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EFFECT OF THE PURIFIED UNSAPONIFIABLE FRACTION OF SOYBEAN IN COMBINATION WITH URSODEOXYCHOLIC ACID ON CHOLESTEROL SATURATION OF BILE AND STONE DISSOLUTION IN GALLSTONE PATIENTS*)

By

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ABSTRACT

The effects of pharmaceutical preparation of the unsaponifiable fraction of soybean (MRG), ursodeoxycholic acid (UDCA) and their combined treatment on lithogenic index (L. I.) and gallstone dissolution were clinically investigated in gallstone patients. When seven patients with cholelithiasis were given UDCA alone at a daily dose of 300 or 400 mg during ca. 5 months, their L. I. were cleary decreased in almost cases and the biliary cholesterol concentration in 6 patients treated by MRG alone also tended to decrease. In another group, four gallstone patients were treated during first ca. one year by UDCA alone at a daily dose of 450 mg, followed by a combined therapy of UDCA (450 mg/day) with MRG (9 g/day) for ave. 6 months. A certain reduction of L. I. through the treatment of UDCA alone was further reinforced by the subsequent simultaneous use of UDCA and MRG. In fourth group, the combined UDCA (300–450 mg/day)-MRG (9 g/day) treatment was performed for 9 patients with gallbladder stones during ca. 4.5 months aiming at the dissolution of gallstones. It must be noted that in three out of nine subjects, the complete dissolution was observed (dissolution percentage 33.3% during ave. 4.5 months) as well as a general decrease of L. I. in most cases of this group. These results indicate that MRG additively or synergistically enhance the clinical potency of UDCA in the dissolution of gallstones

INTRODUCTION

Recentry it is well accepted that ursodeoxycholic acid (UDCA) or chenodeoxycholic acid (CDCA) given to patients with cholelithiasis can induce the dissolution of cholesterol gallstones[1-5]. But generally a fairly long term of drug therapy is needed for the complete dissolution of cholesterol stones in gallbladder. Though the difficulty of gallstone dissolution depends on how large the stones are and on whether they are floating or sunk, a half to two years are generally required for the complete vanishment of gallstones. Accordingly,

*) 梶山梧朗, 川本敏雄, 藤山正道, 丸橋 暉, 三好秋馬：胆石患者におけるウルソデオキシコール酸および大豆純化不鹸化物併用投与の胆汁コレステロール飽和度および胆石溶解に対する効果について

it is clinically of urgent necessity to shorten the duration of therapy for cholelith dissolution. For example, the combination of gallstone dissolution agent with other drugs such as choleretics or hypolipidemic agents has been tried for that purpose. On the other hand, several reports have recently revealed that hypolipidemic agents which clearly decrease the plasma cholesterol level increase reversely cholesterol concentration in bile to aggravate cholelithiasis, which sends out a warning that the clinical means of so-called lipid lowering agents depends on their mode and site of action[6-10].

Since, generally speaking, in patients with cholesterol gallstone the hepatic bile is already supersaturated with cholesterol, what is most important in order to accelerate gallstone dissolution is to lower relative concentration of cholesterol in hepatic bile[11-13]. For this aim, main four factors should be taken into consideration; 1) to increase the amount of bile acids and/or phospholipids in bile. 2) to inhibit hepatic cholesterol biosynthesis. 3) to stimulate cholesterol catabolism in the liver. 4) to decrease cholesterol influx into the liver.

The effective gallstone dissolution by UDCA or CDCA is thought to be due to its abilities to increase directly bile acid pool in enterohepatic circulation and to inhibit cholesterol synthesis in the liver[14,15]. It may be very likely, therefore, to enhance more rapid gallstone dissolution by combination of UDCA or CDCA with those drugs which have the capacity to inhibit intestinal cholesterol absorption or to stimulate cholesterol catabolism in the liver. The purified unsaponifiable fraction of soybean is a representative hypolipidemic drug which has been used clinically for a long time and its main component, phytosterols, has been proved to inhibit intestinal cholesterol absorption[16] and to stimulate hepatic cholesterol oxidation[17]. We report here the results of studies on the effect of the purified unsaponifiable fraction of soybean alone or together with UDCA on fasting duodenal bile saturation and stone dissolution in gallstone patients.

MATERIALS AND METHODS

Materials

Ursodeoxycholic acid (UDCA): Preparation which is aveiable commercially from Tokyo Tanabe Pharmaceutical Co. Ltd. (Tokyo) was used.

Moristerol Granule (MRG): This pharmacon was supplied from Morishita Pharmaceutical Co. Ltd. (Osaka), which contains 3.6 g of the unsaponifiable fraction of soybean, 'Soysterol®", per 9 g of granule. Soysterol® consist of 45% of soybean sterols (SBST), 20% of natural tocopherols, 25% of unsaturated fatty acids and 10% of unknown fraction. SBST, in turn, is composed of 25% campesterol, 30% stigmasterol and 45% β-sitosterol.

Patients and Experimental Design

Experimental groups and design and pertinent feature of each patient are shown in Table 1.

UDCA group

UDCA was administered at a dose of 300 or 400 mg/day during ca. 5 months to 7 patients with cholelithiasis (male 1 and female 6) and biliary lipids before and after treatment were compared.

MRG group

Six patients (male 2 and female 4) ingested a daily dose of 9000 mg of MGR during ca. 4 months and their biliary lipids before and after treatment were determined.

Combined group I

Four patients with cholelithiasis (female) were treated during first ca. one year (8–15 months) by UDCA alone at a daily dose of 450 mg, followed by a combined therapy of UDCA (450 mg/day) with MRG (9 g/day) for ave. 6 months. Changes in biliary lipids during treatment were pursured.

Combined group II

Combined UDCA (300–450 mg/day)–MRG (9 g/day) treatment was performed for 9 patients with gallbladder stones aiming at the dissolution of gallstones during ave. 4.5 months. Biliary lipid composition were also determined.

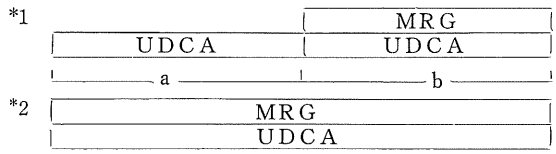
Methods

Duodenal bile was collected at 9 a.m. after overnight fasting and its lipid composition was analyzed instantly by the methods described in previous report[18]. The millimoles concentration of bile, lecithin and cholesterol were converted to the percent of total millimoles concentration constituted by each these components. The lithogenic index was calculated according to Thomas and Hofmann[19]. The presence or absence of stones in gallbladder was observed by both cholecystography and

Table 1. Pertinent Clinical Features of Patients and experimental design

Group	Case No.	Age	Sex	Dose (mg/day)	Therapeutic period (month)	Diagnosis
UDCA	1	63	F	UDCA : 300	7.0	cholelithiasis
	2	43	F	〃	4.0	〃
	3	45	F	〃	5.0	〃
	4	66	F	UDCA : 400	7.0	〃
	5	33	F	〃	3.3	〃
	6	56	F	〃	4.5	〃
	7	47	M	〃	5.0	〃
MRG	8	70	F	MRG : 9000	16.3	cholelithiasis
	9	44	M	〃	14.5	hyperlipidemia
	10	54	M	〃	15.2	〃
	11	51	F	〃	12.0	cholelithiasis
	12		F	〃	11.0	〃
	13	69	F	〃	6.8	〃
Combined group I*1	14	59	F	UDCA : 450, MRG : 9000	a) : 8.3, b) : 4.0	cholelithiasis
	15	43	F	〃 〃	a) : 11.2, b) : 12.2	〃
	16	49	F	〃 〃	b) : 4.3	〃
	17	55	F	〃 〃	a) : 15.3, b) : 3.2	〃
Combined group II*2	18	74	F	UDCA : 400, MRG : 9000	3.5	cholelithiasis
	19	79	F	〃 〃	4.7	〃
	20	67	F	〃 〃	4.5	〃
	21	59	F	UDCA : 450, 〃	4.0	〃
	22	65	F	〃 〃	7.6	〃
	23	42	F	〃 〃	4.3	〃
	24	51	M	〃 〃	3.9	〃
	25	63	F	〃 〃	4.3	〃
	26	48	F	〃 〃	4.1	〃

Protocol of combined groups are as follows.



ultrasonography.

RESULTS

I. Effects on biliary lipid composition and lithogenic index (L. I.).

UDCA group

The relative percentage of biliary lipids of each subject before and after treatment is shown in Table 2 and changes in lithogenic index in Fig. 1. A fair decrease in relative concentration of cholesterol was observed in almost cases, which was shown more clearly in changes of lithogenic index as shown in Fig. 1.

MRG group

Alterations of biliary lipid composition and lithogenic index are shown in Table 3 and Fig. 2, respectively. Except two patients (case No. 12 and 13) who had low concentration of cholesterol and L. I. at starting, there were

Table 2. Biliary lipids before and after UDCA treatment

Case No.	Concentration of Bile lipid					
	before			after		
	CH	PL	TBA	CH	PL	TBA
1	2.1	12.8	83.2	2.0	23.9	74.1
2	8.6	16.5	74.8	6.1	50.2	43.7
3	13.8	28.7	57.5	1.2	49.7	49.2
4	7.5	12.2	80.4	3.4	32.7	63.9
5	8.3	7.9	83.8	2.9	10.1	87.0
6	14.5	36.5	49.1	10.1	33.3	56.7
7	14.1	35.1	50.9	4.6	27.4	68.0
mean ± S.E	9.8 ± 1.7	21.4 ± 4.4	68.5 ± 5.9	4.3* ± 1.1	32.5 ± 5.4	63.2 ± 5.6

Results are given as the relative percentage calculated from bile lipid concentration (μmole/dl).
* : significant at 5% level as compared with value before treatment.

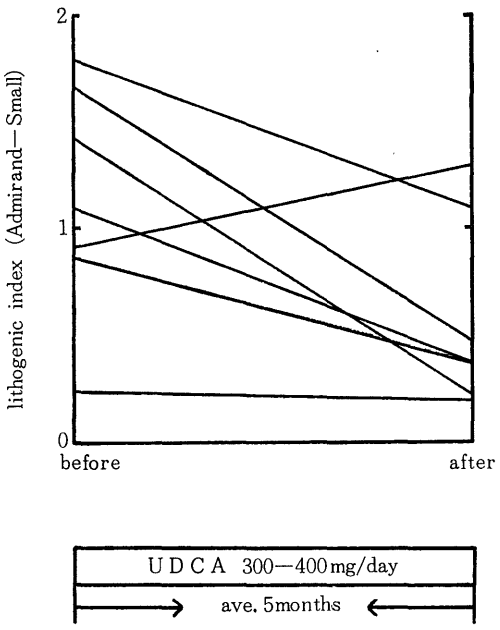


Fig. 1. Changes in lithogenic index of gallstone patients treated with UDCA only

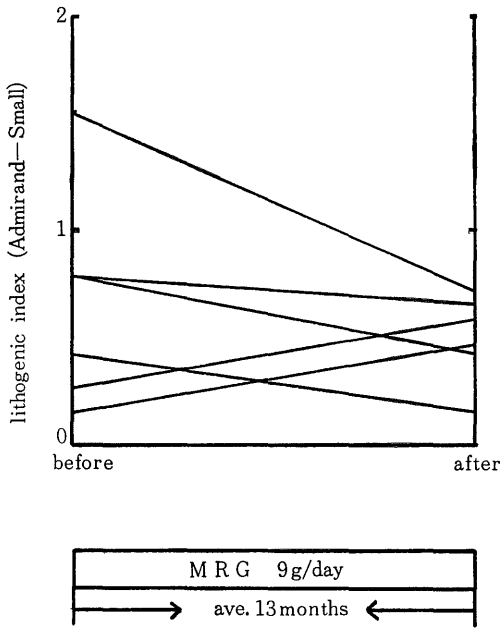


Fig. 2. Changes in lithogenic index of gallstone patients treated with MRG only

observed clear decrease in biliary cholesterol concentration and L. I. which was especially true in case No. 9 who had the largest L. I. at discharge of treatment.

Combined group I

Biliary lipids during treatment with UDCA only and those after the subsequent combined therapy (UDCA+MRG) are shown in Table 4 and changes in L. I. in Fig. 3. For two patients (case No. 16 and 17), biliary lipids were not determined at discharge of treatment by UDCA alone. As seen from the results in case No. 14 and 15, a certain reduction of biliary cholesterol through the treatment of UDCA alone was further reinforced by the simultaneous use of UDCA and MRG, which is seen clearly in Fig. 3.

Table 3. Biliary lipids before and after MRG treatment

Case No.	Concentration of Bile Lipid					
	before			after		
	CH	PL	TBA	CH	PL	TBA
8	6.8	11.4	81.1	2.3	1.0	96.7
9	12.6	40.5	46.9	4.8	4.9	90.3
10	6.0	8.3	85.7	6.3	17.4	76.3
11	2.1	10.9	87.0	5.1	12.1	82.8
12	1.5	18.4	80.1	4.7	21.9	73.4
13	0.4	13.8	85.8	1.3	9.9	88.8
mean ± S.E	4.9 ± 1.9	17.2 ± 4.9	77.8 ± 6.3	4.1 ± 0.8	11.2 ± 3.2	84.7 ± 3.6

Results are given as the relative percentage calculated from bile lipid concentration (μmole/dl).

Table 4. Biliary lipids during treatment with UDCA and MRG (Combined group I)

Case No.	Concentration of Bile Lipid								
	before			middle			after		
	CH	PL	TBA	CH	PL	TBA	CH	PL	TBA
14	16.7	18.2	64.9	16.9	22.6	60.5	7.2	14.5	78.3
15	18.1	25.0	56.9	13.2	24.8	62.0	3.3	8.4	88.2
16	n.d.*	n.d.	n.d.	7.4	16.3	76.5	3.6	11.6	84.8
17	n.d.	n.d.	n.d.	1.0	8.7	90.3	0.7	9.5	89.7
mean ± S.E	17.4 ± 0.7	21.6 ± 3.4	60.9 ± 4.0	9.6 ± 3.5	18.1 ± 3.6	72.3 ± 7.0	3.7 ± 1.3	11.0 ± 1.3	85.2 ± 2.5

Results are given as the percentage calculated from bile lipid concentration (μmole/dl).
* n.d.; not determined.

Combined group II

Changes in biliary lipids and L.I. before and after the conbined treatment of UDCA and MRG are shown in Table 5 and Fig. 4, respectively. In Fig. 4, the three cases in which the complete disolution of gallstones were observed are shown by dotted line. Generally, there were observed a fairly decrease in relative percentage of biliary cholesterol and the higher the pretreatment cholesterol concentration was, the larger decrease in lithogenic index was observed as seen in cases of No. 21, 22 and23.

II. Effect on gallstone dissolution

Gallstone of nine patients in combined group II were closely examined before, during and after treatment by cholecystography and ultrasonography. In three out of nine subjects (case No. 18, 19 and 21), there were observed complete dissolution of gallstones during ave. 4.5 months (dissolution percentage 33.3%). Details of the three effective cases are as follows:

Case No. 18

At starting of therapy, the gallbladder of this patient had been mostly occupied by a large number of gallstone with various sizes as seen in Fig. 5a, which completely disappeared after the combined treatment of UDCA (400 mg/day) and MRG (9 g/day) during four months. Cholecystogram (Fig. 5a) and ultrasonography (Fig. 5c) support the excellent result.

Case No. 19

This patient had a few floating gallstones with a diameter ca. 1 cm, which entirely vanished after the simultaneous use of UDCA (400 mg/day) and MRG (9 g/day) for 4.7 months. No recurrence was observed at 3.3 month after

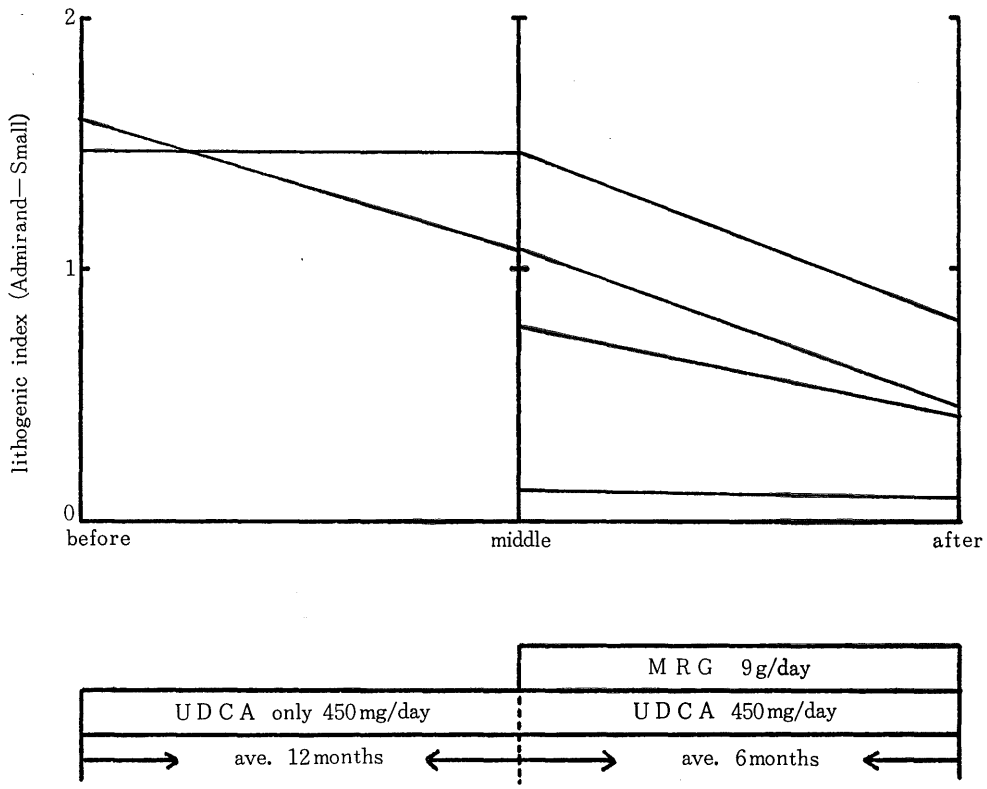


Fig. 3. Changes in lithogenic index of gallstone patients treated with UDCA and MRG (combined group I)

Table 5. Biliary lipids before and after treatment with UDCA and MRG (Combined group II)

Case No.	Concentration of Bile Lipid					
	before			after		
	CH	PL	TBA	CH	PL	TBA
18	1.7	2.9	95.4	0.6	8.2	91.2
19	7.4	8.9	83.7	2.8	18.4	78.8
20	3.6	9.6	86.9	2.9	12.3	84.8
21	16.9	22.6	60.5	7.2	14.5	78.3
22	14.1	11.5	79.5	0.3	0.6	99.1
23	13.2	12.0	74.6	5.4	10.4	84.2
24	2.2	13.2	84.6	2.9	7.3	89.9
25	5.6	2.3	92.2	1.8	5.9	92.3
26	6.9	8.6	84.5	8.5	9.6	81.9
mean ± S.E	8.0 ± 1.8	10.2 ± 2.0	82.4 ± 3.4	3.6 ± 0.9	9.7 ± 1.7	86.7 ± 2.3

Results are given as the relative percentage calculated from bile lipid concentration (μmole/dl).

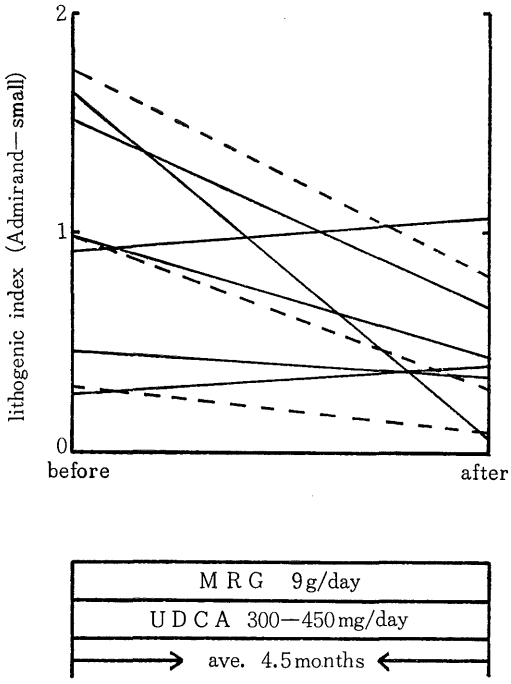


Fig. 4. Changes in lithogenic index of gallstone patients treated with UDCA and MRG (combined group II)

a vanishment of stones. Fig. 6c and 6b show cholecystograms before and after treatment, respectively. Ultrasonograms at pretreatment and 8 months after therapy, namely ca. 3 months after the cure, are given in Fig. 6c.

Case No. 21

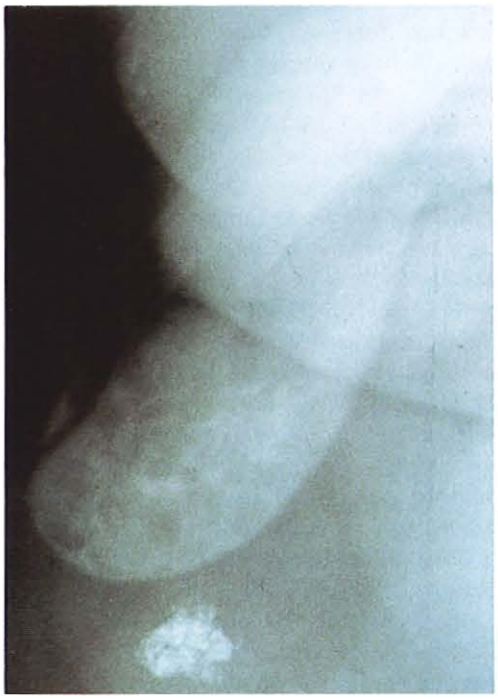
In this case, at discharge of treatment a few floating gallstones with a diameter ca. 7 mm were observed as shown in Fig. 7a, which was thoroughly disappeared after the combined therapy of UDCA (450 mg/day) and MRG (9 g/day) during four months and no recurrence was found even after another four months under same treatment. Cholecystogram (Fig. 7b) and ultrasonograms (Fig. 7c) proved the satisfactory progress.

DISCUSSION

The most reliable parameter to predict a possibility of gallstone dissolution is thought to be lithogenic index (L. I.), a quantitative representation of the cholesterol saturation in bile. L. I. can be calculated by a few different methods. In this report L. I. was computed according to Thomas et al. (Holzbach index)[9].

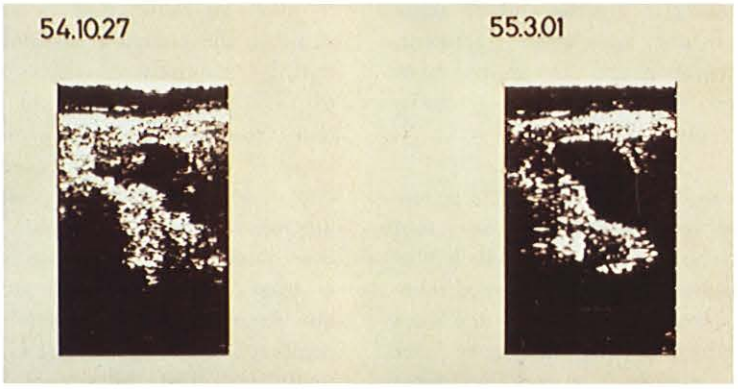
UDCA used clinicaly as a representative choleletic for long time have been recently found to have another activity to dissolve gallstone[4,5]. In present study, too, the effective decrease in L. I. by UDCA was clearly observed as shown in Fig. 1 when administered to patients with gallstones. On the other hand, effect of MRG alone on L. I. was not so pronounced and this may be because of the low L. I. of patients in this group at starting of therapy. In the case No. 9 which had the highest L. I. in this group at discharge of treatment, however, the supersaturated state of bile was clearly improved by MRG alone, which indicate that MRG might also affect favourably the gallstone dissolution. In the combined group I four gallstone patients were treated by UDCA alone for one year but their gallstones were remained undissolved and then they received the combined therapy by UDCA and MRG during about 6 months with a consequence that the combined UDCA-MRG treatment clearly accelerated a decrease in L. I.. To make sure of the combined effect of UDCA with MRG, nine patients with gallstone were subjected to the treatment by UDCA plus MRG (combined group II). It must be noted that in three among nine subjects, the complete dissolution was observed during 4. 5 months as well as a general decrease of L. I. on most cases. In the two effective cases the gallstones were floating but fairly large (7-10 mm) and another effective patient had a large number of stones in gallbladder, among which some might have a diameter over 1 cm and others must be sunk. It could at least be thought that those stones would not dissolve in short term. Nevertheless, the combined treatment of UDCA plus MRG actually dissolved them completely during 4. 5 months (dissolution percentage 33. 3%). It is first time in our clinical experiences that such large number of fair-sized gallstones disappeared entirely in appreciably high percentage during so short term.

For reference, a typical example of gallstone dissolution by UDCA treatment recently observed in this hospital are photographically shown in Fig. 8. This case required two and half years for the complete dissolution by a daily dose of 300 mg of UDCA. Furthermore, the relationship between the duration of therapy and the dissolution percentage of gallstone was



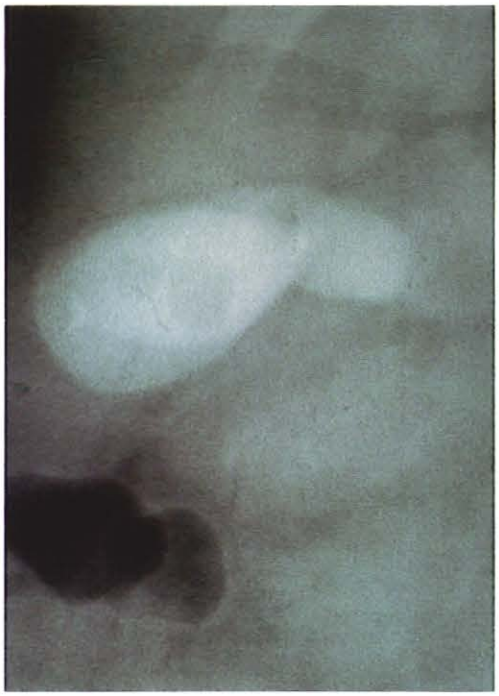
a) : Cholecystogram before treatment

b) : Cholecystogram after treatment

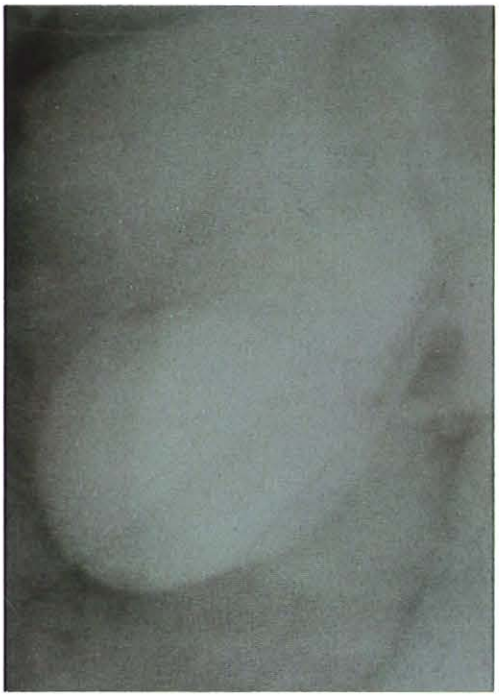


c) : ultrasonograms before (left) and after treatment

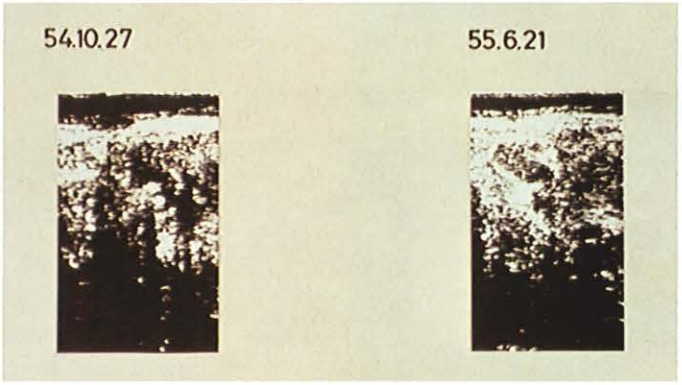
Fig. 5. Cholecystograms and ultrasonograms in case No. 18



a) : Cholecystogram before treatment

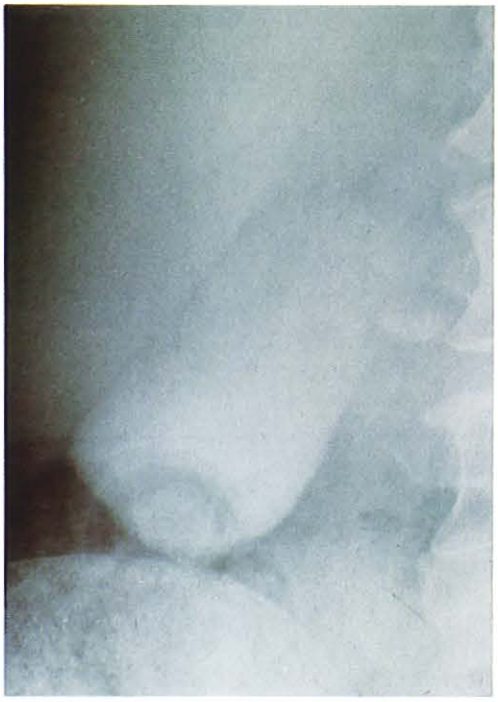


b) : Cholecystogram after treatment

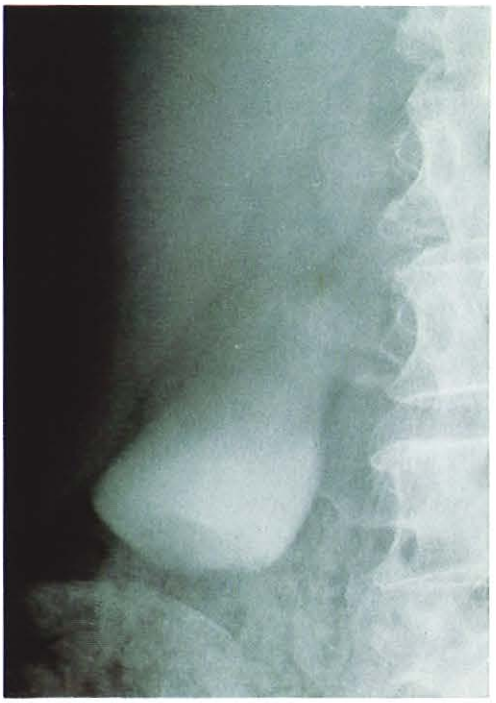


c) : Ultrasonograms before (left) and 8 months after treatment

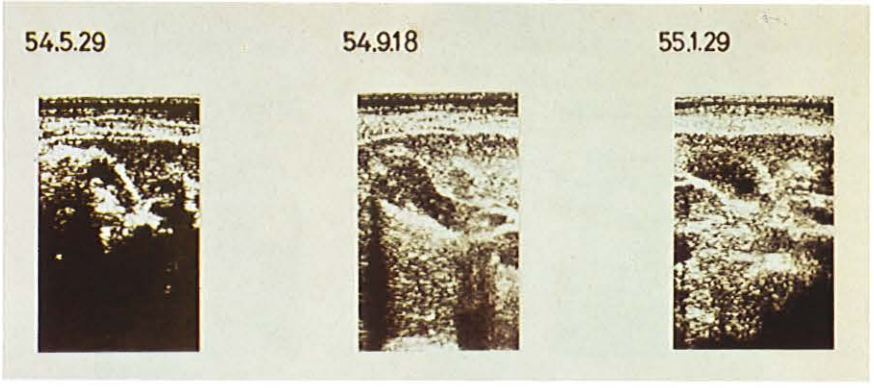
Fig. 6. Cholecystograms and ultrasonograms in case No. 19



a) : Cholecystogram before treatment



b) : Cholecystogram after treatment



c) : Ultrasonograms before (left), 4 months after (center) and 8 months after treatment

Fig. 7. Cholecystograms and ultrasonograms in case No. 21

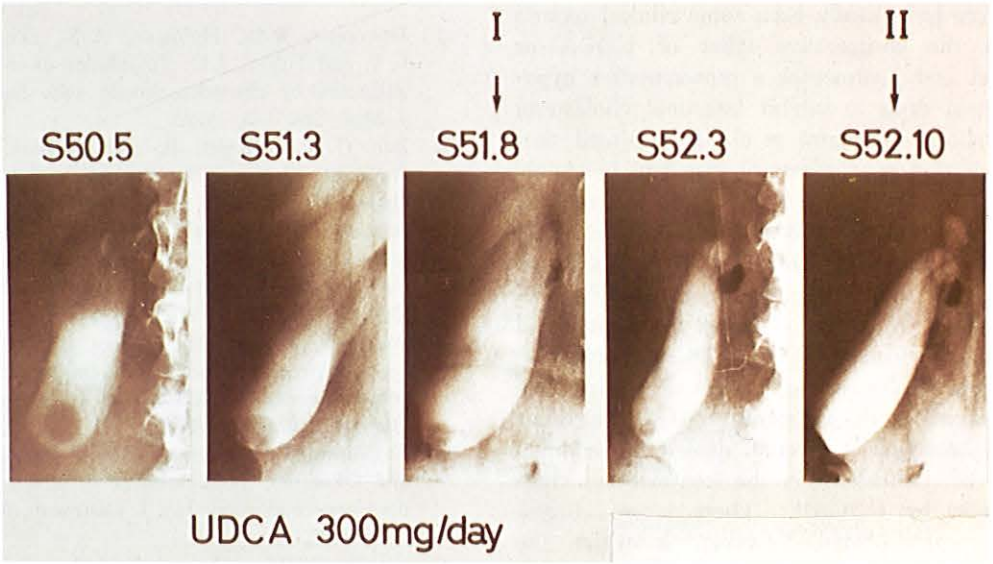


Fig. 8. An example of gallstone dissolution by UDCA treatment during 2.5 years.
I ; partial dissolution. II ; complete dissolution

derived from the data obtained so far in our department. This is shown in Table 6, which indicates that the dissolution percentage strongly depends on the therapeutic period; 23.8% of effective percentage during 0.5 to 1.0 years treatment, 44.0% during 1.0 to 1.5 years and 66.6% during more than 1.5 years. From these data, the tentative dissolution percentage at 4.5 months after treatment would be extrapolated to about 10%, which is far less than that obtained actually in this combined therapy. In addition, others reported the relationship between the dose and the complete dissolution percentage of gallstone with UDCA alone; 26.6% of complete dissolution percentage at a dose of 450 mg/day after 6 months[20] and 24.1% at a dose of 600 mg/day after 9 months or more[21]. In contrast with these data, the dissolution percentage (33.3%) obtained in this combined therapy of UDCA (400-450 mg/day) and MRG during ca. 4.5 months may be excellently high. From these comparative inspection, it may safely be said that MRG additively or synergisticaly enhance the potency of UDCA in the dissolu-

Table 6. The relation between therapeutic period and dissolution percentage in gallstone patients treated with UDCA

	therapeutic period (month)		
	6-12	12-18	18<
	No of patients		
complete dissolution	12	14	19
partial dissolution	3 } 15	8 } 22	9 } 28
ineffectiveness	48	28	14
total	63	50	42
percentage of effectiveness	23.8%	44.0%	66.6%

tion of gallstone.

There has already been some clinical reports about the co-operative effect of UDCA or CDCA and β-sitosterol, a representative hypolipidemic drug to inhibit intestinal cholesterol absorption. Gerolami et al. has claimed that the addition of β-sitosterol (3 g/day) to chenic acid (1 g/day) resulted in a greater rate of gallstone dissolution (30.8% of dissolution percentage during 6.5 months) than that achieved by CDCA alone in gallstone patients[22]. According to Tangedahl T. N. et al. plant sterol increased effectiveness of CDCA therapy in lowering cholesterol saturation of fasting bile in patients with gallstones[23]. On the other hand, Maudgal D. P. et al. denied the additive effect of β-sitosterol in the treatment of cholelithiasis by CDCA[24]. There is no clinical experiment hitherto, however, in which the combination use of hypolipidemic agents with UDCA has been tried in treatment of cholelithiasis.

Although we can not definitely conclude about the mechanism of synergistic effect of MRG in gallstone dissolution by UDCA, soybean sterols, the main active fraction in MRG, has been proved to inhibit specifically the intestinal cholesterol absorption[16] with a consequence of a decrease in the amounts of cholesterol coming into the liver, which may surely affect the hepatic bile components in favour of gallstone dissolution. The amounts of soybean sterols contained in daily dose of MRG (9 g/day), however, are only ca. 1.6 g, which is about one-second of the dose of β-sitosterol used in the clinical experiments[22-24] above mentioned. Nevertheless, MRG seems to have derived better results in gallstone dissolution. Other components in MRG such as tocopherols or/and unsaturated fatty acids may play additive roles in the therapy of cholelithiasis. To clarify the degree of effectiveness and the mode of actions of MRG in gallstone dissolution, several animal experiments have been done by us and co-workers, some parts of which had been already reported elsewhere[25-27]. Although some problems about MRG in relation with gallstone dissolution are still remained to be solved through clinical and animal experiments, it can only be said that a favorable influence of Moristerol upon cholelithiasis and its high safety further expand the range of its clinical utility.

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