contribute to the formation of cholesterol gallstones.

INTRODUCTION
Cholesterol cholelithiasis is one of the common diseases in China. The mechanism of gallstone formation is quite complicated and it has been supposed to be synthesized from a variety of related factors. Among them oversaturation of cholesterol and decrease of bile acids, as well as lecithin in bile might be the fundamental causative factors. Furthermore, these changes are closely related to the disorders of lipoprotein metabolism in liver. However, during the formation of cholesterol gallstones, different links in the disturbance of lipoprotein cholesterol metabolism and their effects in lithogenesis still has much controversies. Besides, the mechanism and regulating elements are still unclear. This study is aimed to investigate the changes of lipid metabolism in plasma, liver tissue and bile in order to find out the relationship between the disturbance of lipid metabolism and the formation of cholesterol gallstones.

MATERIALS AND METHODS

Animals and diets
Male and female white Japanese rabbits were obtained from the Experimental Animal Center of West China University of Medical Sciences. They were...
were divided randomly into a control group (Con) and four experimental groups of ten rabbits each fed with 1.2% cholesterol diet for one week (1 wk), two weeks (2 wk), three weeks (3 wk) and four weeks (4 wk). All animals were maintained in separate cages with free access to water.

**Gallstone evaluation**

At the end of experiment following an overnight fasting, animals were exsanguinated under anesthesia, blood samples were collected for the measurement of plasma lipoprotein cholesterol and apolipoproteins, and portions of liver were removed for the analysis of cholesterol. Bile specimen was aspirated from gallbladder and kept under the microscope, and the gallstones were evaluated according to Juniper’s method. Cholesterol stones proved by infra-red spectrometry were collected.

**Plasma lipid and lipoprotein analysis**

The measurement of plasma triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and its subfractions (HDL2-C, HDL3-C), very low and low density lipoprotein cholesterol (vLDL-C, LDL-C) was taken with standard enzymatic techniques. Apolipoproteins (apo) concentration in plasma were measured by radial immunodiffusion assay for apoA1, apoB100, apoC II and apoC III.

**Analysis of hepatic cholesterol**

Following lipid extraction of liver samples with chloroform and methanol (2:1, v/v) total cholesterol of liver was estimated by the enzymatic method for each animal.

**Analysis of bile cholesterol and bile acids**

Gallbladder bile was extracted with chloroform and methanol (2:1, v/v) total cholesterol of liver was estimated by the enzymatic method for each animal.

Table 1  The results of plasma lipids (mmol/L) and apolipoproteins (mg/L) for each group

|                     | Control | Experimental groups |
|---------------------|---------|---------------------|
|                     |         | 1 wk | 2 wk | 3 wk | 4 wk |
| TC                  | 0.79±0.03 | 4.50±1.86* | 7.23±3.87* | 16.35±3.49** | 17.21±4.78*** |
| TG                  | 0.83±0.21 | 1.10±0.45 | 1.45±0.51* | 1.52±0.41* | 2.87±0.81** |
| PL                  | 0.94±0.29 | 2.37±0.68* | 3.10±1.32* | 6.24±2.43** | 6.71±2.80** |
| HDL-C               | 0.70±0.21 | 0.72±0.30 | 0.52±0.23 | 0.55±0.21 | 0.58±0.24 |
| HDL2-C              | 0.32±0.10 | 0.35±0.11 | 0.25±0.13 | 0.27±0.11 | 0.31±0.16 |
| HDL3-C              | 0.31±0.11 | 0.37±0.17 | 0.26±0.12 | 0.29±0.17 | 0.28±0.15 |
| LDL-C               | 0.05±0.02 | 0.90±0.13* | 1.88±0.97** | 4.60±0.98** | 5.60±1.07*** |
| VLDL-C              | 0.06±0.01 | 0.45±0.18 | 1.22±0.91** | 3.43±0.87** | 3.76±1.01*** |
| apoB100             | 0.15±0.01 | 0.54±0.06* | 0.54±0.10* | 5.36±0.11** | 6.45±0.54*** |
| apoA I              | 20.82±1.57 | 18.10±2.23 | 17.87±2.07 | 18.16±0.74 | 15.96±1.13* |
| apoC II             | 0.05±0.01 | 0.15±0.02* | 0.30±0.04** | 0.25±0.01** | 0.69±0.04** |
| apoC III            | 0.17±0.02 | 0.45±0.06* | 1.22±0.05** | 1.62±0.06** | 2.91±0.07*** |

*P<0.05, vs Con; **P<0.05, vs 1 wk; ***P<0.05, vs 2 wk; ****P<0.05, vs 3 wk.

**Statistical analysis**

Results were shown as X±s (means±SD) and a paired Student’s t test was used for quantitative information, P<0.05 is considered statistically significant. Statistical analysis of the results was carried out using SPSS software in computer.

**RESULTS**

**Gallstones evaluation**

Cholesterol crystal in gallbladder was not found in any animal of the control group fed with routine diet. However, in all the experimental groups fed with dietary cholesterol, cholesterol crystal in gallbladder was found in 2/10 of 1 wk group, 4/10 of 2 wk group, 6/10 of 3 wk group and 7/10 of 4 wk group, suggesting the formation of cholesterol gallstones. Plasma lipids and apolipoproteins (Table 1) Table 1 shows that the concentrations of plasma total cholesterol (TC), triglyceride (TG), phospholipid (pL), VLDL-C, LDL-C, apoB100, apoC II, apoC III gradually increased with the prolonged feeding time of dietary cholesterol (P<0.05). High density lipoprotein cholesterol and its subfractions (HDL-C, HDL2-C, HDL3-C) showed a decreasing tendency, without statistical significance (P>0.05). ApoA1 was reduced with increasing feeding time of dietary cholesterol in rabbits (P<0.05).

**Hepatic and biliary cholesterol and bile acids (Table 2)**

Table 2 shows that the hepatic and biliary cholesterol significantly increased by more than 1.5 times as compared with the control group. The GCA gradually decreased from the control group to the 4 wk group, and GDCA showed an increase at the beginning of dietary cholesterol feeding in 1 week group, then decreased gradually without statistical significance in the other experimental groups.
HDL 2 predominated in hamsters with cholesterol VLDL and LDL cholesterol and a reduced pool of (mol/L).

Recent investigations showed\(^5\) that in cholesterol-fed hamsters with the VLDL-C/HDL-C ratio greater than 1.0, cholesterol gallstone formation occurred easily. Hayes et al\(^4\) found an expanded pool of VLDL and LDL cholesterol and a reduced pool of HDL\(_2\) predominated in hamsters with cholesterol gallstone. Likewise, increased VLDL and decreased HDL, primarily HDL\(_2\), were common in obese persons with gallstones, therefore, elevation of VLDL-C and LDL-C is closely related to excessive dietary cholesterol\(^5\). The decreased HDL, as recently found\(^6\), bears a close relationship to the elevated activity of plasma CETP (cholesteryl ester transfer protein) which promotes the lipoprotein cholesterol of HDL to be transferred to other lipoproteins such as VLDL and LDL in patients with cholesterol gallstones. Our study also showed elevated plasma TC, TG, VLDL-C, LDL-C and a slightly lowered HDL-C and its subfractions (DHL2-C, HDL3-C). Kern Jr\(^7\) reported that the diet-induced cholesterol increase was found in biliary cholesterol secretion in the gallstone subjects, but not in the controls, indicating that dietary cholesterol might be important in the pathogenesis of cholesterol gallstones, and also supported the hypothesis that hepatic metabolism of cholesterol in gallstone patients differs from those without stones. Although the factors that regulate biliary cholesterol secretion are not certain, a number of researches have suggested that most biliary cholesterol were derived from the existed rather than newly synthesized cholesterols.

In epidemiological studies\(^1,2\), the concentrations of plasma lipoprotein cholesterol were various in cholesterol gallstone patients. In normal people, the plasma LDL-C concentration appeared to be related to cholesterol gallstone patients. In normal people, the concentration of plasma apoA1 demonstrates that the concentration of plasma apoA1 might result in a reduced concentration of apoA1 and therefore, elevation of apoA1 and decreased HDL might be closely related to excessive dietary cholesterol. In type IV hyperlipidemias patients with elevated VLDL-C and LDL-C, the incidence of gallstone was high.\(^4\)

In recent studies, Jacks et al\(^8\) found slightly lowered HDL-C and its subfractions (DHL2-C, HDL3-C). Kern Jr\(^7\) reported that the diet-induced cholesterol increase was found in biliary cholesterol secretion in the gallstone subjects, but not in the controls, indicating that dietary cholesterol might be important in the pathogenesis of cholesterol gallstones, and also supported the hypothesis that hepatic metabolism of cholesterol in gallstone patients differs from those without stones. Although the factors that regulate biliary cholesterol secretion are not certain, a number of researches have suggested that most biliary cholesterol were derived from the existed rather than newly synthesized cholesterols. This study shows that dietary, hepatic and biliary cholesterols increased concomitantly, which is consistent with the above conclusion. Robins et al\(^9\) suggested that the precursor cholesterol secreted by the gallbladder was transported directly from plasma through the plasma membrane of hepatocytes to the biliary canaliculi without entering the interior of the cell.

The concentration of apolipoproteins in bile is about 10% of that in plasma. Although apolipoproteins are potential antinucleating protein in bile, their functional role in vivo as a factor in the solubilization of biliary cholesterol is relatively unexplored. In vitro apoA1 in low concentrations can delay the shift from micelles to vesicles, thereby enhancing the cholesterol-solubilizing capacity of bile acids. Another finding\(^10\) is that apoA1 stabilizes phospholipid lamellae and thus prolongs nucleation time in model bile systems. This study demonstrates that the concentration of plasma apoA1 gradually decreased following dietary cholesterol which might result in a reduced concentration of apoA1 and cholesterol crystal formation in bile.

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