Effect of Simvastatin (MK-733) on Sterol and Bile Acid Excretion in Rabbits

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Abstract—The effects of Simvastatin (MK-733), an inhibitor of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, on fecal and biliary excretion of sterols and bile acids were examined using rabbits. Multiple doses of MK-733 (10 mg/kg/day) for 7 days were found to increase fecal concentrations of neutral sterols in cholesterol-fed rabbits, but not to affect those of bile acids. Multiple doses of cholestyramine (750 mg/kg/day), a bile acid sequestrant, for 7 days increased fecal concentrations of neutral sterols and bile acids in normally fed and cholesterol-fed groups. MK-733 did not affect biliary neutral sterols and total bile acids in normally fed and cholesterol-fed groups. Cholestyramine decreased biliary concentrations of neutral sterols in both diet groups. Cholestyramine altered fecal and biliary composition of bile acids, but MK-733 did not. It was considered that MK-733 inhibited the absorption of cholesterol, resulting in an increase of the fecal concentration of neutral sterols in cholesterol-fed rabbits. The mechanism of action of MK-733 in the inhibition of cholesterol absorption is considered to be clearly different from that of cholestyramine. These results confirmed the conclusion in the previous experiment.

Simvastatin (MK-733) (1) is a chemical derivative of lovastatin (MK-803) which is a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase) (2).

In our previous experiment, it was demonstrated that MK-733 inhibited the development of hypercholesterolemia and atherosclerosis in cholesterol-fed rabbits (3). We have already reported that this hypolipidemic effect was related to a marked inhibition of the absorption of exogenous cholesterol from the gastrointestinal wall (4).

There are, theoretically, three mechanisms to control the serum cholesterol pool: 1) decreased absorption of dietary cholesterol, 2) decreased synthesis of cholesterol and 3) increased excretion of either or both cholesterol and its metabolites, the bile acids (5). From the results in our previous study of [3H]-cholesterol absorption and excretion in cholesterol-fed rabbits (4), an increase in the fecal excretion of neutral sterols in rabbits treated with MK-733 would be expected. However, we have not yet examined the effect of MK-733 on sterol balance in rabbits. Bile acid excretion is thought to be a principal route of cholesterol removal and the remainder of the cholesterol is eliminated from the body in the form of biliary cholesterol (6, 7). In the present study, therefore, we examined the effect of MK-733 on biliary and fecal excretion of neutral sterols and bile acids. Cholestyramine, a bile acid sequestrant, has been reported to disrupt the entero-hepatic circulation of bile acids, resulting in the inhibition of cholesterol absorption in several experimental animals (8–11). Therefore, cholestyramine was used as a reference drug.

Materials and Methods

1. Chemicals: MK-733 (Lot No. L-644, 128-000U075, purity 99.1%; Merck Sharp and Dohme Research Laboratories (MSDRL),
Rahway, NJ, U.S.A.) and cholestyramine (Lot No. 126F-0732; Sigma, St. Louis, MO, U.S.A) were used. [2,4-3H]Cholic acid (25.0 Ci/mmol) was purchased from New England Nuclear, MA, U.S.A. Cholesterol was purchased from Sigma and from Wako Pure Chemical Co., Ltd., Osaka, Japan. 5α-Cholestane, campesterol, β-sitosterol, 5β-cholic acid, lithocholic acid, deoxycholic acid and cholic acid were obtained from Sigma. Coprostanol and 12-keto-lithocholic acid were purchased from Steraloids, Inc., Wilton, NH, U.S.A. All other chemicals used were standard commercial high purity materials.

2. Animals: Male Japanese white rabbits aged 16 to 23 weeks and weighing 2.5 to 3.1 kg were used in this study. These animals were purchased from Clean Experimental Animals Co., Ltd. (Saitama, Japan) and maintained under the following environmental conditions: room temperature, 23±2°C; relative humidity, 55±15%; illumination, 12 hr from 6 A.M. to 6 P.M. The rabbits were housed in metal cages individually and were given a normal chow pellet (RC-4, Oriental Yeast, Tokyo, Japan) and water ad libitum for at least 4 weeks in order to acclimatize the animals.

Rabbits were divided into approximately equal weight groups and housed in metabolic cages individually. One hundred grams of the normal diet was given to each animal per 3 kg of body weight in Groups A, B and C throughout the study except for an overnight fasting before the bile duct cannulation, and water was given ad libitum for at least 4 weeks in order to acclimatize the animals.

3. Determination of serum lipid levels: Serum lipid levels were determined on the 8th day. Total cholesterol, free cholesterol and phospholipids were measured with an autoanalyzer (Centrifichem, Encore, Baker Instrument Co., Allenton, PA, U.S.A.), using Determiner TC555 (Kyowa Medex, Tokyo, Japan) Determiner FC555 (Kyowa Medex) and Determiner PL (Kyowa Medex), respectively. Serum total bile acids were determined by an enzymatic method using Total Bile Acids Test Wako (Wako Pure Chemical Co.).

4. Measurement of lipid content in the liver: Lipids in the liver were extracted according to Folch et al. (12). The solvent was evaporated under a stream of nitrogen, and the lipid extract was redissolved with an aliquot of isopropyl alcohol. Total and free cholesterol concentrations were determined as described in the section on determination of serum lipid levels. Phospholipid concentrations were determined using a chemical assay kit (Phospholipid-Test Wako, Wako Pure Chemical Co.).

5. Biliary lipid determination: Bile was collected from the cannulated common bile for 2 hr. Biliary bile acids and neutral sterols were determined according to the method of Imai et al. (13) with some modifications. The bile was extracted with ethanol after the addition of internal standards (5α-cholestane 10 μg/ml bile and 5β-cholic acid 1 mg/ml bile);
one volume of the bile was mixed with 20 volumes of ethanol, boiled for about 5 min and filtered after cooling down to room temperature. Whole filtrate was evaporated to dryness under a stream of nitrogen, and the residue was hydrolyzed in 2 ml of 1.25 M NaOH for 6 hr at 120°C in an autoclave. Neutral sterols were extracted with diethyl ether; bile acids were then extracted with diethyl ether after acidification with 2 M HCl. Neutral sterols were determined by gas liquid chromatography (GLC) on an HP-1 fused silica megabore column (10 m x 0.53 mm, 2.65 μm film thickness, Hewlett Packard, CA, U.S.A.). All GLC analyses were performed on an instrument (GC-9A, Shimadzu, Kyoto, Japan) equipped with a hydrogen flame ionization detector. Bile acids were converted to methyl ester derivatives using trimethylsilyldiazomethane (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) and determined by GLC using an HP-1. Phospholipids were determined by a chemical method using the assay kit as described above. The lithogenic index was calculated from the following formula: neutral sterols (mol)/(phospholipids (mol) + total bile acids (mol)).

6. Fecal sterols and bile acids determination: Fecal neutral sterols and bile acids were determined according to the method of Huff and Carroll (14) with some modifications. Feces were collected for 3 days before the bile cannulation, lyophilized and ground. Four hundred micrograms of 5α-cholestane was added to 0.2 g of freeze-dried feces as an internal standard. The freeze-dried, ground feces (0.2 g) was saponified in 5 ml 1 M NaOH in 90% ethanol for 1.5 hr. The neutral sterols were extracted with 12.5 ml of petroleum ether three times after dilution with 2.5 ml of water and then backwashed with 15 ml of H2O. Neutral sterols were determined by GLC on an HP-1 column. The overall recovery of bile acids was determined by the addition of [2,4-3H]cholic acid. One-half milliliter of 10 M NaOH was added to the lower aqueous phase, and then the solution was autoclaved for 3 hr at 120°C. The sample was acidified, and the bile acids were extracted with chloroform/methanol (2:1, v/v) once and chloroform twice. The bulk of the fatty acids was removed on a Florisil cartridge (Sep-Pack, Waters Associates, MA, U.S.A.). The bile acid fraction was methylated using trimethylsilyldiazomethane. The methyl esters were extracted with diethyl ether after the addition of water. The efficiency of this methylation procedure was determined by the recovery of added [2,4-3H]cholic acid as its methyl ester and was found to be 98%. After adding 5α-cholestane as an internal standard, the bile acid methyl esters were exposed to silylating reagent (N-trimethylsililylimidazole, Pierce Chemical Co., IL, U.S.A.) in order to convert all free hydroxy groups to trimethylsilyl ethers. Silylation of standard samples was confirmed using GC-mass spectrometry. Bile acids were determined by GLC on an HP-1 column. The identity of the neutral sterols and bile acids was determined by comparison to authentic standards.

7. Data analysis: Data from these studies were statistically analyzed using Wilcoxon's rank-sum test (15). The variations in all mean values in the tables and figures are expressed as standard deviation (S.D.).

Results

1. General observations: There was no remarkable difference in the body weight and food intake between the control and drug treated groups. No adverse effect in gross observations was seen in any rabbits in the entire group during the experiment. No visible gross change was observed in any organ or tissue at necropsy.

2. Serum lipid and total bile acid levels: As shown in Fig. 1, multiple administration of MK-733 (10 mg/kg/day) and cholestyramine (750 mg/kg/day) decreased serum total cholesterol levels significantly in normally fed rabbits. Cholestyramine also decreased free cholesterol and phospholipid levels in the normally fed rabbits. Total cholesterol levels were increased to 613.6 mg/dl after the feeding of a 1% cholesterol diet for 7 days. Multiple-dose treatment with MK-733 limited the increase in serum cholesterol levels to 139.9 mg/dl. Cholestyramine inhibited the increase in the cholesterol levels to 46.7 mg/dl. The serum phospholipid levels were also increased by cholesterol feeding for 7 days, and reached 190 mg/dl. MK-733 and cholestyramine limited the increase in phospho-
lipid levels significantly. Serum triacylglycerol concentrations varied during the experimental period in all animals (data not shown). Cholesterol feeding tended to increase serum total bile acid concentrations (Fig. 2). Cholestyramine decreased serum total bile acid levels in the normally fed and cholesterol-fed rabbits. On the other hand, MK-733 did not affect serum total bile acid levels in either diet group.

3. Lipid content in the liver: As shown in Table 1, there was no significant difference in hepatic cholesterol and phospholipid content in the normally fed group except in the cholestyramine group. Almost all cholesterol in

![Fig. 1. Effects of MK-733 and cholestyramine on serum lipid levels. Rabbits in groups A (n=4), C (n=5) and D (n=4) were fed a normal diet. Rabbits in groups E (n=4), G (n=4) and H (n=4) received a 1 % cholesterol diet. MK-733 at a dose of 10 mg/kg/day was administered orally once a day for 7 days in groups C and G. Rabbits in control groups A and E were orally given 0.5% CMC alone. Cholestyramine at a dose of 750 mg/kg/day was administered by incorporation (2.25%) into each diet to rabbits in groups D and H. Serum total cholesterol (TC), free cholesterol (FC) and phospholipids (PL) were determined on the 8th day as described in Materials and Methods. The values represent the mean and S.D. of each group, respectively. Significantly different from the value in each control group: *P<0.05.](image1)

![Fig. 2. Effects of MK-733 and cholestyramine on serum total bile acid levels. MK-733 at a dose of 10 mg/kg/day was administered orally once a day for 7 days in groups C (n=5) and G (n=4). Rabbits in control groups A (n=4) and E (n=4) were orally given 0.5% CMC alone. Cholestyramine at a dose of 750 mg/kg/day was administered by incorporation into each diet to rabbits in groups D (n=4) and H (n=4). Serum total bile acids were determined on the 8th day as described in Materials and Methods. The values represent the mean and S.D. of each group, respectively. Significantly different from the value in each control group: *P<0.05.](image2)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>(n)</th>
<th>Lipid content (mg/g tissue wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal</td>
<td>(4)</td>
<td>FC: 3.83±0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EC: 0.34±0.17</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>PL: 33.3±3.7</td>
</tr>
<tr>
<td>C</td>
<td>Normal+MK-733</td>
<td>(5)</td>
<td>FC: 3.50±0.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EC: 0.07±0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PL: 34.9±1.6</td>
</tr>
<tr>
<td>D</td>
<td>Normal+Cholestyramine</td>
<td>(4)</td>
<td>FC: 2.60±0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EC: 0.01±0.02*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PL: 36.1±1.7</td>
</tr>
<tr>
<td>E</td>
<td>1% Cholesterol</td>
<td>(4)</td>
<td>FC: 6.74±0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EC: 8.49±1.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PL: 33.0±1.2</td>
</tr>
<tr>
<td>G</td>
<td>1% Chol.+MK-733</td>
<td>(4)</td>
<td>FC: 3.98±0.87*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EC: 2.01±1.27*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PL: 32.9±2.7</td>
</tr>
<tr>
<td>H</td>
<td>1% Chol.+Cholestyramine</td>
<td>(4)</td>
<td>FC: 2.64±0.55*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EC: 0.45±0.68*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PL: 32.5±4.2</td>
</tr>
</tbody>
</table>

Lipids in the liver were extracted with chloroform/methanol (2:1, v/v); and free cholesterol (FC), esterified cholesterol (EC) and phospholipids (PL) were determined as described in the text. *: Number of animals in parenthesis. The values represent the mean and S.D. in each group. Significantly different from the value in each control group: *P<0.05.
the liver was present as free cholesterol in the normal diet groups. Cholestyramine decreased esterified cholesterol content. Cholesterol feeding increased the free and esterified cholesterol levels in the liver without affecting the phospholipid content. Multiple-dose treatment with MK-733 inhibited the accumulation of free and esterified cholesterol in the liver. Cholestyramine also inhibited the accumulation of free and esterified cholesterol in the liver. Neither MK-733 nor cholestyramine altered the phospholipid levels. There were fluctuations in triacylglycerol levels in all animals (data not shown).

4. Biliary lipids: The effects of single and multiple doses of MK-733 and multiple doses of cholestyramine on biliary lipids were examined in normally fed and cholesterol-fed rabbits, and the results are shown in Figs. 3 and 4. Cholesterol feeding tended to increase the biliary concentration of neutral sterols, but did not affect the bile flow or the concentration of phospholipids and total bile acids. A single administration of MK-733 did not affect the bile flow or concentrations of neutral sterols, phospholipids and total bile acids in the normally fed or cholesterol-fed rabbits. Cholestyramine decreased the bile flow in the cholesterol-fed rabbits and tended to decrease it in the normally fed rabbits. Multiple-dose treatment with MK-733 did not affect...
The bile was collected from common bile duct for 2 hr after bile duct cannulation. Composition of biliary bile acids was determined by GLC analysis as described in the text. *: Number of animals in parenthesis. The values represent the mean and S.D. in each group. Significantly different from the value in each control group: *P<0.05.

The amount of each biliary bile acid is expressed as a percentage of total bile acids and is shown in Table 2. In the normally fed and cholesterol-fed rabbits, the main bile acid was deoxycholic acid (85.8% and 79.3%, respectively) with small amounts of lithocholic acid and cholic acid. Cholesterol feeding increased the composition ratio of cholic acid and decreased the composition ratio of deoxycholic acid. Multiple-dose treatment with MK-733 did not change the composition of bile acids in the normally fed or cholesterol-fed rabbits. Cholestyramine increased the excretion of the primary bile acid, cholic acid, and decreased the excretion of the secondary bile acid, deoxycholic acid, in the normally-fed and cholesterol-fed rabbits. In the case of deoxycholic acid in cholesterol-fed rabbits, a
significant difference was only observed in the t-test. Cholestyramine also decreased the ratio of lithoholic acid (secondary bile acid from chenodeoxycholic acid) in normally fed and cholesterol-fed rabbits. Multiple-dose treatment with MK-733 decreased the lithogenic index in the normally fed rabbits, but did not affect it in the cholesterol-fed rabbits (Fig. 5). Single-dose treatment with MK-733 did not affect the lithogenic index in normally fed or cholesterol-fed rabbits. Cholestyramine decreased the lithogenic index in normally fed and cholesterol-fed rabbits.

5. Fecal lipids: As shown in Fig. 6, neither MK-733 nor cholestyramine affected the weight of feces in normally fed or cholesterol-fed rabbits. Cholesterol feeding increased the concentration of total bile acids and neutral sterols in the feces significantly. Multiple-dose treatment with MK-733 increased the neutral sterol concentration in the cholesterol-fed rabbits, but decreased it in the normally fed rabbits (only significant in the t-test). Cholestyramine increased the neutral sterol concentration in the normally fed or cholesterol-fed rabbits. MK-733 did not affect the concentration of total bile acids in the normally fed or cholesterol-fed rabbits. MK-733 did not affect the concentration of total bile acids in the normally fed and cholesterol-fed rabbits. The predominant bile acid in the feces was 12-keto-lithoholic acid in the normally fed and cholesterol-fed rabbits (60.7% and 55.4%, respectively) (Table 3). Deoxycholic acid and lithoholic acid were also detected in the feces in the normally fed and cholesterol-fed rabbits. MK-733 did not affect the bile acid composition in normally fed or cholesterol-fed rabbits. Cholestyramine increased the ratio of 12-keto-lithoholic acid and decreased the ratio of deoxycholic acid in the cholesterol-fed rabbits. It also increased the ratio of deoxycholic acid and tended to decrease the ratio of 12-keto-lithoholic acid in normally fed rabbits. Cholesterol and coprostanol were major neutral sterols in the feces in the normally fed and cholesterol-fed rabbits, but there was a great variance in the composition of neutral sterols. Therefore, a significant difference was not detected in neutral sterol composition in either group. Cholesterol absorption was calculated from the difference between the amount of dietary cholesterol administered and the amount of fecal neutral sterols excreted. Cholesterol absorption was 47.2% in the control cholesterol-fed rabbits. MK-733 and cholestyramine decreased the absorption of cholesterol to 29.7 and 26.2%, respectively, but not significantly (data not shown).

The excretion of urinary neutral sterols and bile acids was less than 1% and 10% of fecal excretion, respectively (data not shown).
Table 3. Effects of MK-733 and cholestyramine on the composition of fecal neutral sterols and bile acids

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>(n)</th>
<th>Fecal neutral sterols (%)</th>
<th>Fecal bile acids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>cholesterol</td>
<td>coprostanol</td>
</tr>
<tr>
<td>A</td>
<td>Normal</td>
<td>(4)</td>
<td>60.7± 8.5</td>
<td>39.3± 8.5</td>
</tr>
<tr>
<td>C</td>
<td>Normal+MK-733 (multiple)</td>
<td>(5)</td>
<td>84.1±15.7</td>
<td>35.9±15.7</td>
</tr>
<tr>
<td>D</td>
<td>Normal+Cholestyramine</td>
<td>(4)</td>
<td>50.5± 5.5</td>
<td>49.5± 5.5</td>
</tr>
<tr>
<td>E</td>
<td>1% Cholesterol</td>
<td>(4)</td>
<td>70.4±19.0</td>
<td>29.6±19.0</td>
</tr>
<tr>
<td>G</td>
<td>1% Chol.+MK-733 (multiple)</td>
<td>(4)</td>
<td>47.1±26.5</td>
<td>52.9±26.5</td>
</tr>
<tr>
<td>H</td>
<td>1% Chol.+Cholestyramine</td>
<td>(4)</td>
<td>71.4± 8.9</td>
<td>28.6± 8.9</td>
</tr>
</tbody>
</table>

The feces were collected for 3 days before bile duct cannulation. Compositions of fecal sterols and bile acids were determined by GLC analysis as described in the text. *: Number of animals in parenthesis. The values represent the mean and S.D. in each group. Significantly different from the value in each control group: *P<0.05.
Discussion

In the present experiment, MK-733 was found to increase the fecal neutral sterol concentrations, but not to affect the fecal bile acid concentrations in the cholesterol-fed rabbits. MK-733 did not increase the biliary concentration of neutral sterols, total bile acids or phospholipids, and it did not change the composition of biliary neutral sterols and bile acids in the cholesterol-fed rabbits. We have already demonstrated that MK-733 inhibited \[^3H\]-cholesterol absorption in cholesterol-fed rabbits (4). Treatment with MK-733 (10 mg/kg) clearly reduced the serum \[^3H\]-radioactivity in the cholesterol-fed rabbits. The area under the serum radioactive concentration-time curve (AUC) calculated from the serum radioactivity in the MK-733 group was about 9.6% of that in the control group. The cumulative excretion of the fecal radioactivity of \[^3H\]cholesterol in the MK-733 group was higher than that in the control group. The results in the present study agree well with those in the previous one. Cholestyramine, a bile acid sequestrant, increased fecal concentrations of total bile acids and neutral sterols in the cholesterol-fed rabbits. Cholestyramine decreased the bile flow, the biliary concentrations of neutral sterols and phospholipids. Therefore, the inhibitory mechanism of MK-733 on the absorption of cholesterol was considered to be different from that of cholestyramine. Intestinal acyl coenzyme A:cholesterol acyltransferase (ACAT) activity is thought to participate in the cholesterol absorption. Heider et al. reported that an ACAT inhibitor, 57-118, inhibited cholesterol absorption in cholesterol-fed rabbits, and they concluded that intestinal-mucosal ACAT plays a significant role in cholesterol absorption (18). Recently, MK-733 was found to cause a 70% reduction in microsomal ACAT activity of the intestinal mucosa relative to that observed in concurrent control rabbits (17). MK-733 is thought to inhibit the absorption of cholesterol, because it decreases microsomal ACAT activity in the intestinal mucosa. De novo cholesterol synthesis may be related to the regulation of microsomal ACAT activity. Khan et al. reported that lovastatin (MK-803) may act by mechanisms other than as an inhibitor of cholesterol synthesis to decrease the hepatic pool of cholesterol ester in rats (18). Furthermore, they speculate that newly synthesized cholesterol may be essential to the formation and secretion of very low density lipoprotein (VLDL). MK-733 may suppress the formation and secretion of VLDL and/or chylomicron in the intestinal mucosa by inhibiting de novo cholesterol synthesis. MK-733 is considered to inhibit cholesterol absorption in cholesterol-fed rabbits, resulting in an increase in neutral sterol excretion in the feces. On the other hand, cholestyramine interrupts the entero-hepatic circulation of bile acids and inhibits the absorption of neutral sterols (8-11). Both drugs increased the fecal excretion of neutral sterols, but the mechanism of action of MK-733 and cholestyramine is thought to be clearly different.

MK-733 tended to decrease neutral sterol concentration in the feces in normally fed rabbits. It did not increase the biliary excretion of neutral sterols, total bile acids or phospholipids, and it did not change the composition of neutral sterols and bile acids in normally fed rabbits. Almost all cholesterol excreted in the feces is considered to be derived from endogenously synthesized cholesterol in the normally fed rabbits. Therefore, MK-733 is considered to inhibit de novo cholesterol synthesis in normally fed rabbits, resulting in a decrease in fecal neutral sterol concentrations. Cholestyramine increased the fecal concentration of bile acids in the normally fed rabbits. Cholestyramine decreased the secondary bile acid, 12-keto-lithocholic acid and increased deoxycholic acid in the feces. Huff and Carroll (14) reported that deoxycholic acid, lithocholic acid and 12-keto-lithocholic acid were the major bile acids detected in rabbit feces. These were present in approximately equal proportions in rabbits fed a casein or soy protein diet. They also reported that cholesterol and coprostanol were the major neutral sterols in rabbit feces. We also detected these bile acids and neutral sterols in rabbit feces. However, the ratio of fecal bile acids in our experiment was different from that in the experiment of Huff and Carroll (commercial diet) (14). This may have been caused by the difference in the composition of the diets used in our experiment and their experiment. The
primary bile acid, cholic acid, is converted to the secondary bile acid, deoxycholic acid (19) and then converted to 12-keto-lithocholic acid by intestinal bacteria (20). Cholestyramine is considered to prevent the resorption of bile acids, resulting in an increase in bile acid excretion (8–11). Therefore, the residence time of bile acids in the body via the entero-hepatic circulation is considered to be less in cholestyramine treated rabbits than in control rabbits. So, the ratio of deoxycholic acid/12-keto-lithocholic acid is considered to be increased in feces. Cholestyramine decreased the concentration of biliary bile acids, because it inhibits the entero-hepatic circulation of bile acids. This phenomenon is also proved by the decreased levels of bile acids in the serum in the cholestyramine-treated rabbits. MK-733 did not show any effect on biliary bile acids or neutral sterols.

Hellstrom (21) reported that cholesterol feeding reduced fecal excretion of deoxycholic acid and did not influence the bile acid absorption in rabbits. In our experiment, biliary and fecal excretion of deoxycholic acid was slightly decreased by cholesterol feeding. In bile, cholesterol is solubilized as mixed micelles of bile acids, phospholipids, etc., and the solubility of cholesterol in the bile depends on the relative proportions of these constituents (22–24). Therefore, we examined the lithogenic index in bile. Multiple-dose treatment with MK-733 decreased the lithogenic index in the normally fed rabbits, but did not affect it in the cholesterol-fed rabbits. MK-733 is not considered to affect bile acid excretion directly, because a single dose of MK-733 did not affect the biliary neutral sterol or bile acid concentrations. It has been reported that treatment with pravastatin sodium (CS-514) (25–27), another HMG-CoA reductase inhibitor, at doses of 50 mg/kg for 5 weeks lowered the lithogenic index of bile in dogs (28) and that MK-733 also reduced the lithogenic index of cholecystic bile in humans (29). These findings suggest that treatment with HMG-CoA reductase inhibitors will not predispose an individual to development of cholesterol gallstones.

From these results, it was considered that MK-733 inhibited the absorption of cholesterol, resulting in an increase of the fecal concentration of neutral sterols in cholesterol-fed rabbits. The present results in terms of the sterol balance confirm the conclusion that MK-733 inhibits the absorption of cholesterol, resulting in a suppression of hypercholesterolemia in cholesterol-fed rabbits in our previous experiment (4).

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References


