Effect of Dietary Taurine on Cholesterol Gallstone Formation and Tissue Cholesterol Contents in Mice

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Summary The inhibitory effect of dietary taurine on cholesterol gallstone formation was studied using male mice of Jcl: ICR strain. Mice were provided three kinds of semi-purified diet; a cholesterol-free diet (standard), a lithogenic diet containing 0.5% cholesterol and 0.25% sodium cholate (C-CA), and a lithogenic diet supplemented with 5% taurine. The changes of total cholesterol in serum, cholesterol mass in the liver and the gall bladder, and gallstone incidence were studied as a function of time. Gallstone formation was observed only in the mice fed on the C-CA diet for more than 3 weeks. The changes of serum cholesterol concentration were not consistent with gallstone formation. The cholesterol mass of the liver in taurine-supplemented mice decreased after the 3rd week. Cholesterol content of the gall bladder increased with cholesterol gallstone formation. Therefore, the inhibitory effect of dietary taurine on cholesterol gallstone formation may be related to the decrease in cholesterol content of the liver.

Key Words dietary taurine, cholesterol gallstone, serum cholesterol, liver cholesterol, gall bladder cholesterol

Various animal models for cholelithiasis have been reported to date. Fujihara et al. (1) investigated the incidence of gallstone formation in inbred mice of eight strains using the lithogenic diet as proposed by Tepperman et al. (2). They also found that the development of gallstones is inhibited almost completely by the combined ingestion of taurine with the lithogenic diet (1).

Taurine, one of the non-essential sulfur amino acids, is an important substrate for the formation of the conjugated bile acids as well as glycine in the liver (3).

A previous report (4) has described that cholesterol gallstones are induced in Jcl: ICR male mice by feeding lithogenic diets containing 0.5% cholesterol and
0.25% sodium cholate. When mice were fed on the diet supplemented with 5% taurine, gallstone formation was inhibited and the total cholesterol levels in serum and the liver decreased (4).

However, the mechanism of the inhibition of cholesterol gallstone formation by dietary taurine has not been resolved. The present study was designed to investigate the changes of gallstone formation and the total cholesterol contents of serum, liver and gall bladder as a function of time, and to elucidate which factor is related to the inhibitory effect of dietary taurine on gallstone formation, the possible mechanism being considered.

METHODS

Ninety-nine 4-week-old Jcl: ICR strain mice were obtained from Nihon Clea Inc. (Tokyo). They were kept in an air-conditioned room (23±1°C, 50–60% humidity) lighted for 12 h a day (07:00 h to 19:00 h). After acclimating for about 1 week with a commercial CE-2 chow (Nihon Clea Inc.), 12 mice were sacrificed. Seventy-five mice were divided into 3 groups and kept in groups of 5 in plastic cages. Semi-purified diets as shown in Table 1 and water were provided ad libitum over 5 weeks.

Five mice of each group were anesthetized every 7 days with diethylether and then bled by decapitation. Gallstone formation (6) was observed with the naked eye, the liver and the gall bladder then being excised immediately. In another line of experiment, 10 mice were fed on the CE-2 diet for 5 weeks. They were dealt with using the above-mentioned method.

Further, the distributions of cholesterol and bile acid in the gall bladder were determined using 17 male mice (4 weeks old). They were divided into 3 groups of 5, 7 and 5 mice each. The first two groups of 5 and 7 mice were fed on the CE-2 diet, and the remaining group of 5 mice was fed on the CE-2 diet supplemented by 0.5%

| Constituents (%) | Standard | C-CA | C-CA + 5% Taurine |
|------------------|----------|------|------------------|
| Casein           | 22       | 22   | 22               |
| Mineral mixturea | 3.5      | 3.5  | 3.5              |
| Vitamin mixturea | 1.2      | 1.2  | 1.2              |
| Choline chloride | 0.15     | 0.15 | 0.15             |
| Celluloseb       | 3        | 3    | 3                |
| Cholesterol      | —        | 0.5  | 0.5              |
| Sodium cholate   | —        | 0.25 | 0.25             |
| Soybean oil      | 10       | 10   | 10               |
| Taurine          | —        | —    | 5                |
| Sucrose          | 60.15    | 59.4 | 54.4             |

a AIN-76TM mixture (5). b Solka-floc.

J. Nutr. Sci. Vitaminol.
cholesterol, 0.25% sodium cholate and 5% soybean oil for 5 weeks. After the 5-week period, gall bladders were excised and pooled for every group. Tissue, bile and gallstones of 5–7 mice were separately obtained by washing with distilled water and centrifugation at 3,000 rpm for 10 min.

Serum cholesterol was determined by the enzymatic method (7). Total cholesterol contents of the liver and the gall bladder were quantitated by the enzymatic method as previously described (8). Total bile acid in gall bladder was determined spectrophotometrically (9).

Results were expressed as means ± SEM. Significance was assessed by Student’s t test and p values of less than 0.05 were considered statistically significant.

RESULTS

Body weight, relative liver weight, and food intake

Table 2 shows the body weight, the relative weight of the liver and food intake. There were no differences among the food intakes of the three groups. The relative liver weights of the taurine-supplemented mice lowered significantly as compared with those of mice fed on the C-CA diet after the 4th week.

Gallstone incidence

The incidence of gallstones is given in Table 3. Gallstone formation appeared after the 3rd week in C-CA fed mice, and the

Table 2. Body weight, liver weight, and food intake.

|                  | CE-2   | Standard | C-CA   | C-CA + 5% Taurine |
|------------------|--------|----------|--------|-------------------|
| **Body weight (g)** |        |          |        |                   |
| Initial          | 21.7 ± 0.3 |          |        |                   |
| 1st week         | —      | 31.0 ± 1.7 | 28.4 ± 0.8 | 29.6 ± 0.4       |
| 2nd              | —      | 36.8 ± 0.7 | 37.0 ± 0.7 | 34.8 ± 1.2       |
| 3rd              | —      | 39.2 ± 0.9 | 39.5 ± 1.0 | 38.7 ± 1.6       |
| 4th              | —      | 42.4 ± 1.0* | 39.2 ± 0.5 | 39.6 ± 1.4       |
| 5th              | 38.6 ± 0.7 | 45.7 ± 1.0* | 40.9 ± 0.4 | 38.2 ± 1.1*      |
| **Liver weight (g/100 g body weight)** |        |          |        |                   |
| Initial          | 6.1 ± 0.1 |          |        |                   |
| 1st week         | —      | 7.2 ± 0.2* | 10.2 ± 0.2 | 8.9 ± 0.1*       |
| 2nd              | —      | 5.6 ± 0.2* | 8.9 ± 0.2 | 8.7 ± 0.4        |
| 3rd              | —      | 5.4 ± 0.2* | 9.5 ± 0.3 | 8.4 ± 0.4        |
| 4th              | —      | 5.1 ± 0.2* | 9.4 ± 0.4 | 7.4 ± 0.3*       |
| 5th              | 5.5 ± 0.1* | 4.4 ± 0.1* | 9.0 ± 0.3 | 6.3 ± 0.2*       |
| **Food intake (g/day)** |        |          |        |                   |
| Initial          | —      | 4.4 ± 0.2 | 4.5 ± 0.2 | 4.4 ± 0.2        |

Results are expressed as mean values and standard error of the means. * Differs significantly from the C-CA fed group (p < 0.05).
Table 3. Incidence of gallstone formation (%).

|               | CE-2 | Standard | C-CA | C-CA +5% Taurine |
|---------------|------|----------|------|------------------|
| Initial       | 0    | 0        | 0    | 0                |
| 1st week      |      | 0        | 0    | 0                |
| 2nd           |      | 0        | 0    | 0                |
| 3rd           |      | 0        | 60 (1.0)* | 0                |
| 4th           |      | 0        | 60 (1.8) | 0                |
| 5th           | 0    | 0        | 100 (2.8)| 0                |

*Value in parentheses represents the mean value of gallstone grade (6).

Table 4. Distribution of cholesterol and bile acid in gall bladder.

Seventeen ICR male mice of 4 weeks of age were divided into 3 groups. The first 2 groups of 5 and 7 mice were fed on the commercial CE-2 diet, and the remaining group of 5 mice was fed on the CE-2 diet supplemented with 0.5% cholesterol, 0.25% sodium cholate and 5% soybean oil. Five weeks later, the gall bladders of each group were pooled. Tissue, bile and gallstones were separately obtained by washing with distilled water and centrifugation at 3,000 rpm for 10 min. Cholesterol content was determined by the enzymatic method (7), and total bile acid was estimated spectrometrically (9).

| Diet (number of animals) | CE-2 (5) | CE-2 (7) | CE-2+0.5% cholesterol +0.25% sodium cholate +5% soybean oil (5) |
|-------------------------|----------|----------|---------------------------------------------------------------|
| Cholesterol [μg/mouse]  | Tissue   | 3.93 (81.2)* | 3.96 (58.7) | 9.04 (2.3) |
|                         | Bile     | 0.91 (18.8) | 2.79 (41.3) | 82.64 (20.6) |
|                         | Gallstone | 0 (0)    | 0 (0)    | 308.74 (77.1) |
| Bile acid [μg/mouse]    | Tissue   | —        | —        | 9.46 (5.1) |
|                         | Bile     | —        | —        | 165.24 (89.3) |
|                         | Gallstone | —       | —       | 10.45 (5.7) |

*Value in parentheses represents the percentage of bile acid in gall bladder.

Incidence was 100% at the 5th week. On the contrary, gallstone formation was not observed at all in the taurine-supplemented mice or the mice of the standard group.

**Distribution of cholesterol and bile acid in gall bladder**

The distributions of cholesterol and bile acid in the gall bladder are shown in Table 4. Cholesterol contents of the tissue were very low even if gallstones had been formed. When cholesterol gallstones were formed, the cholesterol contents of bile and gallstone, especially of the latter, increased. As regards bile acid, nearly 90% of total bile acid exists in the bile.
Fig. 1. Changes of total cholesterol concentration in serum as a function of time. Each point is the mean for 5 to 12 animals and vertical extension indicates SEM. *Differs significantly from the C-CA group at each week (p<0.05).

Fig. 2. Total cholesterol mass in liver and its interrelationship with gallstone formation. Solid line indicates the changes of mean value of hepatic cholesterol mass as a function of time. Individual values of mice are also indicated. Closed circles show the hepatic cholesterol value of animals with gallstones. *Differs significantly from the C-CA group at each week (p<0.05).

Total cholesterol contents of serum, liver and gall bladder

Figure 1 shows the total cholesterol concentration of serum. The level increased with time in the standard group. The highest value was observed at the 1st
week in C-CA fed mice and decreased thereafter. Dietary taurine inhibited the increment of serum total cholesterol level significantly in spite of the C-CA feeding at the 2nd to 4th week.

Figure 2 shows the total cholesterol mass in the liver. The level was constantly low in the standard mice. The C-CA fed mice revealed the highest level. Taurine significantly suppressed the hepatic accumulation of cholesterol in spite of the C-CA feeding, but the level was significantly higher than in the standard mice. In contrast to C-CA fed mice, the level for taurine-supplemented mice strikingly decreased after the 3rd week. As shown in Fig. 2, cholesterol gallstone was observed only after the 3rd week when the liver cholesterol content was more than 150 mg.

Figure 3 gives the total cholesterol mass of the whole gall bladder. The content was constantly low in the standard mice in which no stones were found. The mass increased significantly in the C-CA mice after the 3rd week. The cholesterol content of the taurine-supplemented mice was constantly low though it was significantly higher than for the standard group or the CE-2 group.

DISCUSSION

The effect of taurine on cholesterol metabolism has been investigated in rabbit, rat, and man (10). Tsuji et al. (8, 11, 12) reported that the increments of total cholesterol contents in serum and liver were inhibited by dietary taurine in hypercholesterolemic rats.

J. Nutr. Sci. Vitaminol.
The present study was carried out to elucidate the mechanism of the preventive effect of dietary taurine against cholesterol gallstone formation in mice, and several new findings have been made:

First, the increment of serum total cholesterol level by exogenous cholesterol appeared only at the early period (Fig. 1). Changes of the serum total cholesterol level were not consistent with those of gallstone formation (Fig. 3).

Second, gallstone formation could be observed after the 3rd week with possible completion at the 5th week (Table 3). In contrast to the C-CA fed group, dietary taurine completely inhibited gallstone formation during this experimental period.

Third, the cholesterol content of gall bladder tissue was very low (Table 4). When mice were fed on the lithogenic diet and gallstone formation was observed, cholesterol contents of the bile (20.6%) and gallstones (77.9%) were very high. Therefore, the total cholesterol mass of whole gall bladder may be useful as an index of the severity of gallstone formation or of the presence of lithogenic bile supersaturated with cholesterol in the gall bladder.

Fourth, the hepatic cholesterol mass decreased dramatically after the 3rd week in the mice fed on the taurine-supplemented diet in spite of containing C-CA (Fig. 2). Cholesterol gallstone formation was limitedly observed not only at the 3-week feeding of the C-CA diet but also in the case of the hepatic cholesterol mass exceeding 150 mg (Fig. 2). In general, the formation of cholesterol gallstone is induced from abnormal hepatic bile supersaturated with cholesterol (13).

Therefore, our results suggest that the inhibitory effect of dietary taurine on cholesterol gallstone formation is related to the decrease in hepatic cholesterol mass and the following decrease in cholesterol secretion into bile or the decrease in the molar ratio of biliary cholesterol/bile acid. The decrease of hepatic cholesterol mass supports the possibilities that the catabolism of cholesterol to bile acid was stimulated and that the lithogenicity of bile was improved. As regards biliary lithogenicity, Fujihara et al. (1) have already reported in taurine-ingested mice that the biliary cholesterol level decreases while the biliary bile acid level increases, and that the molar ratio of biliary cholesterol/phospholipid and cholesterol/bile acid decreases.

For species other than mice, there are some reports which elucidate the stimulation by dietary taurine in catabolism of cholesterol to bile acid. Kibe et al. (14) reported that the activity of the hepatic cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis, was stimulated with dietary taurine in guinea pig. Also, Tsuji et al. (12) reported that the biliary excretion of newly synthesized bile acids was positively correlated with the biliary cholesterol concentration and the hepatic cholesterol mass in the rat. They suggested that the catabolism of cholesterol to bile acid and biliary cholesterol excretion were stimulated so as to decrease excess cholesterol in the liver.

Further studies are necessary to clarify the relationship among cholesterol metabolism, bile acid metabolism and gallstone formation in taurine-fed mice.
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