Mass Spectrometry——Mass spectral measurements were run on Hitachi Model RMU-6E spectrometer under the following conditions: ionization voltage 70 eV, accelerator voltage 1800 V, temperature of ionization chamber 210°, and width of collector slit 0.4 mm.

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Effect of Taurine on the Capacity of the Bile to Solubilize Cholesterol in Lithogenic Hamsters

Eiichi Fujihira, Takashi Ohshima, Isamu Ushioda, Tadakazu Furuhashi, Norimitsu Takahashi, Michitada Sasajima, and Ichiro Tanaka

Research Laboratory, Taisho Pharmaceutical Co., Ltd.[1]

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It is the recent knowledge that the ratio (bile acids+phospholipids)/cholesterol determines cholesterol solubility in the bile.[2] Whether cholesterol will be in the micellar phase or the crystalline phase can be represented as one point obtained by plotting the relative quantities of the three components on a triangular diagram.

At present, chenodeoxycholic acid treatment seems likely to be the most hopeful in cholelithiasis therapy in order to improve cholesterol solubility in the bile as shown by an increase in the above ratio and by a reduction in the molar ratio of cholesterol.[3] In the preceding paper,[4] the authors reported that taurine-treatment promoted the biliary excretion of intravenously infused deoxycholic acid in the form of the taurine conjugate in normal and vitamin B_6-deficient rats.

Dam and Christensen[5] succeeded in producing experimentally cholesterol-gallstones in hamsters by feeding a diet free of fat and rich in carbohydrate. In the bile of such animals a marked decrease in the ratio bile acids/cholesterol has been noted.[6] On the other hand, a prophylactic effect of taurine on experimentally induced cholelithiasis has been reported in rats and rabbits.[7]

The purpose of the present study is to investigate the effect of administered taurine on gallstone formation and cholesterol solubility in bile in lithogenic hamsters.

Experimental

Male golden hamsters approximately weighing 50 g were kept on the lithogenic diet prepared according to the method of Dam and Christensen[8] for 5 weeks. They were divided into four groups of seven animals

1) Location: *3-34-1, Takata, Toshima-ku, Tokyo.*
2) *a*) W.H. Admirand and D.M. Small, *J. Clin. Invest.*, **47**, 1043 (1968); *b*) D.M. Small, *New Engl. J. Med.*, **284**, 214 (1971).
3) G.D. Bell, B. Whitny, and R.H. Dowling, *Lancet*, Dec. 9, 1213 (1972).
4) E. Fujihira, T. Ohshima, I. Ushioda, and N. Takahashi, *Chem. Pharm. Bull.* (Tokyo), in press.
5) H. Dam and F. Christensen, *Acta Pathol. Microbiol. Scand.*, **30**, 236 (1952).
6) I. Prange, F. Christensen, and H. Dam., *Z. Ernährungswissenschaft*, **3**, 59 (1962).
7) *a*) R.F. Borgman and F.H. Haselden, *Am. J. Vet. Res.*, **30**, 107 (1969); *b*) K. Yoshikawa, *J. Nara Med. Ass.*, **21**, 631 (1970).
8) H. Dam and F. Christensen, *Z. Ernährungswissenschaft*, **2**, 154 (1962).

each and given subcutaneously taurine in the daily doses of 0, 250, 500, and 1000 mg/kg, respectively, during the entire period of the experiment. Seven hamsters of the same lot were free access to a commercial diet supplemented sufficiently with nutrients and served as normal controls. At the end of the period, bile was collected by common bile duct-cannulation and blood was obtained by bleeding through the femoral vein. Color, shape and number of gallstones formed were recorded. Glycine and taurine conjugates of cholic acid and dihydroxy bile acids (chenodeoxycholic and deoxycholic) were separated from the bile by thin-layer chromatography in the solvent system of *n*-BuOH, AcOH, and H_2O (10: 1: 1)[9] and quantified individually by the densitometric method as mentioned previously.[4] Phospholipids and cholesterol in the bile were determined by the method of Osuga and Portman.[10] The Wako-Kits were used for the quantitative determinations of serum cholesterol, phospholipids and triglycerides.

Result

Retardation of growth was observed in the animals fed the test diet and daily administrations of taurine were without effect.

The results of bile analysis are summarized in Table I. Taurine-treatment had no effect on the frequency of gallstone formation. Nevertheless, dark green-brown stones of irregular shape were developed mostly in the highly dosed groups, while white or yellow stones were characteristic in the animals treated at 250 mg/kg and the untreated.

Table I. Incidence of Gallstone(GS) Formation and Data for Biliary Bile Acids(BA), Phospholipids(P) and Cholesterol(Ch) in Lithogenic (Hamsters Receiving) Taurine

Group[a]	Daily dose of taurine (mg/kg)	No. with GS/ No. tested	Conjugated BA (mM)					G/T	P (mM)	Ch (mM)	BA+P/Ch
			Glycine		Taurine		Total BA				
			CA[b]	DiBA[c]	CA[b]	DiBA[c]					
I	1000	3/7	3.90[d]	7.81[e]	1.23[d]	4.97[e]	17.91[e]	1.89	0.71[d]	0.79	23.6
II	500	3/7	4.22[d]	4.19[d]	0.81[d]	2.30	11.52[d]	2.70	0.67[d]	0.86	14.2
III	250	3/7	3.12[d]	4.67[d]	0.53	2.30	10.61[d]	2.75	0.36	1.04	10.6
IV	0	4/7	1.54	2.85	0.58	2.00	6.97	1.70	0.41	0.97	7.6
V[f]	0	0	8.85	3.58	4.04	3.55	20.03	1.64	1.51	0.40	53.8

a) 7 animals each
b) cholic acid
c) dihyroxy bile acids (mostly chenodeoxycholic acid)
d) $p < 0.05$,
e) $p < 0.01$: s significant increase from the level of group IV
f) normal control

Lithogenic hamsters demonstrated a marked decrease in the biliary secretion of total bile acids, especially of cholic acid as compared to normal controls. In the taurine-treated groups of lithogenic animals, however, the reduction of bile acid secretion was to a lesser extent as the doses were increased. The ratio of glycine to taurine conjugated bile acids (G/T) was not different between the normal and the lithogenic groups, and tended to increase more or less in the latter groups following the continuous administrations of taurine.

Organic phosphorous was also reduced markedly in the bile of lithogenic hamsters, while the biliary secretion of cholesterol increased about two times larger than the normal level. Following the administration of taurine to these animals, the reduced secretion of organic phosphorous increased slightly, while the level of cholesterol remained unchanged.

The ratio (bile acids+phospholipids)/cholesterol for the taurine-untreated group was approximately one seventh lower than the normal level. This was improved dose-dependently in the lithogenic animals receiving daily taurine.

9) H. Ganshirt, K.W. Koss, and K. Morianz, *Arznei-Forsch.*, **10**, 943 (1960).
10) T. Osuga and O.W. Portman, *Gastroenterology*, **63**, 122 (1972).

Taurine-treatment had no effect on the concentrations of serum cholesterol, phospholipids and triglycerides in lithogenic animals.

Discussion

Dam and his collaborators[8,11] reported that the addition of hyodeoxycholic acid, or of cholestyramine, a bile acid-sequestering resin, to the lithogenic diet afforded a high degree of protection against cholesterol gallstones in hamsters.

As shown in the present study, taurine-treatment was ineffective in protecting animals from gallstone formation. However, almost the gallstones formed in the groups of the hamsters receiving taurine at higher dosages were dark green-brown in color and sandy in shape. This finding is in good agreement with that of Sǿndergaard, *et al.*[11] on the lithogenic hamsters fed cholestyramine. Dam and Christensen[5] reported that cholesterol content was approximately 50% in dark green-brown stones in contrast to 70.5—94.0% in white or yellow stones.

It is proved in the human that exogenous taurine conjugates readily with bile acids in the liver to bring out a marked decrease in the ratio G/T in the bile.[12] Enhancement of biliary secretion of taurine conjugated bile acids has been proposed as a possible mechanism whereby taurine-treatment can produce a protection against experimental cholelithiasis in rats and rabbits.[7]

In our preliminary experiment, the biliary secretion of radioactivity after administration of taurine-^{35}S into normal hamsters with bile fistula was very slower at rate and smaller in amount than that as would be expected in the human, rat or dog, although radioactivity did not appear at all in the bile when glycine-^{14}C, instead of taurine-^{35}S, was administered. As shown in the present study, any reduction of the ratio G/T does not occur in lithogenic hamsters following daily administrations of large amounts of taurine. Therefore, alterations of bile acid conjugation seem unlikely to explain the taurine-effect on cholesterol solubility in the bile of lithogenic hamsters. The mechanism whereby taurine-treatment improves the cholesterol solubilizing capacity of lithogenic bile is uncertain at present.

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11) E. Sǿndergaard, I. Prange, and H. Dam, *Z. Ernährungswissenschaft*, **9**, 174 (1969).
12) *a*) J. Sjövall, *Proc. Soc. Exp. Biol. Med.*, **100**, 676 (1959); *b*) J.T. Garbutt, K.W. Heaton, L. Lack, and M.P. Tyor, *Gastroenterology*, **56**, 711 (1969).