Effects of Cholestyramine on Hepatic Cholesterol 7α-Hydroxylase and Serum 7α-Hydroxycholesterol in the Hamster

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ABSTRACT: Cholestyramine increases activities of hepatic cholesterol 7α-hydroxylase and serum levels of 7α-hydroxycholesterol. To examine if serum 7α-hydroxycholesterol levels parallel with enzyme activity, 0, 0.5, 1, 2, 4, and 10% of cholestyramine was administered to female golden Syrian hamsters for 28 d in the dose-dependent study, and 2% cholestyramine for 0, 1, 3, 7, 14, 21, and 28 d in the time-dependent study. In the dose-dependent study, hepatic and serum cholesterol levels were significantly decreased dose-dependently when more than 0.5% of cholestyramine was fed for 28 d. Cholestyramine increased the cholesterol 7α-hydroxylase activity in a dose-dependent manner, while the serum 7α-hydroxycholesterol was essentially unchanged. No correlation was found between the serum level and the hepatic enzyme activity. In the time-dependent study, hepatic and serum cholesterol levels markedly decreased when 2% cholestyramine was fed for longer than 3 d. The serum triglyceride level increased significantly for the first 7 d and then decreased. Cholesterol 7α-hydroxylase activity increased significantly as early as day 1, reached maximum activity level on day 7, and then kept the significantly high values until day 28. The serum 7α-hydroxycholesterol level significantly increased for the first 7 d and decreased to the pretreatment level thereafter. 7α-Hydroxycholesterol levels significantly correlated with serum cholesterol and triglyceride levels. We conclude that the serum level of total 7α-hydroxycholesterol may be a good indicator in assessing the enzyme activity and the amount of bile acid synthesized in the liver.

Cholestyramine is a sequestrant of bile acids and accelerates hepatic bile acid synthesis. The activities of cholesterol 7α-hydroxylase and the serum levels of 7α-hydroxycholesterol have been reported to increase with the administration of cholestyramine in rats (15) and humans (18). However, it has not been known if serum 7α-hydroxycholesterol levels reflect hepatic enzyme activity when variable doses of cholestyramine are fed for variable periods. In the present study, we examined dose- and time-dependent effects of cholestyramine on hepatic enzyme activity and serum 7α-hydroxycholesterol levels in hamsters.

MATERIALS AND METHODS

Materials. Cholestyramine (Questran, Bristol-Myers Co., Evansville, IN) was purchased from a commercial source. This preparation contains 4 g of cholestyramine in 9 g of Questran. Cholesterol, EDTA, and dithiothreitol (DTT) were obtained from Sigma Chemical Co. (St. Louis, MO). 7α-Hydroxycholesterol and 5α-cholestan-3β,7β-diol were synthesized as described previously (10). Bond-Elast silica cartridge columns were purchased from Analytech International (Harbor City, CA). TMSI-H (hexamethyldisilazane/tri-methylchlorosilane/pyridine, 2:1:10, by vol) was purchased from Gasukuro Kogyo (Tokyo, Japan). NADPH was obtained

Abbreviations: DTT, dithiothreitol; GC, gas chromatography; GC–MS, gas chromatography–mass spectrometry; LDL, low density lipoprotein.
from Kojin (Tokyo, Japan). All solvents used in the present study were of analytical grade or distilled prior to use.

**Equipment.** A Shimadzu GC15A GC, equipped with a flame-ionization detector, a van den Berg’s solventless injector, and a data-processing system (Chromatopack C-R3A; Shimadzu, Kyoto, Japan), and a Shimadzu Auto GC–MS 9020DF GC–MS system with a data-processing system (SCAP 1123) were employed. A fused-silica capillary column (15 m × 0.2 mm) coated with a 0.25-μm layer of cross-linked methylsilicon (Hicap CBP1; Shimadzu, Kyoto, Japan) was used. Conditions for GC were as follows: column oven temperature, 280°C; injection port temperature, 290°C; detector temperature, 290°C; flow rate of helium carrier gas, 2.7 mL/min. Conditions for determination of 7α-hydroxycholesterol by GC–MS were the same as described previously (10).

**Animal experiment.** Female golden Syrian hamsters (Kyudo, Fukuka, Japan), weighing 90–110 g, were used. The animals were kept in groups of 2–3 animals per cage and had free access to water and standard powder rodent chow (Oriental Yeast Co. Ltd., Tokyo, Japan) under a controlled 12-h light–dark cycle (light period from 8:00 A.M. to 8:00 P.M.) for at least a 1-wk acclimation period. In the dose-dependent study, varying concentrations of cholestyramine (0, 0.5, 1, 2, 4, and 10% in chow) were fed for 28 d to four animals in each group. In the time-dependent study, 2% cholestyramine was fed for various periods (0, 1, 3, 7, 14, 21, and 28 d, 5 to 6 animals in each group). After an overnight fast, the animals were sacrificed under ether anesthesia between 10:00 and 12:00 A.M. Blood was obtained by cardiac puncture and serum was separated and stored at −20°C until analyzed. The liver was excised, rinsed with an ice-cold homogenizing solution, and weighed. This protocol was reviewed and preapproved by the Committee of the Ethics on Animal Experiments in the Faculty of Medicine, Kyushu University, and was carried out under the control of the Guidelines for Animal Experiments of the Institute.

**Determination of hepatic microsomal cholesterol 7α-hydroxylase activity.** Cholesterol 7α-hydroxylase activity was determined by the method previously described (10). Liver homogenate was prepared in 50 mM Tris-HCl buffer (pH 7.4) containing 0.3 M sucrose, 10 mM DTT, and 10 mM EDTA. The homogenate was centrifuged at 100,000 × g for 15 min. The microsomal fraction was obtained by centrifugation of the supernatant fluid at 100,000 × g for 1 h. The microsomal pellet was suspended in the homogenizing medium without DTT and recentrifuged at 100,000 × g for 1 h. The resulting microsomal pellet was resuspended in 0.1 M potassium phosphate buffer (pH 7.4) containing 1 mM EDTA. An aliquot of microsomal suspension was used for protein determination using the method of Lowry et al. (19). The standard assay system consisted of 0.5 mL of the microsomal preparation corresponding to 0.5–1.0 mg of microsomal protein and 0.1M potassium phosphate buffer (pH 7.4) containing 1 mM EDTA and 1 mM NADPH in a total volume of 1.0 mL. The enzyme reaction was conducted for 15 min at 37°C. The reaction was terminated by addition of chloroform/methanol (2:1, vol/vol). 5α-Cholestane-3β,7β-diol was added as an internal standard. After extraction with chloroform/methanol (2:1, vol/vol), the organic phase was evaporated to dryness under a stream of nitrogen. Following purification with Bond-Elut silica cartridge column and TMS derivatization (10), the actual mass of 7α-hydroxycholesterol was analyzed by GC–MS. In the selected ion monitoring mode, the ion at m/z 456 (M − 90) was scanned for the trimethylsilyl ether derivative of 7α-hydroxycholesterol, and m/z 458 (M − 90) for that of the internal standard.

**Assay of serum 7α-hydroxycholesterol.** The serum concentration of 7α-hydroxycholesterol was determined as described previously (13). In brief, 200 pmol of 5α-cholestane-3β,7β-diol dissolved in 50 µL of ethanol was added to 0.5 mL of serum as the internal standard. After the addition of 0.7 mL of 0.9% NaCl solution and 1.8 mL of ethanol, sterols were extracted with n-hexane. The collected n-hexane layer was evaporated to dryness under a stream of nitrogen. Sample was then hydrolyzed in 2.0 mL of 2.5% KOH in 90% ethanol at 55°C for 45 min. After the addition of 1 mL of distilled water, the sterols were extracted with n-hexane and the solvent was evaporated under nitrogen. Following purification and derivatization, the serum total 7α-hydroxycholesterol level was quantitated as described above.

**Determination of hepatic cholesterol and serum lipids.** Approximately 100 mg of the liver was hydrolyzed with 10% KOH in 95% ethanol, containing 20.2 mg of 5α-cholestan as an internal standard, at 70°C for 2 h. Lipids were extracted and the cholesterol content was determined by GC (20). Serum cholesterol and triglyceride concentrations were determined by an automatic multichannel analyzer (Hitachi 736-40 Automatic Analyzer, Hitachi Co., Tokyo, Japan).

**Statistical analysis.** Values were expressed as means ± SEM. The statistical difference was evaluated using the Mann–Whitney U test. Probability values less than 0.05 were considered statistically significant. Correlations between the parameters were examined by Spearman’s rank correlation test.

**RESULTS**

**Dose-dependent study.** The animals ingested approximately 10 g of chow per day, and there were no significant differences in food intake among the groups. Body weight and liver weight are summarized in Table 1. The growth of the animals was suppressed significantly in animals fed 10% cholestyramine. In 4 and 10% cholestyramine groups, one out of 4 and 2 out of 4 animals died during the experiment, respectively. The liver weight decreased significantly in animals fed more than 1% cholestyramine for 28 d. In the 10% cholestyramine group, the liver weight was approximately 50% of the controls. Serum triglyceride, serum cholesterol, and liver cholesterol concentrations are depicted in Figure 1. Serum total cholesterol concentration decreased significantly and was dose-dependent. The serum cholesterol level of animals in the 10% cholestyramine group was only 32% of the control value (186.0 ± 12.6 mg/dL in the control group). Serum triglyceride level became less than that of the control animals (322.3 ± 57.8 mg/dL). In cholestyramine-fed animals, however, a sta-