Hypocholesterolemic Effect of Bile Acid Sulfonate Analogs in Hamsters

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We studied the effects of bile acid sulfonate analogs, namely, 3 α ,7 α ,12 α -trihydroxy-5 β -cholane-24-sulfonate (C-sul), 3 α ,7 α -dihydroxy-5 β -cholane-24-sulfonate (CDC-sul), and 3 α ,7 β -dihydroxy-5 β -cholane-24-sulfonate (UDC-sul), on serum and liver cholesterol levels, cholesterol 7 α -hydroxylase activity, and biliary bile acid composition in hamsters fed cholesterol. Of the three analogs studied, UDC-sul slightly but significantly decreased free, esterified, and total cholesterol concentrations in the serum. UDC-sul and CDC-sul reduced liver total cholesterol levels by 25% and 18%, respectively, particularly in the esterified cholesterol fraction. Analysis of biliary bile acids showed the presence of the administered analogs, indicating that sulfonate analogs efficiently participate in enterohepatic cycling. The proportion of cholic acid was increased in all groups fed sulfonate analogs, but the ratio of glycine to taurine conjugated bile acids (G/T) was elevated only in UDC-sul feeding hamsters. There was no significant change in cholesterol 7 α -hydroxylase activity in hamsters fed C-sul or CDC-sul, while UDC-sul slightly stimulated the enzyme activity compared to the control. The UDC-sul induced decrease in serum and liver cholesterol concentrations may be secondary to enhanced bile acid synthesis. This is supported by the increased cholesterol 7 α -hydroxylase activity and elevated G/T ratio in biliary bile acids observed following UDC-sul administration.

Key words cholesterol; bile acid; hypocholesterolemic; bile acid analog; cholesterol 7 α -hydroxylase

Bile acids are formed from cholesterol in the liver of mammalian species, are excreted into the bile after conjugation with taurine or glycine, are reabsorbed efficiently from the terminal ileum, and participate in enterohepatic cycling.¹⁾ Bile acid synthesis is a major component in cholesterol homeostasis and represents an important pathway for elimination of cholesterol from the body. Cholesterol 7 α -hydroxylase, the first and rate-limiting enzyme in bile acid biosynthesis, is known to be regulated at the transcriptional level by the quantity of bile acids returning to the liver.^{2,3)} Thus, interruption of the enterohepatic circulation is associated with a derepression of cholesterol 7 α -hydroxylase, and this replenishes the bile acid supply by promoting the conversion of hepatic cholesterol to bile acids.

In the course of the development of cholesterol lowering agents, we have attempted to enhance bile acid biosynthesis by release from the negative feedback inhibition. Although bile acid sequestrants have been used for the treatment of hypercholesterolemia, their large dose requirements leave room for improvement. If a bile acid analog can prompt digestion and absorption of fats similarly to endogenous bile acids, suppress cholesterol 7 α -hydroxylase less than endogenous bile acids, and competitively inhibit the reabsorption of endogenous bile acids from the intestine, it should be useful as a cholesterol lowering agent. We have recently shown that bile acid sulfonate analogs, which are a model of non-hydrolyzable taurine conjugated bile acids,⁴⁾ inhibited the absorption of the endogenous bile acid cholytaurine from the intestine.⁵⁾ Further, bile acid sulfonates affected cholesterol 7 α -hydroxylase activity similarly to the corresponding taurine conjugated bile acids in rat primary culture hepatocytes.⁵⁾

To investigate the cholesterol lowering effects of sulfonate analogs of cholic, chenodeoxycholic, and ursodeoxycholic acids, we examined the effects of these sulfonate analogs on serum and liver cholesterol levels, and cholesterol 7 α -hy-

droxylase activity in hamsters fed 0.2% cholesterol.

MATERIALS AND METHODS

Bile Acid Sulfonate Analogs 3 α ,7 α ,12 α -Trihydroxy- (C-sul), 3 α ,7 α -dihydroxy- (CDC-sul), and 3 α ,7 β -dihydroxy-5 β -cholane-24-sulfonates (UDC-sul) were prepared from the corresponding C, CDC, and UDC by the method described previously.⁴⁾

Animal Experiments Male golden Syrian hamsters, weighing 100–120 g (obtained from Hiroshima Experimental Animal Suppliers, Hiroshima, Japan), were maintained for 7 d on commercial rodent chow. The animals were divided into four groups of six animals each. The first group was fed rodent chow containing 0.2% cholesterol. The other three groups received the same diet supplemented with 0.1% C-sul, 0.1% CDC-sul, or 0.1% UDC-sul. The animals were fed these experimental diets for 21 d. At the end of this period (day 21), the animals were anesthetized with ethyl ether, and bile was obtained by needle aspiration from the gallbladder. Then, the animals were killed and samples of blood and liver were obtained for determination of cholesterol concentrations.

Measurement of Liver and Serum Cholesterol Serum total cholesterol, free cholesterol, and high density lipoprotein (HDL)-cholesterol were measured enzymatically using Cholesterol-Test Wako kits. Liver cholesterol was measured by GLC as previously described.⁶⁾

Analysis of Biliary Bile Acids An aliquot of the ethanol extract of gallbladder bile was analyzed by HPLC using a post column containing 3 α -hydroxysteroid dehydrogenase.⁷⁾

Cholesterol 7 α -Hydroxylase Activity Microsomes were isolated from liver homogenates, and the activity of cholesterol 7 α -hydroxylase was determined by GC-MS.⁸⁾

Calculations and Statistics The data are reported as the mean $\pm$ S.D. Student's *t*-test was employed to determine sta-

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tistical significance.

RESULTS

All animals remained healthy throughout the three week experimental period. Body weight gains were similar in all groups, and no difference in liver weight was noted among the 4 groups (Table 1).

Table 1. Initial and Final Body Weights and Liver Weights of Hamsters Fed a Cholesterol Diet or Cholesterol Diet Supplemented with Bile Acid Sulfonates

Diet	Initial body weight (g)	Final body weight (g)	Liver weight (g)
Cholesterol	113.2±1.9	134.2±6.7	8.0±0.6
+C-sul	113.5±8.5	128.4±7.3	7.9±0.6
+CDC-sul	117.5±5.7	135.6±6.1	8.0±0.3
+UDC-sul	116.0±4.4	128.6±6.5	7.2±0.5

Table 2. Serum Lipoprotein Cholesterol Levels in Hamsters Fed a Diet Supplemented with Different Bile Acid Sulfonate Analogs

Diet	Total	HDL (mg/dl)	Non-HDL
Cholesterol	435.0±25.8	128.2±4.5	306.8±21.6
+C-sul	431.7±57.9	126.4±9.2	305.3±51.0
+CDC-sul	398.6±28.6	109.4±6.8*	289.3±30.1
+UDC-sul	380.9±13.9*	113.5±6.8*	267.4±24.3*

Each value represents the mean±S.D. * $p<0.05$ versus cholesterol.

Table 3. Liver Cholesterol Levels in Hamsters Fed a Diet Supplemented with Different Bile Acid Sulfonate Analogs

Diet	Total	Free mg/g liver	Esterified
Cholesterol	114.1±11.3	10.6±0.4	103.5±11.0
+C-sul	130.2±6.0	10.8±0.5	119.4±5.8
+CDC-sul	93.8±8.6*	10.1±0.6	83.7±8.2*
+UDC-sul	85.8±16.8*	9.9±0.7	75.9±16.3*

Each value represents the mean±S.D. * $p<0.05$ versus cholesterol.

Table 4. Bile Acid Composition of Bile from Hamsters Fed Different Bile Acid Sulfonate Analogs

Bile acid	Cholesterol	C-sul	CDC-sul	UDC-sul
		% of total		
C	64.9±3.2	36.8±5.1*	38.4±1.7*	46.8±1.5*
C-tau	44.8±2.1	27.2±2.3*	27.1±1.0*	27.1±4.0*
C-gly	20.1±1.1	9.5±2.8*	11.3±1.4*	19.7±2.9
CDC	24.4±4.3	19.3±3.0	20.9±0.9	24.9±2.4
CDC-tau	18.9±3.9	15.7±2.6	16.1±1.1	15.8±2.0
CDC-gly	5.5±0.5	3.6±0.8*	4.8±0.5	9.1±2.3*
DC	9.1±1.4	8.0±3.2	9.5±2.3	10.1±1.2
DC-tau	8.0±1.2	7.0±2.5	8.0±1.7	7.3±1.3
DC-gly	1.1±0.1	1.0±0.7	1.5±0.6	2.8±1.0*
LC	1.6±0.2	1.2±0.4	1.4±0.3	1.8±0.6
Sulfonate analogs	—	34.7±8.7	29.8±3.3	16.4±1.9
G/T	0.37±0.04	0.28±0.06	0.34±0.04	0.63±0.09*

Each value represents the mean±S.D. * $p<0.05$ versus cholesterol. C, cholic acid; C-tau, cholytaurine; C-gly, cholyglycine; CDC, chenodeoxycholic acid; CDC-tau, chenodeoxycholytaurine; CDC-gly, chenodeoxycholyglycine; DC, deoxycholic acid; DC-tau, deoxycholytaurine; DC-gly, deoxycholyglycine; LC, lithocholic acid. G/T represents the ratio of glycine-conjugated to taurine-conjugated bile acids.

Serum cholesterol levels are shown in Table 2. The addition of C-sul and CDC-sul did not significantly affect total serum cholesterol levels. In contrast, UDC-sul significantly reduced total cholesterol, HDL-cholesterol, and non-HDL-cholesterol levels, and CDC-sul decreased only the HDL-cholesterol level. However, the 4 groups were not significantly different with respect to relative percentages of esterified cholesterol to total cholesterol (73—75%). Table 3 shows liver cholesterol levels. In the two groups fed CDC-sul and UDC-sul, the total cholesterol and esterified cholesterol concentrations were significantly lower than those of the control group.

Biliary bile acids were analyzed by HPLC, and the data are summarized in Table 4. The administered bile acid analogs, C-sul (34.8%) and CDC-sul (29.8 %) became prominent in the bile, while the amount of UDC-sul was relatively small (16.4%) in bile from animals fed UDC-sul. The proportion of cholic acid in the bile of all groups fed bile acid sulfonates (36.8% in C-sul, 38.4% in CDC-sul, 46.8% in UDC-sul) was significantly smaller than that of the control group (64.9 %). The ratio of glycine-conjugated to taurine-conjugated bile acids in animals supplemented with UDC-sul was significantly elevated compared to the other groups.

Figure 1 shows the effects of different bile acid sulfonate

Fig. 1. Specific Activity of Hepatic Microsomal Cholesterol 7 α -Hydroxylase in Hamsters Fed Cholesterol or Cholesterol Supplemented with Bile Acid Sulfonates

* $p<0.05$ versus cholesterol.

analogs on cholesterol 7 α -hydroxylase activity in hamsters fed cholesterol. Supplementation with UDC-sul led to a slight, but significant increase in cholesterol 7 α -hydroxylase activity.

DISCUSSION

It has been shown that most naturally occurring bile acids repress cholesterol 7 α -hydroxylase at the level of gene transcription.^{9,10} Although the molecular basis of the regulation of cholesterol 7 α -hydroxylase has not been completely elucidated, the physicochemical properties of diverse bile acids may affect cholesterol 7 α -hydroxylase activity.^{11,12} Since all administered bile acids are to some extent biotransformed into other bile acids during enterohepatic circulation, it may be hard to evaluate the effects of these bile acids *in vivo*.

We previously synthesized bile acid sulfonate analogs, which are non-hydrolyzable taurine-conjugated bile acids that possess a sulfonic acid moiety in place of a carboxylic acid moiety.⁴ These sulfonate analogs were shown to be absorbed efficiently from the intestine and excreted into the bile as endogenous bile acids, but did not undergo any biological modifications.^{13,14} In addition, these analogs were resistant to bacterial 7-dehydroxylation.¹³ Furthermore, the physicochemical properties of both taurine-conjugated bile acids and sulfonate analogs were very similar⁴ and associated with the structure of the steroid nucleus rather than its side chain.⁵

The present study demonstrated the effects of sulfonate analogs of cholic (C-sul), chenodeoxycholic (CDC-sul), and ursodeoxycholic (UDC-sul) acids on serum and liver cholesterol levels and cholesterol 7 α -hydroxylase activity in hamsters fed a diet containing 0.2% cholesterol. Supplementation with UDC-sul, but not C-sul or CDC-sul, caused an apparent decrease in serum and liver cholesterol levels. Intestinal cholesterol absorption affects serum and liver cholesterol levels. The effects of ursodeoxycholic acid (UDCA) on cholesterol absorption are controversial.^{15–17} Recently, Mikami *et al.* reported that neither UDCA nor its sulfonate analog, UDC-sul, had an effect on cholesterol absorption.¹⁴ Therefore, although cholesterol absorption experiments were not carried out in this study, the possibility that UDC-sul affects cholesterol absorption could be excluded. There is also controversy with respect to whether cholic acid (CA) and chenodeoxycholic acid (CDCA) alter cholesterol absorption in humans and rodents.^{17–20} The present results show that C-sul and CDC-sul exhibited no effect on serum cholesterol and may suggest that these sulfonate analogs seem not to significantly affect the absorption of cholesterol, at least in hamsters.

Since bile acid biosynthesis is a major route for cholesterol elimination from the body, it seems likely that stimulation of conversion of cholesterol to bile acids leads to decreased cholesterol levels in serum and liver. Cholesterol 7 α -hydroxylase, the rate-limiting enzyme in bile acid biosynthesis, is known to be regulated in a negative-feedback manner by bile acids refluxing through the enterohepatic circulation.^{2,3} We have recently shown that CDC-sul and UDC-sul inhibited ileal absorption of cholytaurine.⁵ Furthermore, CDC-sul exhibited remarkable suppression of cholesterol 7 α -hydroxylase activity in primary cultures of rat hepatocytes, and UDC-sul had no effect.⁵ The present studies demonstrated the slight but significant increase of cholesterol

7 α -hydroxylase activity. This may result from the combined effects of UDC-sul on enterohepatic cycling of the endogenous bile acids and on cholesterol 7 α -hydroxylase.

Analysis of biliary bile acids revealed that C-sul and CDC-sul were predominantly present, accounting for 34.7 and 29.8% of total biliary bile acids, respectively, while the proportion of UDC-sul was relatively low (16.4%) and consistent with previously reported results.¹⁴ The present studies also indicated the apparent participation of sulfonate analogs in enterohepatic cycling. Only UDC-sul significantly increased the ratio of glycine-conjugated to taurine-conjugated bile acids. Since an increase in the ratio of glycine to taurine conjugated bile acids indicates elevated bile acid synthesis,²¹ this finding also confirms the stimulatory effect of UDC-sul on cholesterol 7 α -hydroxylase, probably secondary to increased fecal loss of the endogenous bile acids. Supplementation with these three bile acid analogs reduced the proportion of CA in biliary bile acids. Although the reason for this observation is obscure at this time, it is assumed that sterol 12 α -hydroxylase is repressed by sulfonate analogs.

In this paper, we studied the effects of three bile acid sulfonate analogs, C-sul, CDC-sul, and UDC-sul, on cholesterol metabolism using hamsters in which lipoprotein metabolism as well as bile acid composition are similar to those of human. Although CDC- and UDC-sul affected serum cholesterol level, only UDC-sul significantly decreased LDL-cholesterol level. Thus, it is expected that this bile acid sulfonate can be useful as a reagent for hypercholesterolemic diseases.

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