Metabolism of 3,7-Dioxo-5 β -cholanoic Acid in the Biliary Fistula Rodents and Rabbits*

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Intestinal absorption and metabolism of 3,7-dioxo-5 β -cholanoic acid, were studied in the bile fistula rats, hamsters, guinea-pigs and rabbits. The influence of dose (1 and 100 mg/kg) on the absorption and the metabolism was also estimated. The dioxo bile acid was absorbed efficiently from the intestine and quickly excreted into bile in these animals. Large dose did not retard the absorption rate and showed a significant choleretic effect for a few hours. Species differences were observed in the metabolism of this compound. In hamsters and guinea-pigs, most of the metabolites in the bile were conjugated with either taurine or glycine. The proportion of bile acids amidated with glycine was greater with the large dose. In rats, the biliary metabolites were conjugated with taurine, but not with glycine, whereas in rabbits, glycine conjugates were the only recovered metabolites. Unconjugated metabolites were also detected in the bile of the rodents, and the proportion of them rose to 17—29% after the administration of 100 mg/kg quantities. A small part of unchanged 3,7-dioxo-5 β -cholanoic acid was excreted into the bile as both the conjugated and unconjugated forms in these animals. The greater part of this compound administered was metabolized to 7-ketolithocholic acid, chenodeoxycholic acid and ursodeoxycholic acid. In hamsters and guinea-pigs, chenodeoxycholic acid was a greater metabolite of this compound than ursodeoxycholic acid, while in rats and rabbits, the amount of ursodeoxycholic acid exceeded that of chenodeoxycholic acid. In rats, the resulting dihydroxy bile acids were further metabolized to α - and β -muricholic acids.

Keywords — intestinal absorption; metabolism; 3,7-dioxo-5 β -cholanoic acid; choleretic effect; rodent; rabbit

Introduction

3,7-Dioxo-5 β -cholanoic acid (3,7-DOCA) is the 3,7-diketo-derivative of both chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) which are established as cholesterol gallstone dissolving agents.¹⁻⁴⁾ Recent studies suggest that the administration of 7-ketolithocholic acid (7-KLCA), the 7-dehydro derivative of both CDCA and UDCA, may be effective in dissolving cholesterol gallstones.⁵⁾ Therefore, we supposed that 3,7-DOCA might be an effective gallstone dissolving agent.

The chemical structure of 3,7-DOCA resembles dehydrocholic acid, which is the 3,7,12-tridehydro derivative of the naturally occurring primary bile acid, cholic acid. Because

dehydrocholic acid is a nonmicelle-forming bile acid and causes a great choleretic effect,^{6,7)} we reasoned that 3,7-DOCA might, therefore, be an effective choleretic agent.

Prior to test 3,7-DOCA as a therapeutic agent, we carried out preliminary experiments to estimate the intestinal absorption, secretion into bile and hepatic biotransformation of this compound in four different experimental animals, rats, hamsters, guinea-pigs and rabbits.

Materials and Methods

Unlabeled Compounds — 3,7-DOCA was prepared from CDCA as follows. To a solution of CDCA (5.0 g) dissolved in 100 ml of acetone a solution of chromic anhydride (4.0 g) dissolved

* Abbreviations: chenodeoxycholic acid, 3 α ,7 α -dihydroxy-5 β -cholanoic acid; ursodeoxycholic acid, 3 α ,7 β -dihydroxy-5 β -cholanoic acid; 7-ketolithocholic acid, 3 α -hydroxy-7-oxo-5 β -cholanoic acid; cholic acid, 3 α ,7 α ,12 α -trihydroxy-5 β -cholanoic acid; α -muricholic acid, 3 α ,6 β ,7 α -trihydroxy-5 β -cholanoic acid; β -muricholic acid, 3 α ,6 β ,7 β -trihydroxy-5 β -cholanoic acid; lithocholic acid, 3 α -hydroxy-5 β -cholanoic acid; dehydrocholic acid, 3,7,12-trioxo-5 β -cholanoic acid.

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in a mixture of conc. H_2SO_4 (3.5 ml) and water (15 ml) was added with stirring. The reaction mixture was allowed to stand at room temperature for 20 min. Isopropyl alcohol (10 ml) was added to degrade an excess of the oxidant. The reaction mixture was neutralized with 5% NaHCO_3 solution. The solution was filtered and the filtrate was acidified with dil. HCl and extracted with ethyl ether. The ethyl ether extract was washed with water until neutral and the solvent was evaporated off to give a residue (4.8 g). Crystallization from ethyl ether of the residue yielded crystals (3.9 g) of 3,7-DOCA, mp 157–158 °C [reported mp 158 °C].⁸⁾ Proton nuclear magnetic resonance (^1H -NMR, pyridine- d_5): 0.63 (3H, s, 18- CH_3), 0.92 (3H, d, $J = 6.04$ Hz, 21- CH_3), 1.19 (3H, s, 19- CH_3). Mass spectrum (as methyl ester): m/z (relative intensity) 402 (13), 384 (9), 370 (15), 353 (13), 329 (22), 293 (30), 100 (23), 49 (11).

Lithocholic acid, CDCA, UDCA, and cholic acid were purchased from Sigma Chemical Co. 7-KLCA,⁹⁾ α -muricholic acid (α -MC),⁹⁾ β -muricholic acid (β -MC),⁹⁾ 7 α -hydroxy-3-oxo-5 β -cholanoic acid,¹⁰⁾ 7 β -hydroxy-3-oxo-5 β -cholanoic acid,¹⁰⁾ 3 β ,7 α -dihydroxy-5 β -cholanoic acid¹¹⁾ and 3 β ,7 β -dihydroxy-5 β -cholanoic acid¹²⁾ were synthesized in this laboratory according to the reported methods.

Labeled Compounds — [$24\text{-}^{14}\text{C}$]CDCA (4.70 MBq/mg) was purchased from NEN Products (Boston, MA). [$24\text{-}^{14}\text{C}$]3,7-DOCA was prepared from [$24\text{-}^{14}\text{C}$]CDCA as follows: To a solution of the labeled CDCA (0.24 mg, 1.13 MBq) dissolved in 10 ml of acetone, a solution of chromic anhydride (2.5 mg) dissolved in 0.3 ml of conc. H_2SO_4 and 1 ml of water was added and the reaction mixture was allowed to stand at room temperature for 20 min. The product was extracted in the same manner described above for the preparation of unlabeled 3,7-DOCA and chromatographed on an octadecyl silica gel column (15 cm $\times$ 22 mm i.d., Kusano Scientific Co., Tokyo, Japan). Elution with 85% methanol yielded 0.18 mg, 833 kBq (4.63 MBq/mg) of [$24\text{-}^{14}\text{C}$]3,7-DOCA. The purity of the labeled compound was better than 98% as determined by radio-thin-layer chromatography (radio-TLC) with solvent system 2

(described below).

Experimental Animals — Male Wistar rats weighing 200–205 g, male golden Syrian hamsters weighing 100–110 g, male guinea-pigs weighing 245–255 g, and white male Japanese rabbits weighing 1.9–2.0 kg were purchased from Shizuoka Laboratory Animal Center, Shizuoka, Japan. The animals of each species were maintained for at least 14 days on a standard commercial pellet diet (Oriental Kobo Co., Tokyo, Japan; MF for rats and hamsters, RC4 for guinea-pigs and rabbits). The animals were given food and water *ad libitum* and maintained under controlled illumination; the light period was from 6 a.m. to 6 p.m.

Experimental Design — All animals were operated on between 10 a.m. and 11 a.m. Each animal was anesthetized with sodium pentobarbital and ethyl ether, and the bile duct was cannulated with a polyethylene cannula (PE 10 with inside diameter 0.28 mm for hamsters, PE 50 with inside diameter 0.58 mm for rats, guinea-pigs and rabbits). A solution (1.0 ml) of [$24\text{-}^{14}\text{C}$]3,7-DOCA, which was prepared by adding unlabeled 3,7-DOCA to labeled 3,7-DOCA (18.5 KBq, 4 μg), 1 mg or 100 mg/kg (2.57 or 257 $\mu\text{mol/kg}$), was introduced directly into the duodenum of each bile fistula animal as sodium salt. The animals were placed in restraining cages, and had free access to food and water. Bile was collected and their hourly volume measured for a total period of 48 h. An aliquot of the bile sample was removed for radioactivity determination. Second and third aliquots were used for the analysis of chemical forms of the biliary radioactive compounds before and after enzymatic hydrolysis. Urine was also collected for 48 h and an aliquot of the urine sample was removed for radioactivity determination.

Determination of Radioactivity — These were carried out in a Packard TriCarb Model 3255 scintillation spectrometer using Aquasol-2 (NEN Products). Corrections were made for background and quenching.

Radio-TLC — Silica gel 60 F₂₅₄ precoated TLC aluminum sheets (0.2 mm thickness; Merck, Darmstadt, F.R.G.) were used for thin-layer chromatographic analysis. The solvent systems were as follows: system 1 (for conjugated

bile acids), ethyl acetate–acetic acid–water 70:20:10, v/v/v; system 2 (for free bile acids), isooctane–ethyl acetate–acetic acid 5:5:1, v/v/v; system 3 (for free bile acids), benzene–isopropanol–acetic acid 30:10:1, v/v/v; system 4 (for free bile acids), isooctane–isopropanol–acetic acid 30:10:1, v/v/v. Biological samples were applied to the plates as a streak (3–4 cm). Reference bile acids were applied as spots at each side. After development (17 cm), the reference bile acids were visualized by spraying the plates with 50% H₂SO₄ solution followed by heating at 120 °C for 1 min. To localize radioactivity in the developed plates each TLC plate was cut into 7 mm segments from the origin to the solvent front and each segment was put into a scintillation vial together with scintillation solution to determine radioactivity.

Enzymatic Hydrolysis — Samples to be hydrolyzed were dissolved in a mixture of 2.0 ml of sodium acetate buffer (0.025 M, pH 5.6), 0.2 ml of 0.05 M ethylenediamine tetraacetic acid disodium salt solution, and 10 U of cholyglycine hydrolase from *Clostridium perfringens* (Sigma Chemical, St. Louis, Mo.). The mixture

was incubated at 37°C. for 18 h. The reaction mixture was passed through a Sep-Pak C₁₈ cartridge (Millipore Co. Waters Chromatography Div., Milford, MA.). After washing the cartridge with 5 ml of water, bile acids were eluted with 5 ml of methanol.

Results

Cumulative biliary excretion of radioactivity after intraduodenal injection of 1 or 100 mg/kg of [24-¹⁴C]3,7-DOCA to bile fistula animals is shown in Fig. 1. After the administration of a small quantity (1 mg/kg), of the labeled compound, 81, 88, 94 and 97% of the administered radioactivity was excreted into the bile within 24 h, in rats, hamsters, guinea-pigs and rabbits, respectively. When a large quantity (100 mg/kg), of this bile acid was administered to rats and rabbits, the results were almost the same as those obtained in the low dose experiments. In hamsters and guinea-pigs which were given the large quantities of [24-¹⁴C]3,7-DOCA, the radioactivity appeared somewhat more rapidly in the bile rather than that in the small dose experiments.

Fig. 1. Biliary Excretion of Radioactivity in Bile Fistula Animals after Intraduodenal Injection of [24-¹⁴C]3,7-DOCA ▲, 1 mg/kg; ●, 100 mg/kg. Each value represents the mean of 3 animals.

Fig. 2. Bile Flow and Biliary Excretion of Radioactivity in Bile Fistula Animals after Intraduodenal Injection of $[24-^{14}\text{C}]3,7\text{-DOCA}$ (100 mg/kg)

Open bars, bile flow; hatched bars, recovered radioactivity. Each value represents the mean of 3 animals.

The recovered radioactivity in the urine was less than 0.5% of the total activity administered in all the animals experimented.

In order to investigate the choleretic effect of 3,7-DOCA, bile flow after intraduodenal injection of this material was measured (Fig. 2). In the large dose groups in all species of the animals experimented, the bile flows increased for a few

hours after the injection.

The conjugation profiles of the radioactive compounds excreted into the bile of the bile fistula animals which were given $[24-^{14}\text{C}]3,7\text{-DOCA}$ were analyzed by means of radio-TLC using solvent system 1. The results are shown in Table I.

Rats — When the small quantity of $[24-^{14}\text{C}]3,7\text{-DOCA}$ was administered, 98% of the

TABLE I. Distribution of Radioactivity in the Biliary Bile Acids of the Bile Fistula Animals after Intraduodenal Injection of $[24-^{14}\text{C}]3,7\text{-DOCA}$

Species	Dose (mg/kg)	Bile acid composition (% of total) ^{a)}					Glycine/taurine ratio
		TT	TD	GT	GD	UA	
Rat	1	43	55	ND ^{b)}	ND	2	—
	100	12	59	ND	ND	29	—
Hamster	1	2	59	1	35	3	0.59
	100	2	31	7	43	17	1.52
Guinea-pig	1	ND	65	ND	23	12	0.35
	100	ND	17	ND	63	20	3.71
Rabbit	1	ND	ND	ND	100	ND	—
	100	ND	ND	ND	100	ND	—

Each value represents the mean of 3 animals. ^{a)} TT, fraction of taurotrihydroxycholanoates; TD, fraction of taurodi-hydroxycholanoates; GT, fraction of glycotrihydroxycholanoates; GD, fraction of glycodihydroxycholanoates; UA, fraction of unconjugated bile acids. ^{b)} ND, not detected. In solvent system 1: *R_f* value of TC = 0.18; TD = 0.32; GT = 0.55; GD = 0.78; UA > 0.90.

biliary radioactivity was detected in the fractions of taurine-conjugates; taurodihydroxycholanoates (55%) and taurotrihydroxycholanoates (43%), while 2% of the radioactivity was found in the fraction of unconjugated bile acids. In the large dose experiments, the radioactivity found in the unconjugated bile acid fraction increased to 29% and the radioactivity in the taurotrihydroxycholanoate fraction decreased to 12%. No radioactivity was detected in glycine-conjugated bile acid fractions in both the large and small dose experiments.

Hamsters — When the small quantity of the labeled 3,7-DOCA was administered, most of the recovered radioactivity was detected in the fractions of taurine-(59%) and glycine-(35%) conjugates of dihydroxycholanoates. In the large dose experiments, the radioactivity found in the amidated dihydroxycholanoate fractions decreased to 74%, while the activity in the unconjugated bile acid fraction increased to 17%. The ratio of glycine- to taurine-conjugated bile acids (G/T ratio) increased from 0.59 to 1.52, when the dose was increased from 1 to 100 mg/kg.

Guinea-Pigs — When [24-¹⁴C]3,7-DOCA was injected intraduodenally at the low dose, 88% of the recovered radioactivity was detected in taurine-(65%) and glycine-(23%) conjugates of dihydroxycholanoates, and the remainder (12%) was found in the fraction of unconjugated bile acids. In the large dose experiments, the radioactivity found in the unconjugated bile acid fraction increased to 20%. G/T ratio increased from 0.35 to 3.71 when the dose was increased

from 1 to 100 mg/kg.

Rabbits — In both the large and small dose experiments, the biliary radioactivity was detected only in the fraction of glycodihydroxycholanoates.

Radioactive unconjugated bile acids were detected in the bile of rats, hamsters and guinea-pigs. The labeled unconjugated bile acids in the bile of these rodents were analysed by means of radio-TLC using solvent system 2. The results are shown in Table II.

Rats — When the small quantity of [24-¹⁴C]3,7-DOCA was administered, the major radioactive compound in the fraction of unconjugated bile acids was 7-KLCA (44%). However in the large dose experiments, 3,7-DOCA (55%) was predominant in the fraction of unconjugated bile acids.

Hamsters — In both the small and large dose experiments, the major radioactive compound in the fraction of unconjugated bile acids was identified as 3,7-DOCA.

Guinea-Pigs — The major labeled compound in the unconjugated bile acid fraction was 7-KLCA, in both the large and small dose experiments.

The labeled compounds obtained after the enzymatic hydrolysis of the bile from the animals which were given the labeled 3,7-DOCA were analyzed by means of radio-TLC using solvent systems 2—4. The results are shown in Table III.

Rats — When the small quantity (1 mg/kg) of 3,7-DOCA was administered, the major metabolites had *R_f* values consistent with those of 7-KLCA (31%), UDCA (22%) and β -MC

TABLE II. Distribution of Radioactivity in the Biliary Unconjugated Bile Acids of the Bile Fistula Animals after Intraduodenal Injection of [24-¹⁴C]3,7-DOCA

Species	Dose (mg/kg)	Bile acid composition (% of total)			
		3,7-DOCA	7-KLCA	CDCA	UDCA
Rat	1	28	44	10	18
	100	55	27	6	12
Hamster	1	67	17	12	4
	100	77	16	5	2
Guinea-pig	1	10	62	20	8
	100	18	58	17	7

Bile samples were analyzed by TLC with solvent system 2. Each value represents the mean of 3 animals. In solvent system 2: *R_f* value of 3,7-DOCA = 0.64; 7-KLCA = 0.48; CDCA = 0.43; UDCA = 0.39.

TABLE III. Distribution of Radioactivity in the Hydrolysate of the Biliary Bile Acids of the Bile Fistula Animals after Intraduodenal Injection of [24-¹⁴C]3,7-DOCA

Species	Dose (mg/kg)	Bile acid composition (% of total)					
		3,7-DOCA	7-KLCA	CDCA	UDCA	β -MC	α -MC
Rat	1	ND ^{a)}	31	4	22	40	3
	100	24	37	4	23	11	1
Hamster	1	2	35	56	6	1	ND
	100	27	27	34	6	6	ND
Guinea-pig	1	1	88	8	3	ND	ND
	100	4	77	14	5	ND	ND
Rabbit	1	1	49	14	36	ND	ND
	100	3	51	12	34	ND	ND

Bile samples were analyzed by TLC with solvent systems 2 and 3. Each value represents the mean of 3 animals. *a)* ND, not detected. In solvent system 2: *Rf* value of 3,7-DOCA = 0.64; 7-KLCA = 0.48; CDCA = 0.43; UDCA = 0.39. In solvent system 3: *Rf* value of α -MC = 0.39; β -MC = 0.43; CDCA = 0.70; UDCA = 0.71.

(40%). CDCA (4%) and α -MC (3%) were also detected as the minor metabolites. In the large dose experiments, considerable amounts (24%) of unchanged 3,7-DOCA were excreted into the bile and the formation of radioactive β -MC decreased to 11%.

Hamsters — The major metabolites in the small dose experiments were CDCA (56%) and 7-KLCA (35%). When the dose was increased from 1 to 100 mg/kg, the proportion of 3,7-DOCA increased from 2% to 27%.

Guinea-Pigs — In both the large and small dose experiments, the major metabolite in the bile was identified as 7-KLCA. Lesser amounts of CDCA and UDCA were also found. Unchanged 3,7-DOCA amounted to only 1% and 4% of the recovered activity, in the small and large dose experiments, respectively.

Rabbits — In the small dose experiments, the biliary radioactivity was composed of 7-KLCA (49%), UDCA (36%) and CDCA (14%). The increased dose did not exert significant influence on the biotransformation of 3,7-DOCA.

In all species of the animals experimented, after hydrolysis of the bile by cholyglycine hydrolase, radioactive metabolites with *Rf* values corresponding to sulfated or glucuronidated bile acids were not found. Neither radioactive 3-oxo, 7-hydroxy bile acids (7 α -hydroxy-3-oxo-5 β -cholanoic acid, *Rf* value = 0.64 in solvent system 4; 7 β -hydroxy-3-oxo-5 β -cholanoic acid, *Rf*

value = 0.46 in solvent system 4) nor 3 β ,7 α -dihydroxy-5 β -cholanoic acid (*Rf* value = 0.51 in solvent system 4) were detected in any of the animals.

Discussion

The intestinal absorption, hepatic uptake and biliary excretion of CDCA and UDCA have been well studied. However, no information is available concerning such behaviours of 3,7-DOCA, the diketo-derivative of these naturally occurring bile acids. In this study we have demonstrated that 3,7-DOCA was absorbed efficiently from the intestine, was processed by the liver, and was excreted quickly into the bile in all the animals experimented, rats, hamsters, guinea-pigs and rabbits. No significant differences in the absorption rate of 3,7-DOCA were observed between the small and large dose groups, although in hamsters and guinea-pigs the large dose recovered somewhat more rapidly than the small dose. Since nonionic diffusion played a significant or even predominant role in the intestinal absorption of unconjugated bile acids,¹³⁾ we consider that this unconjugated bile acid was absorbed mainly by passive nonionic diffusion in the intestine.

The present study showed that the administration of a large quantity of 3,7-DOCA caused considerable choleresis for a while after the intraduodenal administration in all the animals

tested. It is, however, not clear whether the choleric effect of 3,7-DOCA is due to the administered compound itself or not, because the dioxo bile acid is transformed into several metabolites during its hepatic passage. When 100 mg/kg of [24- 14 C]3,7-DOCA was administered to rats, hamsters and guinea-pigs, considerable amounts of the radioactivity were found in the fraction of unconjugated bile acids. This fact may have relation to the choleric effect. The bile flow in the isolated perfused rat or hamster liver after bolus injection of unconjugated dihydroxy bile acids correlates with the presence of unconjugated dihydroxy bile acids in the bile.¹⁴ The choleric effects of unconjugated dihydroxy bile acids were greater than those of conjugated dihydroxy bile acids.^{15,16} In the present study, 3,7-DOCA was the main component of the unconjugated bile acid fraction. Relatively lesser percentages of amidated 3,7-DOCA

were found in these species. The results suggest that the hepatic conjugation of 3,7-DOCA is less efficient than that of other bile acids in these rodents.

The present results showed that in hamsters and guinea-pigs, 3,7-DOCA and its metabolites were conjugated with either taurine or glycine. The G/T ratio increased in the large dose experiments. This may be explained as the exhaustion of the taurine pool in the liver. When the large quantity of 3,7-DOCA was administered to these species, glycine was mainly used for conjugation. Previous studies from other laboratories have shown that in situations characterized by increased conjugation requirement, such as during bile acid therapy,¹⁷ cholestyramine feeding¹⁸ and external biliary drainage,¹⁹ glycine conjugation increases because of an abundant source of glycine precursors whereas taurine conjugation remains constant. In rats no radi-

Fig. 3. Possible Metabolic Pathways of 3,7-DOCA

I, 3,7-DOCA; II, 7-KLCA; III, CDCA; IV, UDCA; V, α -MC; VI, β -MC.

oactivity was found in the fractions of glycine conjugated bile acids even in the dose of 100 mg/kg of [24-¹⁴C]3,7-DOCA. Hoshita *et al.* reported that when 500 mg/kg of UDCA was administered to bile fistula rats, more than 90% of the administered bile acid was recovered as the taurine conjugate in the bile.²⁰⁾ These results indicate that rat liver has a quite large pool of taurine. In contrast to these three species, in rabbits, all the labeled bile acids secreted in the bile were completely conjugated with glycine but not taurine. It has been found that the enzyme system for the conjugation in the rabbit liver has a high affinity for glycine and a poor affinity for taurine.²¹⁾

Figure 3 shows possible metabolic pathways of 3,7-DOCA (I). In rats, the composition of the biliary metabolites of 3,7-DOCA (I) resembles that of 7-KLCA (II) reported elsewhere.^{22,23)} In all the animals experimented, considerable amounts of labeled 7-KLCA (II) were excreted into the bile, whereas 3-oxo, 7-hydroxy bile acids were not detected. These results suggest that the reduction of 3-oxo group to 3-hydroxy group would precede the reduction of 7-oxo group in 3,7-DOCA. The 3-oxo group was reduced exclusively to 3 α -hydroxy group. Therefore, we suppose that a small part of unchanged 3,7-DOCA was excreted into bile as both the amidated and unconjugated forms, and the greater part of this compound administered was converted into 7-KLCA (II) by reduction of its 3-oxo group. A part of the resulting 7-KLCA (II) was secreted into the bile as the conjugated and unconjugated forms. The other 7-KLCA (II) was reduced to form either CDCA (III) or UDCA (IV). In hamsters and guinea-pigs, the reduction was favoring the 7 α -hydroxy epimer, CDCA (III), whereas in rats and rabbits the 7 β -hydroxy epimer, UDCA (IV), was the predominant product. Since 3-oxo and 7-oxo groups of dehydrocholic acid were reduced by the hepatic enzyme after intravenous administration,²⁴⁾ we consider that the biotransformation of 3,7-DOCA (I) occurred also in the liver. It is well known that the rat liver has 6 β -hydroxylase activity. Hence CDCA (III) and UDCA (IV) were further metabolized to α - and β -muricholates (V and VI) in rats.^{25,26)} In the present study, la-

beled β -MC (VI) was found in the bile of the hamsters which had received [24-¹⁴C]3,7-DOCA (I). Tateyama *et al.* reported that a small portion of CDCA (III) administered to hamsters was converted to β -MC (VI) in the liver.²⁷⁾

Several previous studies on the hepatic biotransformation of 7-KLCA (II) in man have revealed that 7-KLCA (II) was extensively reduced to CDCA (III) and, to a lesser extent, to UDCA (IV).^{28,29)} Since human liver has neither 6 α - nor 6 β -hydroxylase activity, CDCA (III) and UDCA (IV) could not be transformed into muricholic acids (V and VI) in man. Hence, we guess that 3,7-DOCA (I) administered to a man would be converted to CDCA (III) and UDCA (IV) *via* 7-KLCA (II), and CDCA would be the predominant product. That is to say, the results of this study imply that administration of 3,7-DOCA to man would be similar to a combination feeding of CDCA and UDCA. A number of studies have confirmed the efficacy and the utility of treatment with a combination of CDCA and UDCA for gallstone dissolution.^{30–32)} CDCA–UDCA combination therapy reduced cholesterol saturation index of the gallbladder bile more prominently than the single administration of either CDCA or UDCA, and lowered the degree of the side effects (diarrhea *etc.*).^{30–32)}

CDCA used for gallstone dissolution is chemically synthesized from cholic acid. However, since CDCA is hard to crystallize, its purification is difficult.³³⁾ Another agent for dissolving cholesterol gallstones, UDCA, is chemically synthesized from CDCA by several processes. Thus, UDCA therapy is more expensive than CDCA therapy. 3,7-DOCA can be prepared easily by a single step reaction to oxidize crude CDCA in the synthetic process of CDCA. In addition, purification of 3,7-DOCA is easy since it can be crystallized readily.⁸⁾ Therefore, 3,7-DOCA therapy, if possible, should be provided much more economically than CDCA or UDCA therapy.

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