Improved Intestinal Absorption of an Enteric-Coated Sodium Ursodeoxycholate Formulation

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A new enteric-coated formulation of sodium ursodeoxycholate was prepared and administered to man. The barrier film disintegrates and releases the drug only at pH ≥5.5. The sodium salt of glycoursodeoxycholate was also prepared and encapsulated like ursodeoxycholate. Serum levels of ursodeoxycholate and glycoursodeoxycholate were measured by specific enzyme immunoassay after oral administration of their sodium salts in an enteric-coated formulation at equimolar doses of 475 and 540 mg. The same subjects also received in separate experiments ursodeoxycholic acid, sodium ursodeoxycholate, and glycoursodeoxycholic acid in gelatin capsules. The mean area under the curve (μmol/L · hr) following administration of enteric-coated sodium ursodeoxycholate (45 ± 8) was significantly higher than that of either ursodeoxycholic acid (26 ± 5; $P < 0.01$) or sodium ursodeoxycholate (25 ± 6; $P < 0.001$) administered in a conventional gelatin capsule. No differences were found when glycoursodeoxycholic acid was administered as an enteric-coated sodium salt or in acid form in gelatin capsules. Ursodeoxycholic was administered at a dose of 10 μmol/min/kg over 1 hr to bile fistula rats both intraduodenally (i.d.) and intravenously (i.v.). The experiment included administration of the sodium salt in solution and the acid as a suspension. A similar experiment was performed with glycoursodeoxycholic acid. The ratio of the amount recovered from bile in the i.d. to that in the i.v. experiment is almost 1 for the sodium salt of ursodeoxycholate in solution, while it drops to 0.55 for ursodeoxycholic acid. No differences were found between i.v. and i.d. administration when glycoursodeoxycholic acid was administered in acid form and as a soluble sodium salt. The results in rats point out that the limiting factor for ursodeoxycholic acid intestinal absorption is its poor solubility and the high pH (8.4) it requires for micellar solubilization. On the other hand, glycoursodeoxycholic acid is well absorbed either in acid form or as a sodium salt because of its higher solubility at lower pH (6.4). The new enteric-coated sodium ursodeoxycholate formulation resulted in complete solubilization and increased absorption.

KEY WORDS: ursodeoxycholic acid; glycoursodeoxycholic acid; bile acid intestinal absorption; cholesterol gallstones dissolution; liver disease.

INTRODUCTION

The chronic administration of ursodeoxycholic acid (UDCA) has proven to be effective in the dissolution of cholesterol gallstones (1–3), and the therapeutic effectiveness of UDCA in patients with cholestatic liver diseases has recently been reported (4–7). However, UDCA is often poorly absorbed in the small intestine, and after a single oral dose of 300 mg, more than 50% is lost in the stool (8,9). On the other hand, its 7α epimer, chenodeoxycholic acid, is almost completely absorbed (10,11).

The poor intestinal absorption of UDCA is probably due to its critical micellar pH (CMpH), which accounts for the difference in critical micellar concentration (CMC) (12–14). Only at a pH of 8.4 can UDCA be solubilized and passively absorbed along the intestinal tract. This high pH is usually reached only postprandially with sustained duodenal and pancreatic secretion.

An improvement in UDCA bioavailability is thus required to obtain a higher concentration in bile for a given dose. This is even more important in those patients with a gastric hypersecretion or a decreased duodenal bicarbonate secretion as a consequence of pancreatic disease or cholestatic syndrome (15). The objective of the present study is, therefore, to evaluate the role of UDCA solubility on its intestinal absorption and bioavailability and to design a new formulation that is more effectively absorbed. The sodium salt of ursodeoxycholic acid (NaUDC) was prepared and encapsulated into an enteric-coated formulation which disintegrates only at pH ≥5.5.

Two independent studies, carried out in bile fistula rat and man, were performed. To test the working hypothesis, we administered UDCA and glycoursodeoxycholic acid (GUDCA). GUDCA was included in the present study since it is the main chemical form accumulating in bile after chronic feeding of UDCA (1,2). Moreover, it is extremely water-insoluble in acid form, like UDCA (12), but requires a lower pH to be solubilized (6.4 vs 8.4), due to its lower CMC and pK_a (16). The experimental protocol included the administration of UDCA and GUDCA intraduodenally to bile fistula rat both in acid form (suspension) and as a sodium salt (in solution). UDCA and GUDCA were also administered at the same dose intravenously. The amount recovered in bile was then evaluated by HPLC.

In the human study, each subject received UDCA, GUDCA and NaUDC in gelatin capsules as well as NaUDC and enteric-coated sodium glycoursodeoxycholate (NaGUDC). The intestinal absorption of UDCA, GUDCA, and their sodium salts was evaluated by their serum levels vs time, measured by enzyme immunoassay.

MATERIALS AND METHODS

Chemicals

UDCA was kindly supplied by ALFA Wasserman SpA, Bologna, Italy, and was more than 99.5% pure, as assessed by thin-layer chromatography (TLC) and HPLC. GUDCA was supplied by Sigma (St. Louis, MO) and was found to be more than 99% pure. The sodium salts of the two bile acids were prepared by adding an equimolar amount of sodium bicarbonate in water and mixing under ultrasound agitation until the solutions were clear. The solutions were then

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freeze-dried and the percentage of bile salt in the powder was determined by HPLC. The sodium salts were more than 99% pure.

Enteric-Coated Capsules

The enteric-coated formulation was a two-barrier coating depot form. The first polimeric film was constituted by hydroxypropylmethylcellulose and polyethylene glycol (PEG) 6000; the second was formed by hydroxypropylmethylcellulose phthalate and acetylated monoglycerides. The capsule was prepared as reported elsewhere (European Patent publication EP0510404, 28.10.92). The film coat had a pH-dependent solubility: it was stable for more than 2 hr at pH $\leq$3.5, dissolved in 15 min at pH 5.5, and dissolved in 5 min at pH 6.5. Once disintegrated, NaUDC or NaGUDC was ready to solubilize and diffuse in the intestine.

Animal Study

Sprague Dawley male rats 250–300 g in body weight were used. The animals were given free access to water and food 12 hr before the start of the study. The rats were anesthetized with pentobarbital at a dose of 50 mg/kg and the bile duct was cannulated with PE-10 tubing (Clay-Adams, NJ). Baseline bile collections were performed at 30-min intervals for 2 hr. UDCA or GUDCA was then infused.

UDCA and GUDCA were i.d. and i.v. infused at a dose of 10 μmol/min/kg over 1 hr to the bile fistula rat (n = 6 for each study). Bile was collected at 30-min intervals for 6 hr, and the amount of UDCA plus its metabolites determined. In the i.d. study the acid forms of UDCA and GUDCA were administered as a fine suspension obtained by 5 min of sonication of the saline solution. Their sodium salts were dissolved in saline solution. In the i.v. study the bile acids (BA) were administered in saline solution containing 3% of bovine serum albumin. In the i.d. study the intestinal perfusate volume and flow were chosen in the physiological range to ensure an optimal, if present, active transport.

Human Study

Experimental Design

The study group consisted of six healthy, nonobese subjects with normal liver function tests. All had given informed consent and our work had ethical committee approval (University of Bologna, S. Orsola Hospital, Italy). UDCA and GUDCA and their sodium salts were administered orally to each subject in five separate experiments performed at 10-day intervals and in randomized order. Specifically, the patients received the following formulations:

- 450 mg of UDCA in a gelatin capsule,
- 475 mg of NaUDC in a gelatin capsule,
- 475 mg of enteric-coated NaUDC,
- 515 mg of GUDCA in a gelatin capsule, and
- 540 mg of enteric-coated NaGUDC.

The excipients used in each formulation were the same. The drug was administered at 12 AM, after a standard meal which consisted of 40 g of boiled rice, seasoned with butter and cheese, 120 g of chicken, 1 slice of bread, 1 stewed apple, and 1 glass of water. Blood samples were taken at 30-min intervals for 8 hr.

Analytical Methods

Bile Acid Analysis in Serum

The serum concentrations of the drugs were evaluated by quantitative solid-phase competitive enzyme immunoassay (17). Policlonal antibodies for UDCA and its amidates GUDCA and tauroursodeoxycholic acid (TUDCA) were raised in rabbit using a C-24-UDCA-bovine serum albumin conjugate (18). The antibodies were evaluated for their specificity, titer, and affinity, purified, and immobilized on polystyrene microtiter plates. A suitable UDCA horseradish peroxidase (HRP) derivative was synthesized from UDCA using a mixed anhydride method, purified, and used as enzymatic tracer. The developed method is of a competitive type which allows direct analysis of total UDCA on less than 10 μL of serum (17).

Since the method is specific for both UDCA and GUDCA, the assay was preceded by a bile acid class separation since we needed to measure in serum the administered molecule and not the hepatic metabolites which could be formed during each enterohepatic cycling. The free and the glycoconjugated fractions were separated by solid-phase extraction on C-18 and BE-SAX cartridges (Analytichem Int., Harbor City, CA) as reported previously (19).

The free and/or glycine BA fractions obtained by solid-phase separation were dried under vacuum and reconstituted with an appropriate amount of 0.1 M phosphate buffer, pH 7.2, for the enzyme immunoassay. To 100 μL of the diluted serum, 100 μL of the UDCA-HRP enzymatic tracer was added. A standard curve ranging from 0.001 to 0.100 μM was prepared. The microtiter plates were left to incubate at 37°C for 1 hr and washed three times with 0.1 M phosphate buffer, pH 7.4. Two hundred microliters of chromogenic substrate (H_2O_2/*o*-phenilendiamine) in 0.1 citrate/borate buffer, pH 6, was added, and after 30 min the enzymatic reaction was stopped with 100 μL of 4 N H_2SO_4. The absorbance was measured with a microtiter reader at 490 nm. The concentration of the sample was calculated by the calibration curve and is expressed as micromolar.

The precision of the assay was assessed using serum pools at high (40 μM), medium (5 μM), and low (0.5 μM) concentrations for UDCA and GUDCA. The inter- and intraassay variance was calculated by performing the assay in 10 consecutive assays and results are expressed as mean values ± SD.

Bile Acid Analysis in Bile

Bile acid composition in rat bile was performed by HPLC (Water 600E multisolvent delivery system) with an evaporative light-scattering mass detector (ELSD II, Varex Corporation, Burtonsville, MA) according to a previously described method (20). Total bile acid concentration was evaluated enzymatically by using 3α-hydroxysteroid dehydrogenase as described previously (21).

Data Presentation

From the serum concentration vs time after administra-

tion of the bile acids (μM), we calculated the area under the 8-h serum bile acid concentration–time curve (AUC), the maximum concentration (C_{max}), and the time at which it occurred (T_{max}). Mean values were compared and a two-way analysis of variance (ANOVA) was used. In the animal study data are expressed as biliary secretion rate (μmol/min/kg) and the mean maximum secretion rate (S_{max}) was calculated from the three highest mean secretion rate values. The cumulative recovery over 6 hr of the studied formulation and their major metabolites (evaluated by HPLC) was also calculated.

RESULTS

Animal Study

When UDCA was infused intraduodenally in the bile fistula rat in acid form at a dose of 10 μmol/min/kg over 1 hr (Fig. 1), its biliary secretion rate slowly increased vs time, and after 6 hr of bile collection, an appreciable amount of UDCA was still secreted. On the contrary, intraduodenal infusion of a solution of NaUDC caused a rapid increase in UDCA secretion, which reached a maximum value after 2 hr, then fell quickly, and after 4 hr, a small amount was still recovered in bile (Fig. 1). The S_{max} (±SD) following i.d. NaUDC administration (3.44 ± 0.87 μmol/min/kg) was significantly higher than when UDCA was administered in acid form (2.12 ± 0.40 μmol/min/kg; $P < 0.001$). In both cases UDCA was secreted as TUDCA and, to a lesser extent, as GUDCA as evaluated by HPLC analysis of bile samples.

When the same experiment was performed with GUDCA and NaGUDC (Fig. 2), no significant differences between the two studies were observed. The maximum BA secretion was reached after 2 hr in both the GUDCA and the NaGUDC i.d. studies; the S_{max} values are, respectively, 3.85 ± 0.64 and 3.92 ± 0.72 μmol/min/kg. The S_{max} reached in the GUDCA study is similar to that obtained in the NaUDC study and much higher than that during UDCA administration in acid form.

In Figs. 1 and 2 biliary BA secretion after i.v. infusion of NaUDC and NaGUDC is also reported. The S_{max} values are, respectively, 3.81 ± 0.34 and 4.45 ± 0.86 μmol/min/kg.

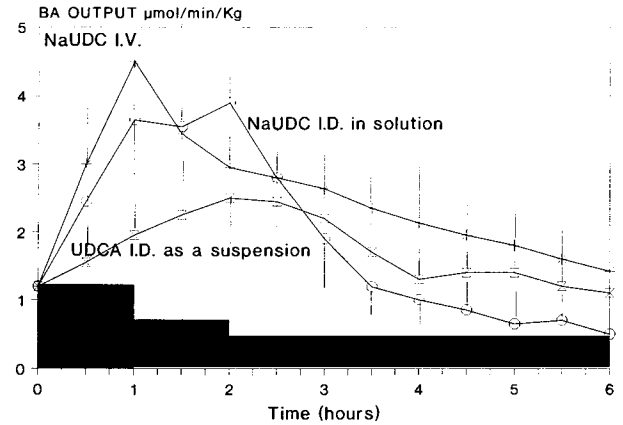

Fig. 1. The effect of UDCA infusion at a dose of 10 μmol/min/kg over 1 hr on total bile acid output. Black area represents the control rat study (n = 6 rats for each study).

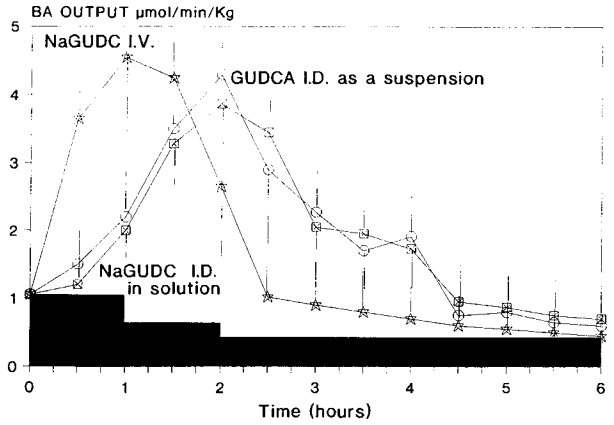

Fig. 2. The effect of GUDCA infusion at a dose of 10 μmol/min/kg over 1 hr on total bile acid output. Black area represents the control rats study (n = 6 rats for each study).

When the i.d. and i.v. data were compared, the cumulative biliary recovery after i.d. infusion of the NaUDC was similar to the i.v. recovery; a significantly lower ($P < 0.001$) recovery is observed for the UDCA i.d. study (Table 1). As shown in Fig. 2 and Table I, in the GUDCA study, no significant difference was found between the percentage of the administered dose recovered in bile during the i.d. infusion of NaGUDC and that during infusion of GUDCA.

Human Study

UDCA Study

After administration of a conventional single dose of UDCA in a gelatin capsule, the mean serum UDCA showed a first peak after 1 hr and a higher peak after 4 hr (Fig. 3), reaching low levels only after 6–8 hr. The intersubject variability was very high for C_{max} (Table II), which varied from 4.2 to 12.2 μM, while the AUC values were less variable (range, 18.9–30.2 $\mu M \cdot$ hr).

When NaUDC was administered in a conventional gelatin capsule, no significant difference in mean C_{max}, T_{max}, and AUC was found compared to those for the acid form (Table II). On the contrary, the pharmacokinetics of the NaUDC when administered with an enteric coating was completely different. The serum UDCA level remained 0 for almost 2 hr, then rapidly increased, reaching a maximum

Table I. Percentage of Administered Dose Recovered in Bile After i.v. and i.d. Infusion (10 μmol/min/kg over 1 hr) of UDCA, GUDCA (as Protonated Insoluble Acids), and Their Sodium Salts

		% dose recovered[a]	
Route	Bile acid	i.v.	i.d.
Suspension	UDCA[b]	—	55 ± 4
Solution	NaUDC	95 ± 5	80 ± 4
Suspension	GUDCA[b]	—	88 ± 6
Solution	NaGUDC	95 ± 7	89 ± 7

[a] Mean values ± SD of six experiments.
[b] UDCA and GUDCA were administered only i.d. as protonated acid in suspension.

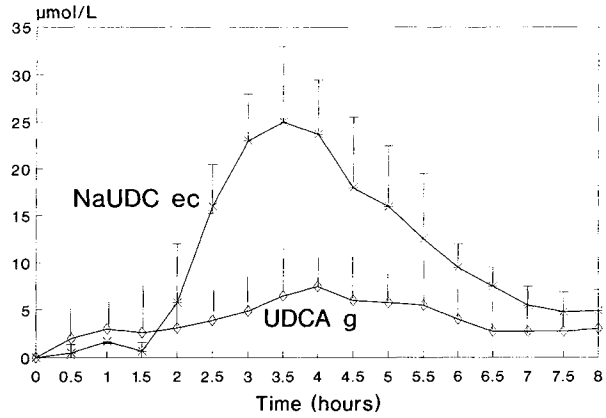

Fig. 3. Time profile of mean serum ursodeoxycholic acid concentrations after a single-dose administration of UDCA and enteric-coated sodium ursodeoxycholate. Each point is the mean value ± SD of six experiments.

value after 3–4 hr (Fig. 3). The C_{max} was significantly higher ($P < 0.05$) than that obtained during administration of both UDCA and NaUDC in conventional gelatin capsules (Table II). The mean AUC ($\mu M \cdot$ hr), following administration of enteric-coated NaUDC (44.8 ± 8.2), was significantly higher than those for both UDCA (25.6 ± 4.8; $P < 0.01$) and NaUDC (25.2 ± 6.2; $P < 0.01$) when administered in a conventional gelatin capsule. Variability between subjects is present (Fig. 4) for two representative subjects. The variability is, in this case, mainly for T_{max}; similar AUC and C_{max} values were found.

GUDCA Study

Mean serum levels after administration of GUDCA in gelatin capsules and as its enteric-coated sodium salt are reported in Fig. 5. After administration of the acid form, GUDCA is present in serum from the first 30 min, reaching a peak after 2.5 hr, while in the enteric-coated NaGUDC formulation, serum GUDCA levels begin to be present after 1.5–2 hr, reaching significantly higher levels between 4 and 5 hr ($P < 0.05$). No significant differences were found in the AUC obtained in the two studies despite the significantly ($P < 0.01$) higher C_{max} observed after administration of the enteric-coated NaGUDC. After 8 hr appreciable amounts of serum GUDCA are present (5–7 μM) in both studies as a result of the recycled GUDCA.

DISCUSSION

Animal Study

The amount of UDCA recovered in rat bile is highest when UDCA is infused in the intestine in solution as NaUDC. This is not surprising since, in solution, the drug is ready for passive absorption in the small intestine. The poorer absorption of UDCA infused in acid form is due mainly to the high pH required for its intestinal solubilization and, consequently, to the amount of UDCA remaining undissolved. A pH of 8.4, required for its solubilization, is difficult to obtain under physiological conditions, and possible only with an elevated pancreatic secretion. The ratio of the dose recovered in bile after i.d. and that after i.v. administration is 0.84 for NaUDC and 0.57 for UDCA, further confirming the efficient absorption of the NaUDC salt.

The working hypothesis was further demonstrated by the results obtained in the GUDCA study. In this case, no significant differences were found between the two sets of experiments, i.e., GUDCA infused i.d. in acid form and as NaGUDC in solution. In this case, the ratios of the dose recovered after i.d. to that after i.v. administration are 0.92 and 0.93, respectively.

The results obtained fit very well with the CMpH of GUDCA, which, due to its lower pK_a, is 6.3, two unities lower than that of UDCA, and show that, even administered in acid form, GUDCA can be readily dissolved and absorbed.

Human Study

Biological and Analytical Variability

The use of serum levels for evaluating bile acid intestinal absorption is rather imprecise as shown by the variability observed between subjects. Many factors contribute to the variability of the data, which are related to gallbladder emptying, intestinal motility, gastric emptying, and portal vein flow. These factors affect the maximum concentration (C_{max}), the time at which it occurs (T_{max}), and, to a lesser extent, the area under the curve (AUC).

Because the serum levels of bile acids are derived from the amount of bile acid not taken up by the liver in a single pass, this parameter must be considered the same for all subjects studied to obtain quantitative information on BA absorption. However, to minimize biological variability, the study was performed on the same subject taking all the different studied formulations. From these data, only the AUC is considered an accurate parameter to be used for comparative studies.

The developed quantitative enzyme immunoassay for UDCA showed adequate analytical performance in terms of sensitivity, and the imprecision of the assay, in both the intra- and the interassay studies, was not higher than 8%.

Serum Levels of UDCA and GUDCA

UDCA Study. The mean AUC after the administration of enteric-coated NaUDC was significantly higher than all the studied UDCA formulations ($P < 0.01$). These results agree with those obtained in the animal study and, further, show complete solubilization of NaUDC salt released as such once bypassing the stomach. The profile of the serum UDCA levels is also in agreement since UDCA in serum is almost absent for 2–3 hr and then quickly increases as a result of capsule disintegration. The relatively high C_{max} found, could be of benefit for the hepatocyte in light of the results found in an animal model on the protective effect of UDCA for hepatic toxicity induced by detergent bile acids, such as taurochenodeoxycholic acid (22).

The main variability found with this formulation is in the T_{max} required for its release in the intestine. The main determinant for this variability is gastric emptying; the capsule designed as enteric barrier depot forms follows the fate of the solid in the release from the gastric content, which is de-

Table II. Peak Serum UDCA Concentration (C_{max}; μM), Time to Peak Serum Concentration (T_{max}; min), and Area Under the Curve to 8 hr ($\mu M \cdot$ hr) in the Same Subject Receiving (UDCA g) 450 mg of UDCA in a Gelatin Capsule, (NaUDC g) 475 mg of NaUDC in a Gelatine Capsule, and (NaUDC ec) 475 mg of Enteric-Coated NaUDC

Formulation	1	2	3	4	5	6	Mean ± SD
UDCA g							
C_{max}	7.5	8.4	4.2	12.2	6.5	7.9	6.6 ± 3.9
T_{max}	4.0	3.2	2.8	4.1	2.8	3.0	3.8 ± 0.6
AUC	30.2	27.6	20.5	18.9	26.6	30.2	25.6 ± 4.8
NaUDC g							
C_{max}	8.7	12.5	7.2	6.5	10.6	6.8	8.7 ± 2.4
T_{max}	2.6	2.4	2.4	2.8	1.7	2.5	2.4 ± 0.4
AUC	31.4	29.5	20.8	17.6	20.7	31.6	25.2 ± 6.2
NaUDC ec							
C_{max}	30.5	28.9	24.4	18.7	24.7	26.8	25.5 ± 9.1
T_{max}	3.0	2.5	4.1	4.1	2.9	3.8	3.4 ± 0.7
AUC	53.2	46.6	36.7	32.7	48.2	51.6	44.8 ± 8.2

layed, and the data of 2–4 hr found account for this hypothesis. To minimize the gastric emptying variability, a definitive choice of the ideal formulation, i.e., barrier coating or microencapsulation, requires further detailed extensive studies.

The designed formulation was effective in all patients studied and, particularly, in subjects who showed a very low AUC after the administration of conventional UDCA (subjects 3, 4, and 5) but a normal AUC after enteric-coated NaUDC. This result is important since it has been reported that some patients could be affected by impaired pancreatic secretion or by gastric hypersecretion with the result of a persistent low intestinal pH (23,24). In this case, the sodium salt will dissolve, while UDCA will remain completely insoluble and, consequently, partially malabsorbed.

We have recently reported that in patients with cystic fibrosis and evidence of liver disease (24) receiving UDCA for 2 months at a dose of 15 mg/kg body wt/day, biliary UDCA accounts only for 25% of the total BA and a large amount of UDCA (12 to 67%) is excreted as such in the stool.

GUDCA Study. After oral administration of GUDCA at a single dose of 475 mg (equimolar with UDCA), GUDCA serum levels rapidly increase as the result of efficient absorption of GUDCA, probably with both a passive and an active mechanism, which has been demonstrated previously in animal models (25).

Since GUDCA is not metabolized by the liver, its serum levels during the day are the result of the intestinal output coming from the first administration and that coming from the subsequent GUDCA input, which undergoes enterohepatic cycling. This accounts for steady-state levels at the end of the study as a result of the complete accumulation of GUDCA in the enterohepatic circulation.

When enteric-coated NaGUDC was administered to the same subject, the serum pharmacokinetics was different. GUDCA remains practically 0 for 2 hr, then suddenly increases as a result of the disintegration of the enteric-coated capsule. The C_{max} was higher, while the AUC was practically the same. These results show that in the case of GUDCA, thanks to its low CMpH, a pH of 6–7 is enough to ensure complete dissolution of the acid form, and this accounts for the similar results obtained with GUDCA and NaGUDC.

An intercomparison between serum UDCA and GUDCA levels is not theoretically possible since the determinant of each serum concentration is different. The first-pass hepatic uptake of GUDCA is much faster and efficient than that of UDCA; for example, in rat we have found that the first-pass hepatic uptake of UDCA is 48–50%, while that

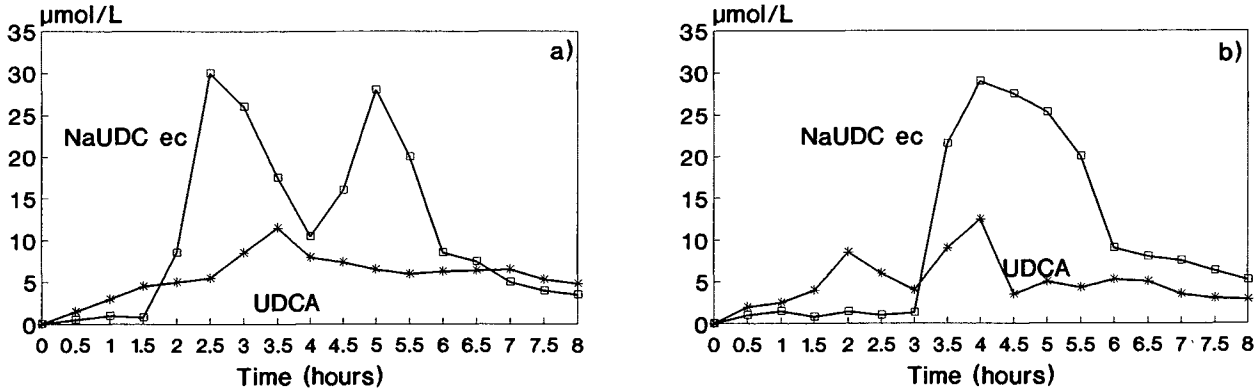

Fig. 4. Intersubject variability of the time–serum ursodeoxycholic acid concentration profile in two subjects taking UDCA and NaUDC, enteric coated.

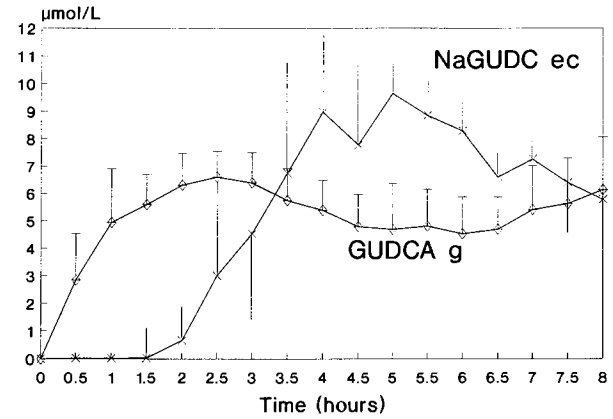

Fig. 5. Time profile of mean serum glycoursodeoxycholic acid concentrations after a single-dose administration of GUDCA and NaGUDC, enteric coated. Each point is the mean value ± SD of six experiments.

of GUDCA is 60–80% (26). As a result, for the same amount of the two BA reaching the liver, a greater spillover, with consequent higher serum levels, occurs for UDCA. As a consequence, higher UDCA serum levels do not mean higher intestinal absorption with respect to GUDCA. Bioequivalence studies between different BA, even with the same BA in free and amidated form, are not correct. A quantitative evaluation of the overall pharmacokinetics must be carried out to quantify hepatic uptake, metabolism, residence time, and biliary secretion properly. We report these two studies only to show that between the two BA, with different physicochemical properties, the sodium salt offers an improvement only for UDCA.

In conclusion, the results obtained confirm the previous ones (8,9) on partial intestinal absorption of UDCA. When enteric-coated NaUDC is administered, intestinal absorption increases significantly. The passive intestinal absorption of UDCA is also determined by lipophilicity, which is slightly lower than those of other dihydroxy BA (27); this explains the incomplete UDCA absorption even if it is well formulated. As far as GUDCA is concerned, both animal and human studies show complete intestinal absorption of either the acid form or the enteric-coated sodium salt, results which further support solubilization as one of the critical steps for UDCA intestinal absorption.

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