Inhibition of Ileal Na⁺/Bile Acid Cotransporter by S-8921 Reduces Serum Cholesterol and Prevents Atherosclerosis in Rabbits

Junko Higaki, Seijiro Hara, Nobuo Taku, Kanya Tonda, Kenji Miyata, Tsutomu Shke, Kiyoshi Nagata, Takuji Mizui

Abstract—The ileal Na⁺/bile acid cotransporter (IBAT) plays an important role in the enterohepatic circulation of bile acids. We investigated the effects of IBAT inhibition on the maintenance of serum cholesterol level by using a novel IBAT inhibitor, S-8921, in rabbits. Administration of S-8921 by its incorporation into the diet (0.01% to 0.1%) for 1 to 2 weeks in heterozygous Watanabe heritable hyperlipidemic rabbits decreased serum cholesterol by 29% to 37% and increased fecal excretion of measured bile acids by 60% to 180% compared with control rabbits. Liver microsomal cholesterol 7α-hydroxylase and 3-hydroxy-3-methylglutaryl coenzyme A reductase activities were increased by 75% to 84% and 84% to 89%, respectively, with S-8921 treatment. S-8921 administration (0.1% in the diet) to normal New Zealand White rabbits for 2 weeks resulted in increased hepatic low density lipoprotein receptor expression, which was assessed by Northern blot analysis. In cholesterol-fed New Zealand White rabbits, S-8921 treatment (0.003% to 0.1% in the diet) for 10 weeks dose-dependently inhibited the development of hypercholesterolemia. It also inhibited the accumulation of cholesterol in the aortic arch and reduced the severity of coronary atherosclerosis. These results indicate that IBAT inhibition by S-8921 affects serum cholesterol, liver enzymes, low density lipoprotein receptor activity, and atherosclerosis in the same manner as bile acid sequestrants. We suggest that an IBAT inhibitor such as S-8921 could be useful in the treatment of hypercholesterolemia.

Key Words: ileal bile acid cotransporter ■ serum cholesterol ■ S-8921 ■ LDL receptor ■ cholesterol 7α-hydroxylase

Hypercholesterolemia has been recognized as a major risk factor for coronary heart disease (CHD). In clinical trials, reducing serum LDL cholesterol has been demonstrated to decrease the incidence of CHD and to reverse atherosclerotic lesions.1-4 Two main classes of clinically useful hypocholesterolemic agents are the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors and the bile acid sequestrants. Both induce hepatic LDL receptor activity by increasing hepatic cholesterol demand.5-8 Because the major determinant of serum cholesterol level is hepatic LDL receptor activity, these agents share a common mechanism leading to reduction of cholesterol.

In the case of bile acid sequestrants such as cholestyramine and colestipol, they are nonspecific anion-exchange resins, and patients have complained of their bulkiness.9 The mechanism of action of a bile acid sequestrant is to inhibit the enterohepatic circulation of bile acids. Bile acids are synthesized from cholesterol in the liver and secreted into the bile flow to facilitate the digestion and absorption of lipids, followed by nearly quantitative reabsorption from the intestine.11 The ileal Na⁺/bile acid cotransporter (IBAT) maintains the reabsorption of bile acids from the intestine,12,13 and thus, its inhibitor is expected to exhibit pharmacological effects similar to those of bile acid sequestrants.

Some compounds have been shown to inhibit IBAT in vitro and to decrease serum cholesterol in vivo in rats loaded with cholesterol and bile acid or in cholesterol-fed guinea pigs.14,15 Recently, we reported that a new IBAT inhibitor, S-8921, could markedly decrease serum cholesterol in non–cholesterol- and bile acid–loaded normal hamsters16 as well as in cholesterol- and bile acid–loaded rats.17 However, only limited knowledge concerning the impact of IBAT inhibition on cholesterol metabolism is available at present. In this study, we investigated the mode of action of S-8921, a novel IBAT inhibitor, by using several rabbit models to evaluate the efficacy of an IBAT inhibitor as a hypocholesterolemic and antiatherosclerotic agent and to clarify the mechanisms leading to cholesterol reduction after IBAT inhibition.

Methods

Chemicals
S-8921 [methyl-1-(3,4-dimethoxyphenyl)-3-(3-ethylvaleryl)-4-hydroxy-6,7,8-trimethoxy-2-naphthoate] was synthesized at Shionogi Research Laboratories. Cholestyramine was purchased from Sigma Chemical Co. [3-14C]HMG-CoA (2.2 GBq/mmol), [1-14C]cholesterol (2.1 GBq/mmol), [1,2,6,7-3H(N)]cholesteryl oleate (2886 GBq/mmol), and Na[131I] (644 GBq/mg) were from Dupont–New England Nuclear.

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Animals
Male heterozygous Watanabe heritable hyperlipidemic (WHHL) rabbits (8 weeks old) and male New Zealand White (NZW) rabbits (18 or 9 weeks old) were obtained from Kitayama Labes Co, Ltd (Nagano, Japan). They were housed individually in metal cages and given standard chow pellets (LRC-4 for WHHL rabbits and RC-4 for NZW rabbits; Oriental Yeast Co, Ltd) and water ad libitum for 2 weeks before the experiments began. During the experimental period, 100 g of chow pellets per day was given to each animal, and water was given ad libitum. The animals were fasted overnight before they were killed. On the day that they were killed, the animals were bled from the abdominal vein under pentobarbital anesthesia. All animal experiments were approved by the Shionogi Animal Care and Use Committee.

Experimental Design

Study With Heterozygous WHHL Rabbits
Eighteen heterozygous WHHL rabbits (10 weeks old) were divided into 3 groups (n=6 each) so that each group had a similar baseline serum cholesterol concentration. Next, the animals were either kept on the control diet or switched to a diet supplemented with S-8921 at concentrations of 0.01% and 0.1% (corresponding to 4.0 to 4.6 and 42 to 47 mg/kg, respectively) for 2 weeks. Blood samples were collected from the marginal ear vein to determine serum lipids at 0, 1, and 2 weeks of the study without fasting. Feces were collected over 24 hours and lyophilized to measure bile acid contents at 2 weeks of the study. The liver was immediately removed after the animal was killed, and its microsomal fraction was prepared. The microsomal fractions were stored at −80°C until determination of enzyme activities. Their protein contents were determined as described by using BSA as a standard.

Study With Normal NZW Rabbits
Eight NZW rabbits (20 weeks old) were divided into 2 groups (n=4 each) so that each group had a similar baseline serum cholesterol concentration. The animals were either kept on the control diet or switched to a diet supplemented with S-8921 at a concentration of 0.1% (corresponding to 31.1 to 31.4 mg/kg) for 2 weeks. Blood samples were collected from the marginal ear vein after overnight fasting on the day before diet administration and on the day that the animals were killed. After euthanasia, the liver was immediately perfused with 120 mL of ice-cold saline through a cannulated portal vein. Small pieces of tissues were rapidly frozen in LN2 and stored at −80°C until RNA extraction. Other pieces of liver tissue (≈2 g) were resected and homogenized with a Physcotron homogenizer. Four samples from each group were combined, and the membranes that sedimented between 8000 g and 100 000 g were prepared and stored in LN2 until the 19H-LDL binding assay.

Study With High-Cholesterol Diet–Fed (HCD) NZW Rabbits
Thirty-seven NZW rabbits (11 weeks old) were fed a diet containing 0.5% cholesterol (high-cholesterol diet; HCD) for 1 week. They were then divided into 6 groups so that each group had a similar serum cholesterol concentration. The animals were either continued on the HCD (n=7) or switched to an HCD supplemented with S-8921 at concentrations of 0.003%, 0.01%, 0.03%, and 0.1% (corresponding to 1.0 to 1.2, 3.3 to 4.2, 10 to 12, and 33 to 42 mg/kg, respectively; n=6) or with cholestyramine at a concentration of 1.5% (corresponding to 510 to 640 mg/kg; n=6) for 10 weeks. Blood samples were collected from the marginal ear vein every other week without fasting. The liver, heart, and aorta were removed after the animals were killed. A small piece of liver was stored at −20°C until the determination of lipid contents was made. The heart and longitudinally opened aorta were fixed in 10% phosphate-buffered formalin until evaluation of atheromatous plaque and the determination of cholesterol contents. Four NZW rabbits were fed an ordinary diet (RC-4) throughout the experimental period, killed, and then treated as described above.

Analysis of Lipids and Bile Acids

Serum Lipids
Serum total cholesterol, triglyceride (TG), and phospholipid were determined by using commercial kits Steryose Auto-545 (Fujirebio, Inc), L-type Wako TG H, and L-type Wako PLH (Wako Pure Chemical Industries), respectively. Serum lipoprotein fractions were separated by ultracentrifugation. LDL was determined as the d<1.006 g/mL fraction, and HDL was determined as the d>1.063 g/mL fraction. LDL was calculated by subtracting the d>1.063 g/mL fraction from the d>1.006 g/mL fraction.

Lipids in the Liver and Aorta
The liver was homogenized with ethanol, and then lipid extraction was performed by refluxing for 20 minutes. The extract was evaporated under N2 gas and dissolved with isopropanol. The contents of total and free cholesterol, TG, and phospholipid were determined by using commercial kits Determinar TC 555 and Determinar FC 555 (Kyowa Medex Co, Ltd), triglyceride E-test (Wako), and phospholipid B-Test (Wako), respectively. Lipid measurement was performed with a Cobas-Fara centrifugal analyzer (Roche Diagnostics). Esterified cholesterol was calculated by subtracting free cholesterol from total cholesterol. The aorta was frozen in LN2 and pulverized. Lipids were extracted from these samples with chloroform-methanol (2:1, vol/vol) and filtered. The contents of total and free cholesterol were determined as described above.

Fecal Bile Acids
Bile acids were obtained from the lyophilized feces after 3 ethanol extractions and purified with piperidinoxyhydroxypropylxetan gel (Shimadzu Corp). These samples were analyzed by high-performance liquid chromatography (HPLC). The amounts of the 3 major bile acids (12-oxo-lithocholic acid, deoxycholic acid, and lithocholic acid) were determined and summed.

Activity of Liver Microsomal Enzymes

Cholesterol 7α-Hydroxylase
Cholesterol 7α-hydroxylase activity in microsomes was measured by the HPLC method. 7α-Hydroxylcholesterol generated from endogenous cholesterol during the incubation of microsomes for 15 minutes at 37°C was labeled with 1-anthryl cyanide (Wako), and its content was quantified by HPLC with fluorometric detection.

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**TABLE 1. Effects of 2-Week Treatment With S-8921 on Body Weight (BW) and Serum VLDL, LDL, and HDL Cholesterol, TG, and Phospholipid (PL) in Heterozygous WHHL Rabbits**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW, kg</th>
<th>VLDL, mmol/L</th>
<th>LDL, mmol/L</th>
<th>HDL, mmol/L</th>
<th>TG, mmol/L</th>
<th>PL, mmol/L</th>
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<tr>
<td>Control</td>
<td>6</td>
<td>2.44±0.05</td>
<td>0.057±0.018</td>
<td>1.02±0.20</td>
<td>0.76±0.07</td>
<td>0.72±0.12</td>
<td>1.22±0.10</td>
</tr>
<tr>
<td>S-8921 (0.01%)</td>
<td>6</td>
<td>2.50±0.08</td>
<td>0.041±0.013</td>
<td>0.58±0.07</td>
<td>0.57±0.05*</td>
<td>0.76±0.07</td>
<td>1.10±0.08</td>
</tr>
<tr>
<td>S-8921 (0.1%)</td>
<td>6</td>
<td>2.41±0.02</td>
<td>0.026±0.005</td>
<td>0.47±0.09*</td>
<td>0.64±0.03</td>
<td>0.80±0.14</td>
<td>1.07±0.09</td>
</tr>
</tbody>
</table>

Data represent mean±SE.

*Significantly different from control group (P<0.05, Dunnnett’s multiple comparison test).
Figure 1. Effects of S-8921 on serum total cholesterol (A) and fecal bile acid contents (B) in heterozygous WHHL rabbits. S-8921 was administered by its incorporation into the diet, and concentrations of 0.01% and 0.1% corresponded to 4.0 to 4.6 and 42 to 47 mg/kg, respectively. Data represent mean±SE of 6 animals. *,**Significantly different from control group at P<0.05, P<0.01, respectively, by Dunnett’s multiple comparison test.

Figure 2. Effects of S-8921 on liver microsomal cholesterol 7a-hydroxylase (A), HMG-CoA reductase (B), and ACAT (C) activities in heterozygous WHHL rabbits. S-8921 was administered by its incorporation into the diet for 2 weeks, and liver microsomal enzyme activities were determined as described in Methods. S-8921 concentrations of 0.01% and 0.1% corresponded to 4.0 to 4.6 and 42 to 47 mg/kg, respectively. Data represent mean±SE of 6 animals. *,**Significantly different from control group at P<0.05, P<0.01, respectively, by Dunnett’s multiple comparison test.

Figure 3. Induction of hepatic LDLR mRNA by S-8921 in normal NZW rabbits. S-8921 was administered by its incorporation into the diet at a concentration of 0.1%, which corresponded to 31.1 to 31.4 mg/kg for 2 weeks. Northern blot analysis of hepatic LDLR was performed as described in Methods. A, Typical autoradiogram of Northern blotting. B, Densitometric analysis of bands corresponded to LDLR and GAPDH. Data represented mean±SE of 4 animals. *Significantly different from control group at P<0.05 by Student’s t test.

The membrane was hybridized to a 32P-labeled synthetic oligonucleotide probe for the LDLR [LR55, 5'-GCCAC(A/G)TCATCCTC CAGGCTGACCATCTGTCT(C/T)GAGGGGTAGGTGTAGCCG TCCT-3'] or a human GAPDH cDNA probe (CLONTECH Laboratories, Inc) overnight at 65°C in 0.5 mol/L sodium phosphate (pH 7.5), 10 mg/mL BSA, 1 mmol/L EDTA, and 7% SDS, followed by 2 washes for 30 minutes at 65°C in 2× SSC (1× SSC is 0.16 mol/L NaCl and 0.016 mol/L sodium citrate) and 0.1% SDS. Bands corresponding to LDLR and GAPDH in an autoradiographic film of the probed membrane were densitometrically quantified and normalized as the ratio of LDLR to GAPDH.

Evaluation of Atheromatous Plaque

Aortic Lesions

The fixed aorta was cut into 3 segments (aortic arch, thoracic aorta, and abdominal aorta), and each intimal surface was photographed. Enlarged prints of the photographs were analyzed with an image analyzer (Cosmo Zone ISA system, Nikon). Total surface area and the area covered with plaque were determined, and the percentage of the atheromatous plaque area was calculated.

Coronary Artery Lesions

The fixed heart was cut into cross sections at 5-mm intervals. Five cross sections from each heart were embedded in paraffin. The samples were stained with elstica–van Gieson’s stain. All coronary arteries visible in the 5 cross sections from each heart (average, 110 arteries) were analyzed by using the image analyzer (Cosmo Zone), and the severity of intimal thickening was classified into 3 levels: none or mild, 0% to 30% stenosis; moderate, 31% to 60% stenosis; and severe, 61% to 100% stenosis. The percentage of each stenosis level was calculated.

Statistical Analysis

Data are expressed as mean±SE. Statistical significance of differences was determined by using Student’s t test, paired t test for paired data between two groups, or Dunnett’s multiple comparison test when more than 3 groups were compared. P values <0.05 and <0.01 were considered significant.
TABLE 2. Effects of 10-Week Treatment of S-8921 on Body Weight (BW) and Serum VLDL, LDL, and HDL Cholesterol, TG, and Phospholipid (PL) in HCD NZW Rabbits

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol, mmol/L</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
<th>TG, mmol/L</th>
<th>PL, mmol/L</th>
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<tr>
<td>HCD control</td>
<td></td>
<td>37.50±2.29*</td>
<td>18.03±1.45*</td>
<td>1.11±0.15</td>
<td>1.12±0.09*</td>
<td>7.69±1.00*</td>
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<tr>
<td>+S-8921 (0.003%)</td>
<td></td>
<td>30.33±4.47</td>
<td>12.54±1.70†</td>
<td>1.10±0.15</td>
<td>0.97±0.12</td>
<td>6.74±0.74</td>
</tr>
<tr>
<td>+S-8921 (0.01%)</td>
<td></td>
<td>15.87±3.18§</td>
<td>6.78±1.40§</td>
<td>0.88±0.15</td>
<td>0.51±0.08§</td>
<td>3.65±0.48§</td>
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<tr>
<td>+S-8921 (0.03%)</td>
<td></td>
<td>20.53±4.35§</td>
<td>8.34±2.32§</td>
<td>1.05±0.20</td>
<td>0.91±0.24</td>
<td>4.74±0.88‡</td>
</tr>
<tr>
<td>+S-8921 (0.1%)</td>
<td></td>
<td>12.28±3.42§</td>
<td>5.76±0.65§</td>
<td>1.06±0.13</td>
<td>0.61±0.08§</td>
<td>3.31±0.43§</td>
</tr>
<tr>
<td>+Cholestyramine (1.5%)</td>
<td></td>
<td>20.13±3.09§</td>
<td>7.99±0.65§</td>
<td>0.68±0.04</td>
<td>0.66±0.10</td>
<td>4.29±0.40§</td>
</tr>
<tr>
<td>OD control</td>
<td></td>
<td>0.05±0.01</td>
<td>0.12±0.02</td>
<td>0.55±0.13</td>
<td>0.44±0.04</td>
<td>0.84±0.08</td>
</tr>
</tbody>
</table>

Data represent mean±SE.

*Significantly different from ordinary diet (OD) control (P<0.01, Student’s t test), †Significantly different from OD control, P<0.05, Student’s t test.
‡, §Significantly different from HCD control group (P<0.05, P<0.01, respectively, Dunnett’s multiple comparison test).

Results

General Results

All animals treated with S-8921 in this report were in good health throughout the study, and apparent side effects such as diarrhea were not observed in S-8921–treated animals as well as control animals.

Study With Heterozygous WHHL Rabbits

To confirm the ability of S-8921 to inhibit IBAT in vivo in the rabbit model, we first characterized the effect of S-8921 administration on heterozygous WHHL rabbits. Food consumption during the 2-week experimental period in each group was 100%. The body weight of the rabbits was not affected by S-8921 treatment (Table 1). S-8921 treatment for 1 to 2 weeks caused a decrease in serum total cholesterol concentrations, with 0.01% S-8921 (4.0 to 4.6 mg/kg) being almost maximally effective (Figure 1A). The serum cholesterol decrease was accompanied by increased fecal excretion of measured bile acids (Figure 1B), indicating that this drug could inhibit IBAT in vivo in rabbits. S-8921 treatment for 2 weeks decreased serum cholesterol especially in the LDL fraction but did not affect serum TG and phospholipid concentrations (Table 1).

The effects of S-8921 on the activities of 3 liver microsomal enzymes are shown in Figure 2. The free cholesterol contents in microsomes of control, 0.01% S-8921, and 0.1% S-8921 groups were 25.9±0.8, 26.1±1.8, and 23.7±2.0 μg/mg protein, respectively, which were not significantly different from each other. S-8921 treatment for 2 weeks clearly increased cholesterol 7α-hydroxylase and HMG-CoA reductase activities (Figure 2A and 2B) but did not affect ACAT activity (Figure 2C).

Study With Normal NZW Rabbits

Food consumption during the 2-week experimental period in the control group and the 0.1% S-8921–treated group was 96.5±3.5% and 99.5±0.5%, respectively. The body weight of S-8921–treated rabbits, 3.16±0.08 kg, was not significantly different from that of control animals, 3.20±0.10 kg.

Treatment with S-8921 for 2 weeks decreased serum cholesterol in normal rabbits. The serum total cholesterol value after S-8921 treatment, 0.48±0.06 mmol/L, was significantly lower than that before S-8921 treatment, 0.68±0.09 mmol/L (P<0.01, paired t test). Serum total cholesterol values of the control group were not significantly different before versus after the experiments (data not shown).

Northern blot analysis revealed that the amount of liver mRNA for the LDLR was increased 1.7-fold by S-8921 treatment (Figure 3). The ratio of LDLR to GAPDH in the S-8921–treated group, 0.907±0.077, was significantly higher than that in the control group, 0.522±0.072 (P<0.05, Student’s t test). S-8921 treatment also increased 125I-LDL specific binding to liver membranes. The specific binding of 125I-LDL at 1.5 and 6 μg/mL was 2.9 and 5.5 ng/mg protein for the control group and 4.3 and 10.4 ng/mg protein for the S-8921–treated group, respectively. The significance of differences was not determined because we used 1 preparation from each group of rabbits as described in Methods.

Study With HCD NZW Rabbits

In this experiment, treatment with S-8921 and cholestyramine was initiated after feeding of a diet containing 0.5% cholesterol for 1 week. Food consumption during the next 10-week experimental period in control, 0.003% S-8921, 0.01% S-8921, 0.03% S-8921, 0.1% S-8921, and 1.5% cholestyramine groups was 95.4±1.8%, 96.6±2.4%, 97.1±1.9%, 99.6±0.3%, 99.0±0.0%, and 97.3±1.9%, respectively. There were no significant differences between control and drug-treated groups. The body weight of rabbits was not affected by S-8921 and cholestyramine treatment (Table 2). The control group developed further hypercholesterolemia during the experimental period (Figure 4). S-8921 treatment suppressed this effect dose-dependently, and 0.01% S-8921 (3.3 to 4.2 mg/kg) was the minimum dose needed for a significant decrease (Figure 4). A high dose of cholestyramine (1.5%, 510 to 640 mg/kg) moderately suppressed the development of hypercholesterolemia (Figure 4). S-8921 treatment decreased serum cholesterol in the VLDL and LDL fractions and also decreased serum phospholipid, but its effects on TG were not clear (Table 2). An HCD resulted in accumulation of cholesterol in the liver. S-8921 decreased the...
Contents of esterified cholesterol but did not change other lipid contents in the liver (Table 3). Aortic lesions were evaluated on the basis of total cholesterol and the area covered with atheromatous plaque. The analysis was performed for 3 segments of the aorta (aortic arch, thoracic aorta, and abdominal aorta). Figure 5 shows the results for the aortic arch. S-8921 treatment significantly decreased total cholesterol contents and also tended to decrease the lesioned area, although the differences were not statistically significant (Figure 5). Almost the same results were obtained for the thoracic and abdominal aorta, although the differences in both cholesterol contents and lesioned areas were not statistically significant (data not shown).

Atherosclerotic lesions of coronary arteries in cholesterol-fed rabbits were varied (Figure 6). We classified the severity of each lesion into 3 levels as described in Methods. All 3 levels of intimal thickening were observed in all experimental groups. S-8921 treatment significantly decreased the appearance of severe stenosis and increased the cases of no or mild stenosis (Figure 7). Cholestyramine showed similar effects (Figure 7).

### Discussion

Interruption of the enterohepatic circulation of bile acids by attenuating intestinal bile acid reabsorption is considered to be one of the best ways to lower serum cholesterol levels. Intestinal bile acid reabsorption can be prevented by using bile acid sequestrants, such as cholestyramine and colestipol, or by partial ileal bypass surgery. Both methods lead to lower serum cholesterol levels. Because IBAT plays an important role in the maintenance of intestinal bile acid reabsorption, its inhibition is expected to exhibit a hypocholesterolemic effect by interrupting the enterohepatic circulation of bile acids. The aim of this study was to assess the impact of IBAT inhibition on serum cholesterol level and atherosclerosis and to clarify the underlying mechanisms leading to the cholesterol decrease after IBAT inhibition. S-8921 has been demonstrated to be a novel IBAT inhibitor.

### Table 3. Effects of 10-Week Treatment of S-8921 on Liver Weight, Liver Total Cholesterol (TCH), Free Cholesterol (FC), Esterified Cholesterol (EC), TG, and Phospholipid (PL) in HCD NZW Rabbits

<table>
<thead>
<tr>
<th>Liver Weight, g/kg</th>
<th>Liver Lipid, mg/g Wet Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCH</td>
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<tr>
<td>HCD control</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
</tr>
<tr>
<td>+S-8921 (0.003%)</td>
<td>6</td>
</tr>
<tr>
<td>+S-8921 (0.01%)</td>
<td>6</td>
</tr>
<tr>
<td>+S-8921 (0.03%)</td>
<td>6</td>
</tr>
<tr>
<td>+S-8921 (0.1%)</td>
<td>6</td>
</tr>
<tr>
<td>+Cholestyramine (1.5%)</td>
<td>6</td>
</tr>
<tr>
<td>OD control</td>
<td>4</td>
</tr>
</tbody>
</table>

Data represent mean ± SE.

*Significantly different from ordinary diet (OD) control (P < 0.01, Student’s t test).
††Significantly different from HCD control group (P < 0.05, Dunnett’s multiple comparison test).
in hamsters and rats. S-8921 was shown to inhibit Na+-dependent \(^{3}H\)taurocholate uptake in COS cells, which constitutively expressed the hamster IBAT gene and rat ileal brush-border membrane vesicles in vitro, and was also shown to markedly decrease serum cholesterol in normal hamsters and rats loaded with cholesterol and bile acid. Thus, we decided to use S-8921 as an IBAT inhibitor that could operate in vivo. We used rabbits as the animal models because of the resemblance of their lipid metabolism and atherosclerosis to those of humans.

We first characterized the effect of S-8921 administration on heterozygous WHHL rabbits to confirm the ability of S-8921 to inhibit IBAT in vivo in the rabbit model. Treatment with S-8921 clearly produced serum cholesterol reduction accompanied by an increase in fecal excretion of measured bile acids (Figure 1), suggesting that this drug could inhibit IBAT in vivo in rabbits. Because IBAT inhibition reduces the amount of bile acid return to the liver, increased conversion of cholesterol to bile acid and increased cholesterol synthesis in the liver was expected. To confirm this, we measured the activities of cholesterol 7α-hydroxylase and HMG-CoA reductase, which are the rate-limiting enzymes of the conversion of cholesterol to bile acid and of cholesterol synthesis, respectively. Both of these liver microsomal enzyme activities were increased by S-8921 treatment (Figure 2), suggesting compensating alterations of cholesterol metabolism. Hepatic LDLR activity is considered to be the major determinant of serum cholesterol level, and 2 main classes of clinically useful hypocholesterolemic agents, HMG-CoA reductase inhibitors and bile acid sequestrants, were reported to induce it. S-8921 treatment in normal rabbits clearly showed increased expression of the hepatic LDLR (Figure 3) as well as the reduction of serum cholesterol. Thus, IBAT inhibition by S-8921 exhibited alterations of liver enzymes and receptor activities related to cholesterol metabolism; both could be expected from the interruption of the enterohepatic circulation of bile acids. Bile acids are known to be reabsorbed from the intestine by both passive and active mechanisms. Because S-8921 is an IBAT inhibitor and could not inhibit passive absorption of bile acids, only inhibition of the active absorption of bile acids may be sufficient to change liver cholesterol metabolism and reduce serum cholesterol.

The effect of IBAT inhibition on diet-induced hypercholesterolemia and atherosclerotic lesion formation was assessed in cholesterol-fed rabbits. Administration of S-8921 to HCD NZW rabbits clearly suppressed the development of hypercholesterolemia (Figure 4). Because 0.01% S-8921 suppressed the development of hypercholesterolemia more strongly than did 1.5% cholestyramine, S-8921 was >150× more efficacious than cholestyramine. The development of hypercholesterolemia in HCD NZW rabbits can be maintained by dietary cholesterol intake. S-8921 was reported to inhibit the absorption of cholesterol that might be due to the inhibition of bile acid reabsorption; thus, the hypcholesterolemic effect of S-8921 observed in this study may have resulted from the inhibition of cholesterol absorption. S-8921 treatment also decreased the cholesterol content of the aortic arch, which may be the result of serum cholesterol reduction, although the suppressive effects of S-8921 on atheromatous plaque area were not statistically significant (Figure 5). Atherosclerosis of coronary arteries plays an important role in myocardial infarction and ischemic heart disease. S-8921 treatment dramatically reduced the severity of coronary atherosclerosis in HCD NZW rabbits (Figure 7), with the improvement being more evident than that in the aorta. Serum cholesterol reduction by pravastatin, an HMG-CoA reductase inhibitor, was reported to prevent coronary atherosclerosis but not aortic atherosclerosis in WHHL rabbits. Serum cholesterol reduction may have a greater influence on the coronary arteries than on the aorta in general.

Concerning the effect of the IBAT inhibitor on serum TG level, S-8921 did not cause any increase of serum TGs in heterozygous WHHL rabbits (Table 1) and HCD NZW rabbits (Table 2). Bile acid sequestrants have been reported to increase serum TG values in human patients. In our study, cholestyramine did not increase serum TG levels in HCD NZW rabbits (Table 2). In former studies using other rabbit models, cholestyramine was reported to decrease serum TG in normal rabbits but to have no effect in heterozygous and homozygous WHHL rabbits. These findings suggest that bile acid sequestrants might not raise serum TG levels in rabbit models. It would be important to determine the effects of IBAT inhibitor on serum TG level in humans.
In conclusion, the current study investigating the impact of IBAT inhibition with the use of S-8921 produced 2 important findings. One is that IBAT inhibition changed liver cholesterol metabolism, i.e., upregulated cholesterol 7α-hydroxylase and HMG-CoA reductase activities, and most important, it increased LDLR expression, which can reduce serum cholesterol. The other is that IBAT inhibition efficiently suppressed diet-induced hypercholesterolemia and atherosclerosis. We suggest that IBAT inhibitors such as S-8921 can be useful in the treatment of hypercholesterolemia.

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References


Inhibition of Ileal Na⁺/Bile Acid Cotransporter by S-8921 Reduces Serum Cholesterol and Prevents Atherosclerosis in Rabbits

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