EFFECT OF TAURINE AND HOMOTAURINE ON BILE ACID METABOLISM IN DIETARY HYPERLIPIDIDEMIC RATS

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The effects of taurine (2-aminoethanesulfonic acid) and its homologue, homotaurine (3-aminopropanesulfonic acid), on biliary and fecal excretion of bile acids were investigated in dietary hyperlipidemic rats. Taurocholic acid was a major component in the bile of rats on laboratory chow and the ratio between glycine and taurine conjugated bile acids (G/T ratio) was 0.14. In feces, more free bile acids were present than in bile and the G/T ratio increased to 1.49. The biliary bile acid level increased approximately 2-fold in rats fed a diet containing 0.5% cholesterol and 1.0% cholic acid for 10 days. The glycine conjugated bile acid level increased approximately 7.5-fold, so that the G/T ratio was reversed to 1.17. The level of fecal bile acids increased approximately 19-fold while the G/T ratio remained at 1.43. Taurine or homotaurine (500 mg/kg/d each) was orally administered for 10 d concurrently with the cholesterol diet. Taurine suppressed elevation in the glycine conjugated bile acid level so the bile acid composition was roughly similar to that of the rats on laboratory chow and the G/T ratio became 0.06. In fecal excretion, the bile acid level increased significantly 1.2-fold over that of the control and the G/T ratio was 0.45. Homotaurine did not significantly alter the biliary bile acid level or the composition. These results indicated that taurine stimulated the excretion of bile acids, resulting in a reduction in serum cholesterol. Homotaurine did not influence bile acid metabolism.

Keywords — taurine; homotaurine; hyperlipidemic rat; biliary bile acid; fecal bile acid; HPLC

INTRODUCTION

Cholesterol is mainly catabolized to bile acids in the liver. Bile acids are necessary for intestinal absorption of cholesterol and modulate cholesterol biosynthesis and metabolism; they are thus closely related to the serum cholesterol level.1) On the other hand, bile acids are excreted in the bile after conjugation with glycine or taurine. The ratio of these two conjugates (G/T ratio) is known to vary depending on the species of animal.2,3) It has been suggested that the conjugation pattern of biliary bile acids may influence the serum cholesterol level.4) In general, taurine conjugation of bile acids is associated with low serum cholesterol level and glycine conjugation with high serum cholesterol level.5) It was reported that treatment with taurine alters the G/T ratio of bile acids.5–7) Cholesterol 7ɑ-hydroxylase, which is a rate-limiting enzyme in the metabolism of cholesterol to bile acids, is induced by taurine.7,8) However, the effects of taurine on biliary bile acid composition and fecal excretion of bile acids are yet to be determined.

Using dietary hyperlipidemic rats, we previously suggested that taurine suppressed elevation in the serum cholesterol level.9) Taurine inhibited intestinal absorption of cholesterol and, while this was one reason for the marked reduction in serum cholesterol level, the inhibition was too weak to totally account for the change.

Therefore, in this report we present results of our studies on the effect of taurine and homotaurine on biliary and fecal bile acids in dietary hyperlipidemic rats in order to determine the acceleration of metabolism of cholesterol and excretion of bile acids.

MATERIALS AND METHODS

Animals — Male Wistar rats (Shizuoka Laboratory Animal Center) weighing 110–120 g were divided into groups of 6 animals each and maintained in an air conditioned room (24 ± 1 °C, 55 ± 5% in humidity). Rats were fed a diet containing 0.5% cholesterol and 1.0% cholic

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acid for 10 d. Taurine or homotaurine dissolved in water was administered orally at a dose of 500 mg/kg/d in parallel with the cholesterol diet. Diets and water were fed ad libitum.

**Measurement of Biliary Bile Acids** — Eleven days after beginning the experiments, rats were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and the bile ducts cannulated with polyethylene tubing (size FR 2, IMC®, Imamura Rubber Co., Ltd.). Bile was collected exactly 3 h from the start of cannulation. After measurement of the bile volume, a methanol-acetone (1 : 1) extract was prepared with four volumes of methanol-acetone for each volume of sample. After centrifugation, the supernatant fluid was evaporated under a nitrogen stream and the residue was dissolved in 1.0 ml of methanol. Appropriate aliquots of this preparation, usually 10 to 25 μl, were used for measurement.

The amount of total, free and conjugated bile acids was determined by thin-layer chromatographic (TLC) separation and a 3α-hydroxysteroid dehydrogenase technique. TLC was carried out with 0.5 mm layers of silica gel G (E. Merck) on plates (20 × 26 cm). Development was made with triple runs employing isooctane-ethyl acetate-acetic acid (5 : 5 : 1), isooctane-diisopropyl ether-acetic acid-n-butanol-isopropanol-water (10 : 5 : 5 : 3 : 1) and chloroform-methanol-water (70 : 25 : 2) as successive solvent systems. The areas corresponding to \( R_f \) 0.05—0.31 and \( R_f \) 0.31—0.65 gave the taurine and glycine conjugated bile acids, respectively. The G/T ratio was then calculated.

The main conjugated bile acids, taurocholic acid (TCA), taurochenodeoxycholic acid (TCD), taurodeoxycholic acid (TDA), glycocholic acid (GCA), glycochenodeoxycholic acid (GCD) and glycodeoxycholic acid (GDA), were measured by high performance liquid chromatography (HPLC)(LC-3A; Shimadzu Seisakusho Ltd.) equipped with a Zorbax-C₈ column (4.6 mm i.d. × 250 mm, Du Pont). Acetonitrile-0.05 M sodium phosphate buffer (pH 5.8) 3 : 7 was used as the mobile phase. The isobaric flow rate was 1 ml/min and the column compartment temperature was 40 °C. The bile acids were monitored at 205 nm. A reference mixture of bile acids containing TCA, TCD, TDA, GCA, GCD and GDA was prepared and chromatographed to establish retention time characteristics. The chromatogram of the standard conjugated bile acids is shown in Fig. 1. The amount of each bile acid present in the bile sample solution was determined by comparison of peak areas with those from the corresponding standards. The standard curves were linear over the range 0.01—0.20 μmol. The lower limit of detection was 5 nmol.

Blood samples were taken from the ophthal-mic vessels, after the bile collection, and were centrifuged at 3,000 rpm for 10 min to separate the serum. Total cholesterol was measured by the method of Zak.

**Measurement of Fecal Bile Acids** — Eleven days after beginning the experiments, rats were caged individually in a specially designed cage according to Uchida et al. and one-day feces

![FIG. 1. Separation of Standard Major Conjugated Bile Acids by HPLC](image-url)

**Conditions:** Zorbax-C₈ column (4.6 mm i.d. × 250 mm, Du Pont); mobile phase, acetonitrile-0.05 M phosphate buffer (pH 5.8) 3 : 7, 1.0 ml/min, 45 kg/cm², 40 °C; monitored at 205 nm.
TABLE 1. Effects of Taurine and Homotaurine on Serum Cholesterol and Biliary Bile Acids in Cholesterol-Fed Rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Taurine</th>
<th>Homotaurine</th>
<th>Laboratory chow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cholesterol (mg/100 ml)</td>
<td>443 ± 14.2</td>
<td>229 ± 12.6$^{b)}$</td>
<td>368 ± 26.3$^{a)}$</td>
<td>79 ± 4.4</td>
</tr>
<tr>
<td>Bile flow (ml/rat/h)</td>
<td>0.73 ± 0.050</td>
<td>0.77 ± 0.073</td>
<td>0.60 ± 0.047</td>
<td>0.53 ± 0.030</td>
</tr>
<tr>
<td>Bile acids (mmol/l)</td>
<td>30.91 ± 2.190</td>
<td>31.75 ± 1.942</td>
<td>29.43 ± 2.653</td>
<td>16.93 ± 0.864</td>
</tr>
<tr>
<td>Taurine conjugate (mmol/l)</td>
<td>11.23 ± 1.573</td>
<td>20.75 ± 1.865$^{b)}$</td>
<td>11.23 ± 1.138</td>
<td>13.16 ± 0.820</td>
</tr>
<tr>
<td>Glycine conjugate (mmol/l)</td>
<td>13.14 ± 2.057</td>
<td>1.20 ± 0.263$^{b)}$</td>
<td>9.10 ± 0.708</td>
<td>1.75 ± 0.307</td>
</tr>
<tr>
<td>Free (mmol/l)</td>
<td>8.62 ± 4.406</td>
<td>11.27 ± 2.227</td>
<td>10.38 ± 1.429</td>
<td>2.51 ± 0.548</td>
</tr>
<tr>
<td>G/T ratio</td>
<td>1.17 ± 0.061</td>
<td>0.06 ± 0.010$^{b)}$</td>
<td>0.84 ± 0.093</td>
<td>0.14 ± 0.023</td>
</tr>
</tbody>
</table>

Rats were fed laboratory chow or cholesterol diet (control) for 10 d. Taurine and homotaurine were administered orally at a dose of 500 mg/kg/d in parallel with the cholesterol diet. Results are expressed as mean ± S.E. of 6 rats. Significant difference from the control ($a)p < 0.05$, $b)p < 0.01$).

were collected under fasting conditions. Feces were dried at 25 °C and homogenized in 20 ml absolute ethanol. Extraction of bile acids was carried out twice at 85 °C for 1 h. Extracts were combined, evaporated and the residue was dissolved in 1.0 ml of methanol. Free and conjugated bile acids were separated by TLC. The silica gel which contained the bile acids was scraped and extracted with 20 ml hot methanol twice. The pooled extracts were evaporated and the residue was dissolved in 1.0 ml of the mobile phase of HPLC. Measurement of bile acids was

![Graph](image reference)

**FIG. 2. Typical High-Performance Liquid Chromatograms of Major Conjugated Biliary Bile Acids**

Rats were fed laboratory chow or cholesterol diet (control) for 10 d. Taurine and homotaurine were administered orally at a dose of 500 mg/kg/d in parallel with the cholesterol diet. Conditions as in Fig. 1.
FIG. 3. Effects of Taurine and Homotaurine on Biliary Bile Acids in Cholesterol-Fed Rats

Rats were fed laboratory chow or cholesterol diet (control) for 10 d. Taurine and homotaurine were administered orally at a dose of 500 mg/kg/d in parallel with the cholesterol diet. [●], cholic acid; [▪], deoxycholic acid; [▴], chenodeoxycholic acid. Results are expressed as mean value with S.E. of 6 rats. Significant difference from the control (a) p < 0.01).

carried out as with the biliary bile acids.

Statistical Analysis — Results were evaluated by Student’s t-test.

RESULTS

Table I shows the effects of taurine and homotaurine on serum cholesterol, bile secretion and biliary excretion of bile acids. The cholesterol diet feeding caused an increase in serum cholesterol level. Taurine and homotaurine significantly inhibited the increase by 48.3% and 15.3%, respectively.

Typical high-performance liquid chromatograms of major conjugated biliary bile acids are shown in Fig. 2 and biliary excretion of these acids calculated from the peak area is summarized in Fig. 3.

TCA was a major component of the biliary bile acids of rats fed on laboratory chow. In control rats fed the cholesterol diet containing cholesterol and cholic acid, bile secretion was increased approximately 1.4-fold and biliary excretion of total bile acids was increased approximately 2-fold in animals on laboratory chow. Glycine conjugated bile acids were increased approximately 7.5-fold, and GCD and GDA were detected.

The concurrent administration of taurine with the cholesterol diet caused an increase in taurine conjugated bile acids and a decrease in

| TABLE II. Effect of Taurine on Fecal Bile Acids in Cholesterol-Fed Rats |
|-----------------------------|-----------------------------|-----------------------------|
|                             | Control                     | Taurine                     | Laboratory chow              |
| Feces (g/rat/d)             | 0.69 ± 0.043                | 0.70 ± 0.035                | 1.43 ± 0.238                 |
| Bile acids (mmol/g)         | 177.12 ± 14.754             | 215.99 ± 6.996              | 9.43 ± 0.730                 |
| Taurine conjugate           | 27.84 ± 2.730               | 63.71 ± 4.618               | 0.98 ± 0.125                 |
| Glycine conjugate           | 39.03 ± 3.954               | 27.99 ± 1.777               | 1.44 ± 0.186                 |
| Free                        | 118.16 ± 10.215             | 124.28 ± 3.201              | 7.01 ± 0.553                 |
| G/T ratio                   | 1.43 ± 0.160                | 0.45 ± 0.010                | 1.49 ± 0.124                 |

Rats were fed laboratory chow or cholesterol diet (control) for 10 d. Taurine was administered orally at a dose of 500 mg/kg/d in parallel with the cholesterol diet. Results are expressed as mean ± S.E. of 6 rats. Significant difference from the control (a) p < 0.05, (b) p < 0.01).
glicine conjugated bile acids. The high-performance liquid chromatogram of major conjugated biliary bile acids were therefore similar to those of the rats on laboratory chow. The G/T ratio was decreased markedly but bile secretion and biliary excretion of total bile acids did not significantly differ from that of the control rats fed the cholesterol diet alone.

Homotaurine decreased the G/T ratio of biliary bile acids but did not alter biliary excretion of glycine and taurine conjugated bile acids. Bile secretion and biliary excretion of total bile acids did not differ from those of the control and the high performance liquid chromatogram was similar to that of the control.

Table II shows the effect of taurine on fecal excretion of bile acids. This excretion, calculated from the peak area of the high-performance liquid chromatogram, is summarized in Fig. 4.

In rats on laboratory chow, the difference between biliary and fecal bile acids was markedly increased in free bile acids. The cholesterol diet feeding caused an approximately 19-fold increase in fecal excretion of total bile acids. Conjugated bile acid was increased approximately 28-fold and GCA, which was not detected in the rats on laboratory chow, was very evident. The G/T ratio, however, did not significantly differ in animals fed the two diets.

The concurrent administration of taurine with cholesterol diet caused an increase in fecal excretion of total bile acids. An increase in taurine conjugate and a decrease in glycine conjugate was the same as shown in biliary bile acids.

DISCUSSION

Taurine is not only utilized in the formation of bile acid conjugate in the liver, but also increases cholesterol 7α-hydroxylase activity which is a rate-limiting step in the metabolism of cholesterol to bile acids.7-8

Rats on laboratory chow had predominantly taurine conjugated bile acids in their bile. In control rats fed the cholesterol diet, glycine conjugate increased to the same level as the taurine conjugate (Table I, Fig. 3). Taurine is preferentially utilized in the formation of bile acid conjugate in rat liver.15,16 Increased bile acids resulting from the diet containing cholesterol and cholic acid were conjugated with taurine, so that the taurine pool available for bile acid conjugation in liver was depleted and glycine was then utilized in bile acid conjugation. On the contrary, concurrent administration of taurine with the cholesterol diet caused an increase in the taurine pool in the liver, so that the taurine conjugate was increased to equal that of rats on laboratory chow. It has been suggested that treatment with taurine also alters the G/T ratio of bile acids in human5,6 and guinea pigs.7

Treatment with taurine, however, did not alter the bile secretion or the biliary excretion of bile acids (Table I). In the ileum, bile acids are extensively absorbed by an active transport sys-
tem and return to the liver vein. The relatively small losses of bile acids into the feces are replaced by further hepatic synthesis which is regulated by a negative feedback control to keep the size of the bile acid pool constant.\(^{1,7,18}\) Provided that the metabolism of cholesterol to bile acids is stimulated by taurine, treatment with taurine should increase the fecal excretion of bile acids. In addition, a close relationship between the amount of bile acids excreted in the feces and the blood level of cholesterol was reported.\(^ {19,20}\) We therefore measured the fecal excretion of bile acids.

In the feces, the ratio of conjugated bile acids and bile acids identified as TCA, TCD, TDA, GCA, GCD and GDA by HPLC were decreased as compared with biliary excretion (Table II, Fig. 4). This was because most conjugated bile acids were hydrolyzed and dehydrated by bacteria in the intestines and then excreted to the feces.\(^ {21}\)

The control rats were supplied cholesterol and cholic acid, thereby increasing fecal excretion of bile acids 19-fold. Conjugated cholic acid, which was not detected in the feces of rats on laboratory chow, was much in evidence.

The concurrent administration of taurine with cholesterol diet caused a significant 1.2-fold increase of fecal excretion of bile acid, showing the close relationship between such excretion and serum cholesterol level. It was reported that cholesterol 7α-hydroxylase which was a rate-limiting enzyme in the metabolism of cholesterol to bile acids, was induced by taurine in rats\(^ {8}\) and hyperlipidemic guinea pigs.\(^ {7}\) These facts suggest that taurine stimulates the metabolism of cholesterol to bile acids and the excretion of bile acids.

Homotaurine, a propane homologue of taurine, has a structure and pharmacologic action essentially similar to that of taurine.\(^ {22-27}\) In fact, aminomethanesulfonic acid, a methane homologue of taurine, binds to bile acid in vitro.\(^ {24}\) However, in our present investigation, homotaurine did not affect the composition of biliary bile acids. Previously, we reported that although the inhibitory effect of homotaurine on intestinal absorption of cholesterol was greater than that of taurine, the reduction of serum cholesterol level was less.\(^ {9}\) These facts suggested that homotaurine exerted an action different from taurine and its primary action was presumed to be the inhibition of the intestinal absorption of cholesterol.

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REFERENCES


