Effect of administration of ursodeoxycholic acid at bedtime on cholesterol saturation of hepatic bile in Japanese patients with gallstone

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Abstract : The administration of a single, daily 600 mg dose of ursodeoxycholic acid (UDCA) at bedtime and 3-200 mg doses per day at mealtime was conducted for 6 patients with gallstone and choledocholithiasis who were undergoing biliary drainage for the purpose of improving jaundice. Hepatic bile was collected from a drainage tube after a lapse of time in order to compare the bile acid compositions and cholesterol saturation index (SI) in bile for the 2 protocols. A significant increase in UDCA levels in hepatic bile was observed after both UDCA administration at bedtime and mealtime, but the effect of bedtime administration was significantly greater than that of mealtime administration. Whereas levels of cholic acid and chenodeoxycholic acid (CDCA) decreased for the case of bedtime administration, this was not detected for mealtime administration, although no significant differences among the mean interval values were observed. A significant in difference decreased SI was observed during UDCA bedtime administration, but not during mealtime administration, compared to the SI before administration. This suggests a decreased cholesterol excretion into the bile. Based on these findings and from the point of view of compliance, bedtime administration of UDCA appears to be an effective method. J. Med. Invest. 45 : 115-122, 1998

Key words **:** *ursodeoxycholic acid, gallstone, bedtime, cholesterol saturation*

INTRODUCTION

The oral administration of the bile acids chenodeoxycholic acid (CDCA) (1) and ursodeoxycholic acid (UDCA) (2,3) reduces the cholesterol saturation index (SI) in fasting gallbladder bile and aids in the solubilization of cholesterol-derived gallstones. Northfield and co-workers reported a significant increase in the rate of gallstone dissolution in patients who were administered CDCA at bedtime, along with a low cholesterol diet, compared with CDCA administration at mealtime, along with an unrestricted diet. (4) Moreover, when the latter investigators compared the results of CDCA administration for mealtime vis-a-vis bedtime administration, as well as for UDCA, along with an unrestricted diet, bedtime administration significantly enhanced the rate of gallstone dissolution. They also proposed the bedtime regimen as an appropriate strategy for reducing the minimum effective dose of CDCA required to desaturate gallbladder bile with cholesterol, (5) and demonstrated the utility of bedtime UDCA administration in dissolution therapy in a randomized study. (6) Lanzini *et al*. reported that a reduced UDCA dose (7mg/kg/day) given at bedtime was as effective as the conventional 10mg/kg/day UDCA given at mealtime for reducing the cholesterol SI in gallbladder bile in individual patients. (7) Moreover, UDCA is generally preferred over CDCA for use in gallstone dissolution in contrast to the latter bile acid, (8, 9) because it does not cause diarrhea and

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hypertransaminasemia. (10)

Therefore, in the U.S. and Europe, bedtime UDCA administration is generally thought to be more effective than mealtime administration of the same total daily dose in enhancing the rate of dissolution of gallstone. However, in Japan, UDCA at 600 mg/day is conventionally divided into 3 doses, which is given at mealtimes. Moreover, gallstones which are composed largely of calcium bilirubinate are generally more common than in western countries, and pigment stone formation is especially prominent. (11) These findings suggest that Asian subjects may respond differently to western subjects, in terms of cholesterol SI, to the administration of UDCA. However, little information on the cholesterol SI in hepatic bile in Japanese patients with gallstone is available for comparison between bedtime and mealtime administration of UDCA, although Shibata *et al*. (12) reported on the utility of bedtime administration.

The present paper describes a study in which the bedtime administration of UDCA is alternated with mealtime administration in Japanese patients with gallstone and choledocholithiasis who were undergoing biliary drainage. The patients received a routine hospital diet, and hepatic bile was collected from a drainage tube retained retrograde in the common bile duct, after an appropriate time lapse, and the cholesterol SI and the bile acid composition were compared for the two protocols.

SUBJECTS AND METHODS

Subjects

Six patients [3 men and 3 women ; mean age (±SD), 60±11 yr ; mean body weight (±SD), 53±6 kg] with gallstone and choledocholithiasis were studied (Table1). All patients were treated to improve jaundice by undergoing biliary drainage at Second Department of Internal Medicine, Tokushima University Hospital, and Tokushima Municipal Hospital. For biliary drainage, endoscopic nasotracheal biliary drainage was carried out by retaining a drainage tube (Create Medic Co., Tokyo, Japan, 7F) retrograde in the common bile duct. Prior to the study, the objectives were explained to each patient and written informed consent was obtained. The analytical schedule was started for each patient when his/her aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were 100 IU/L or less and serum total bilirubin was 3 mg/dl or less, and no other serious functional disorder was observed. During the experimental course all patients received a standard hospital diet.

Methods

UDCA was synthesized by Tokyo Tanabe Pharmaceutical Co. (Tokyo, Japan), and was at least 99% pure, as evidenced by gas liquid chromatography and thin layer chromatography. Fig.1 shows the protocol for UDCA administration and the schedule for bile collection from the biliary drainage tube. On a body weight basis, the daily UDCA dose ranged from 10.0 to 12.8mg/kg for the 6 individual subjects, with a mean value (±SD) of 11.3±1.1 mg/kg/day (Table 1). On the first day of study when UDCA was not administered, bile was collected 13 times every 3 h for a total period of 39 h (Before UDCA). UCDA at 600 mg was administered once at bedtime the next day. On the third day, bile was also collected 13 times every 3 h (Bedtime UDCA). At 58 h after the bedtime administration of UDCA, another 600 mg, divided into 3 doses of 200 mg each,

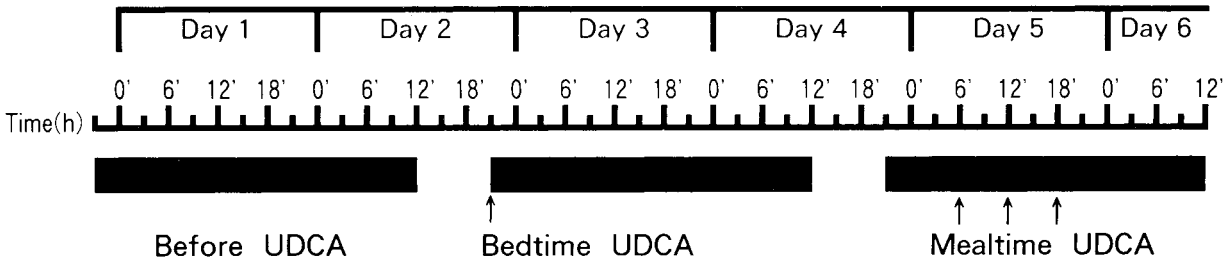

Fig.1. UDCA administration method and the schedule for bile collection from the biliary drainage tube. The drainage tube was retained retrograde in the common bile duct in individual subjects. Hepatic bile was collected 13 times at 3 h intervals during a 39 h period without UDCA administration (Before UDCA). A 600 mg dose of UDCA was given once the next day at bedtime, and hepatic bile was collected as described above (Bedtime UDCA). From 58 h onwards, another 600 mg which was divided into 3-200 mg doses a day at mealtime was given, and hepatic bile was again collected during the 39 h period (Mealtime UDCA). All subjects received a routine hospital diet during the course of bile collection.

Table 1. Patients studied with gallstone and choledocholithiasis

Patient no.	Age Yr	Sex	Dose of UDCA mg/day	mg/kg/day	Disease
1	68	M	600	12.8	Gallstone and Choledocholithiasis
2	43	M	600	10.9	Gallstone and Choledocholithiasis
3	73	F	600	11.1	Gallstone and Choledocholithiasis
4	68	M	600	12.5	Gallstone and Choledocholithiasis
5	58	F	600	10.0	Gallstone and Choledocholithiasis
6	52	F	600	10.7	Gallstone and Choledocholithiasis

All patients were treated by undergoing endoscopic nasobiliary drainage for the purpose of improving jaundice and received a routine hospital diet during the course of biliary drainage.

was administered at mealtimes. Bile was again collected 13 times every 3 h from the 5 th to the 6 th day (Mealtime UDCA). Bile obtained from the drainage tubes was frozen and stored at -80℃ until assayed.

For determination of the cholesterol SI of hepatic bile, total cholesterol (13) and total bile acids (14, 15) were measured enzymatically. Phospholipids were extracted (16) and determined colorimetrically. (17) The SI was calculated by the formula established by Thomas and Hofmann, (18) based on the limits of cholesterol solubility defined by Hegardt and Dam (19) and Holzbach *et al*. (20) The determination of individual bile acids was carried out by high-performance liquid chromatography (HPLC) with a post-column enzymatic reaction and fluorescence detection. (21)

Statistics

Data obtained from each collection period before UDCA administration, during bedtime administration and mealtime administration were expressed as the mean (±SD) of the individual bile collected 13 times over an interval of 3 h to 39 h unless otherwise indicated. Statistical significance was tested with the Mann-Whitney U test, and p values <0.05 were accepted to indicate significant differences.

RESULTS

Changes in bile acid composition

Changes in the differential amount of bile acid in hepatic bile before UDCA administration, and during bedtime administration and mealtime administration in patients with gallstone and choledocholithiasis are shown in Figs. 2 and 3. Prior to UDCA administration, the concentrations of UDCA in bile ranged from 0.0 to 0.08 mol/l for the 6 individual subjects with a mean value of 0.01±0.03 mol/l. The UDCA level increased rapidly for 12 h after the bedtime administration and returned to baseline by at least 36 h after bedtime administration (Fig.2). Thus, there was no additive influence of the initially administered UDCA on bile acid compositions at 58 h after bedtime administration, at which time an additional 600 mg was administered in 3-200 mg doses at mealtimes. The total bile acid (TBA) levels in bile before administration, and during bedtime administration and mealtime administration were 85.6±35.4, 108.6±31.2 and 120.5±48.0 mol/l, respectively, and no significant differences among the three interval values were observed. The respective values of cholic acid (CA) were 45.7±15.5, 40.7±12.2 and 53.0±21.9 mol/l ; CDCA, 37.9±13.3, 29.7±11.4 and 47.6±15.4 mol/l ; deoxycholic acid (DCA), 1.3±1.3, 0.7±0.5 and 0.8±0.6 mol/l ; UDCA, 0.3±0.3, 37.3±10.6 and 18.6±6.5 mol/l ; lithocholic acid (LCA), 0.03±0.04, 0.0 and 0.0 mol/l. The levels of DCA and LCA during the experimental course were negligible. The levels of CA and CDCA tended

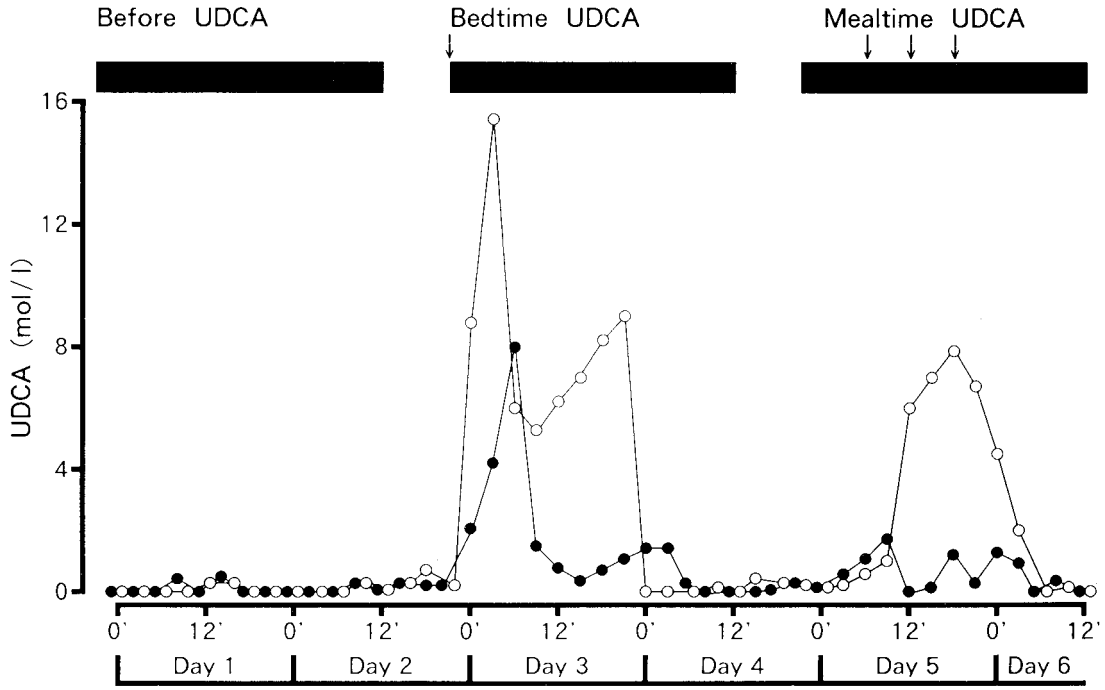

Fig.2. Profiles for UDCA levels at intervals of 3 h in hepatic bile from 2 subjects (○, patient No.1 ; ●, patient No.4) studied on 6 successive days.

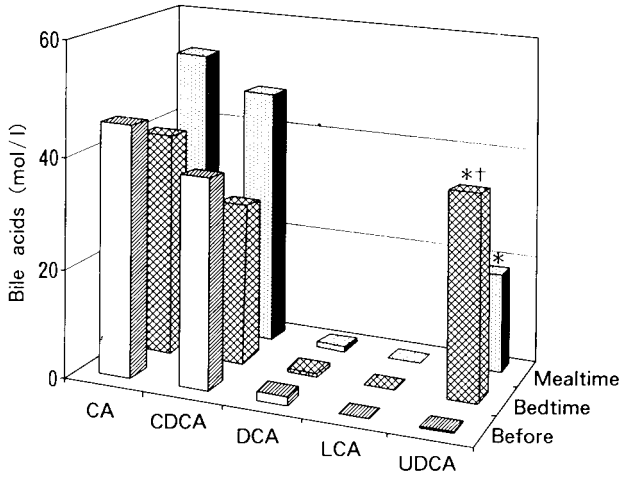

Fig.3. Mean interval values of bile acid compositions in hepatic bile from 6 subjects before and during UDCA administration at bedtime and mealtime. Bile was collected 13 times at 3 h intervals over a 39 h period before UDCA administration and during UDCA administration at bedtime and mealtime. Bile acid composition in the collected bile was determined by HPLC with a post-column enzymatic reaction and fluorescence detection. *Significant difference between values before and during UDCA administration ($P<0.05$). †Significant difference between values during UDCA administration at bedtime and mealtime ($P<0.05$). □, Before administration ; ▩, bedtime administration ; ▥, mealtime administraion.

to decrease during bedtime UDCA administration, but not during mealtime administration, although the differences in the mean interval values of CDCA or CA were not significant. While the UDCA levels increased significantly during both bedtime and mealtime administrations compared to those before administration, the effect of bedtime administration was significantly greater (Fig.3).

Alterations in conjugated bile acids

The alterations in conjugated CA and CDCA in hepatic bile before and during UDCA administration at bedtime and mealtime are shown in Fig.4. The glycine conjugated CA levels before administration, and during bedtime administration and mealtime administration were 34.0±11.7, 29.1±10.1 and 37.7±12.8 mol/l, respectively. The respective values of taurine conjugates were 11.7±4.6, 9.0±3.5 and 12.7±5.8 mol/l and those of the non-conjugation variety were 0.02±0.03, 2.6±1.4 and 2.6±1.6mol/l. In the case of CDCA, the respective values of the glycine conjugates were 30.8±12.9, 22.7±8.9 and 38.2±13.4 mol/l ; the taurine conjugates, 7.1±2.9, 5.0±1.8 and 9.1±3.4 mol/l ; non-conjugation type, 0.1±15.8, 16.0±17.9 and 2.0±16.9 mol/l. While the levels of non-conjugated CA and CDCA had a tendency to increase, the levels of glycine-conjugated CA and CDCA tended to decrease during bedtime UDCA administration (Fig.4), although the differences were not significant.

Alterations in cholesterol SI

Changes of the cholesterol SI in hepatic bile

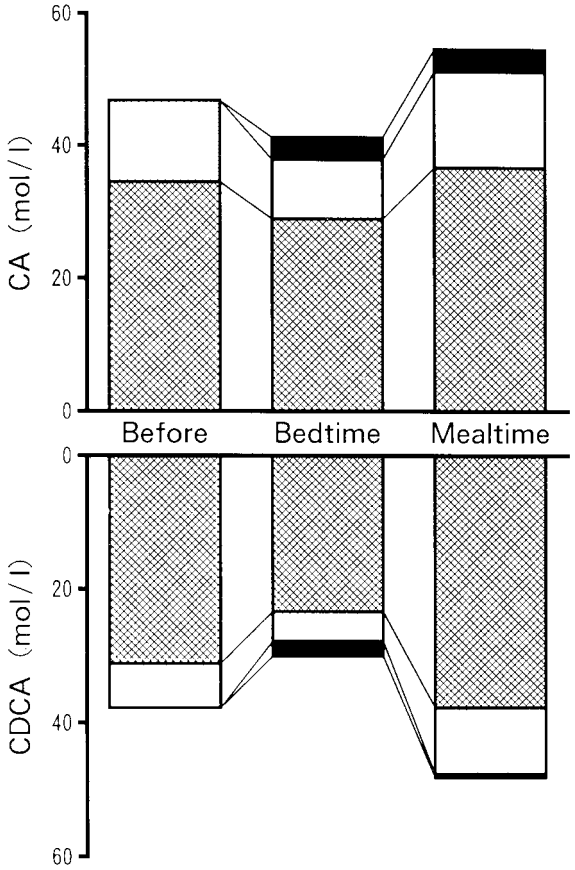

Fig.4. Mean interval values of conjugated CA (upper panel) and CDCA (lower panel) in hepatic bile from 6 subjects before and during UDCA administration at bedtime and mealtime. Bile was collected 13 times at 3 h intervals over a 39 h period before UDCA administration and during UDCA administration at bedtime and mealtime. Conjugated bile acids in the collected bile were determined by HPLC with a post-column enzymatic reaction and fluorescence detection.
▨, Glycine conjugation variety ; ▥, Taurine conjugation variety ; ■, Non-conjugation variety.

before and during UDCA administration at bedtime and mealtime are shown in Figs.5 and 6. The mean interval values for cholesterol SI during the period of 39 h in all the 6 subjects, before administration, during bedtime administration and during mealtime administration were 2.4 ± 0.5, 1.6 ± 0.3 and 1.9 ± 0.4, respectively. The mean values decreased during UDCA administration both at bedtime and mealtime : the difference between the values for before and during bedtime administration were significant, but the difference between the values before and during mealtime was not.

DISCUSSION

It has been reported that, for the case of bile acid composition after UDCA administration to patients with gallstones, UDCA concentrations in the fasting gallbladder bile generally increase in proportion to the dose, and that levels of CA, CDCA and DCA decrease while LCA levels remain unchanged. (18) The results of this study also indicate a significant increase in UDCA levels in hepatic bile obtained from patients with gallstone and choledocholithiasis after UDCA administration, both at bedtime and at mealtime. The effect of bedtime administration was significantly greater than that of mealtime administration. It is worth noting that the hepatic bile levels of CA and CDCA tended to decrease during bedtime UDCA administration, but not during mealtime administration, and that the levels of DCA and LCA were negligible throughout the experiment, although the differences between the mean interval values were not significant. The findings herein are consistent with findings reported by Makino and Nakagawa, who reported analyses of gallbladder bile from Japanese patients. (22)

In regard to CA and CDCA, we investigated differences in the levels of glycine- and taurine-conjugated type levels, as a result of UDCA administration at bedtime and mealtime. The levels of glycine-conjugated CA and CDCA were not significantly decreased during bedtime UDCA administration. Tanimura *et al*. (23) also reported that, compositionally, no difference in the levels of conjugated bile acids between cholesterol gallstone patients and normal controls was evident. These findings suggest that conjugated bile acid composition is not affected by UDCA administration or gallstone formation.

To simply illustrate the correlation between bile acid, cholesterol and phospholipid in bile, Admirand and Small proposed a triangular drawing, the sides of which represent the molar ratio of these 3 parameters. (24) In addition, Metzger *et al*. were able to calculate the extent of gallstone formation from the dissolved status of these 3 parameters in terms of cholesterol SI, (25) and Thomas and Hofmann (18) derived a formula for the calculation of SI. In the present study, the cholesterol SI in hepatic bile decreased after UDCA administration at bedtime and mealtime, which is consistent with the results of studies on gallbladder bile reported to date in western countries. (26-28) It is noteworthy that the SI in hepatic bile was significantly lower for bedtime administration, compared to mealtime administration. Coyne (29) and Maton *et al*. (30) reported that treatment with bile acids induced a decrease in HMG-CoA reductase, a rate determining enzyme in the biosynthesis of cholesterol in the liver, as one of the pharmacological effects of bile acids, that

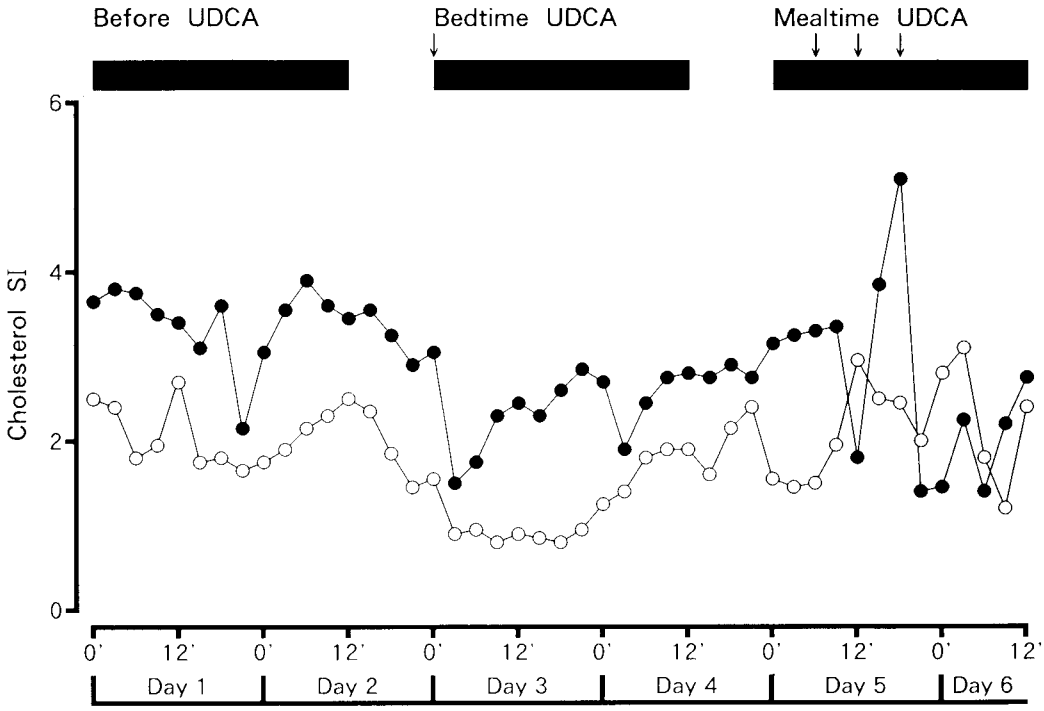

Fig.5. Profiles for cholesterol SI at intervals of 3 h in hepatic bile from 2 subjects (○, patient No.1 ; ●, patient No.4) studied on 6 successive days.

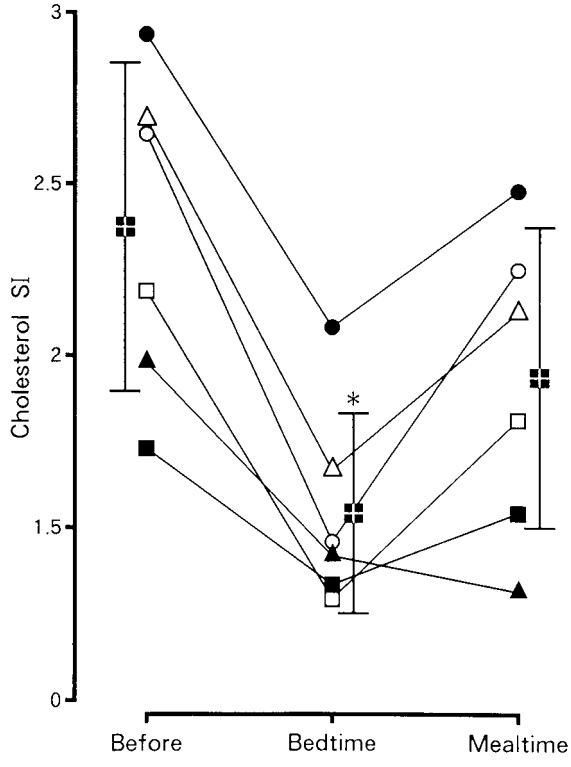

Fig.6. Mean interval values [■, ±SD (⊢⊣)] of cholesterol SI in hepatic bile from 6 subjects before and during UDCA administration at bedtime and mealtime. Bile was collected 13 times at 3 h intervals over a 39 h period before UDCA administration and during UDCA administration at bedtime and mealtime. Cholesterol SI in the collected bile was calculated by the formula established by Thomas and Hofmann. (17). *Significant difference between values before and during UDCA administration ($P<0.05$).
○, patient No.1 ; □, patient No.2 ; △, patient No.3 ; ●, patient No.4 ; ■, patient No.5 ; ▲, patient No.6.

cholesterol excretion in the liver was decreased in patients with cholesterol gallstones, and that this improved the SI and induced litholysis. Moreover, Northfield *et al*. reported that bile acid treatment was more efficient when the administration was daily at bedtime because hepatic bile with a higher SI is secreted at night. (5) Lanzini *et al.* (7) concluded that an increase in biliary cholesterol saturation could be expected as a result of bedtime administration, an observation which could lead to dose reduction. A statistical significance for the decreased cholesterol SI in hepatic bile was observed in this study during UDCA bedtime administration, as compared to mealtime administration, suggesting a decreased cholesterol excretion into hepatic bile. However, it should be noted that cholesterol solubility in hepatic bile is now known to be largely determined by cholesterol-phospholipid vesicles (31, 32). In fact, the cholesterol SI is nearly always in excess of 1 in hepatic bile, indicating that mixed micelles are always supersaturated in a diluted system, such as hepatic bile. These vesicles are then micellized by bile salts as bile becomes concentrated in the bile ducts and further in the gallbladder. Although the cholesterol SI in gallbladder bile is considered to be a lithogenic index in patients with cholesterol gallstone, the significance of the cholesterol SI in hepatic bile in the formation of cholesterol gallstone remains to be elucidated.

Based on these, and other findings together which show a diminished SI in bile, UDCA administration at bedtime is more useful in patients with gallstone and choledocholithiasis both in Japan and western countries. Accordingly, it is reasonable to assume that this effect of bedtime UDCA administration may account not only for its greater potency in comparison with equimolar mealtime UDCA administration but also for the reduction of the minimum effective dose in achieving consistent desaturation of gallbladder bile with cholesterol. In Japan, many patients with gallstone for which litholysis treatment is indicated, have silent stones, which require the intake of bile acids over a prolonged period. For this issue, the problem is how to improve compliance and to support gallstone dissolution with minimum incidences of dose-related side effects. Although no data on the minimum effective dose of UDCA is available at present, compared with administration 3 times per day, a single, daily administration at bedtime appears to be a more effective protocol, since better compliance would be expected for this protocol.

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