

BIOAVAILABILITY STUDY OF A NEW, SINKING, ENTERIC-COATED URSODEOXYCHOLIC ACID FORMULATION

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A new enteric-coated ursodeoxycholic acid (UDCA) formulation which sinks in the stomach and releases the drug only at a $\text{pH} \geq 6.5$ was developed. In 12 healthy subjects we measured, using a specific enzyme immunoassay, the serum levels of UDCA after a single oral dose of 450 mg of UDCA in three different formulations; enteric coated sinking tablet, stomach-floating enteric coated hard gelatin capsule and conventional gelatin capsule. The drug was given after a meal.

Results are expressed as mean \pm SD. The area under the curve [AUC, $\mu\text{mol l}^{-1}$ (8 h)] following oral administration of enteric-coated, sinking UDCA (39.0 ± 8.5) was significantly higher than that obtained after both conventional UDCA (30.5 ± 4.9) and floating enteric coated UDCA (29.3 ± 3.4). Moreover, the maximum UDCA serum concentration (C_{max}) was significantly higher with the enteric coated sinking UDCA formulation when compared to the other two formulations, while the time of maximum UDCA serum concentration (t_{max}) occurred later. These results may be explained by the hypothesis that the sinking tablet is expelled in the latter phase of gastric emptying along with the solid content. It therefore reaches the intestine at the highest alkalization phase caused by sustained biliary and pancreatic secretions. When released, the protonated insoluble UDCA is promptly solubilized by the alkaline pH thus giving a higher UDCA concentration gradient which facilitates its passive absorption. On the other hand, the floating capsule reaches the intestine too early, still in presence of an acidic pH; and in this condition UDCA is almost insoluble and consequently may be malabsorbed.

The new formulation of UDCA seems to be an improvement with respect to commercially available UDCA formulations, where UDCA is only partially absorbed (30–40% of the administered dose). The increased serum AUC indicates an increased UDCA intestinal absorption and bioavailability that should lead to better accumulation of the drug in the enterohepatic circulation and a more effective displacement of endogenous bile acids.

KEY WORDS: ursodeoxycholic acid, intestinal absorption, enzyme immunoassay, bioavailability, enteric coated formulation.

INTRODUCTION

Ursodeoxycholic acid (UDCA) is a naturally occurring bile acid (BA) that is well established as a therapeutic agent having been in use for over 15 years [1–3]. It is used mainly as a solubilizing drug for cholesterol gallstones, and more recently it has been introduced for the treatment of chronic cholestatic liver diseases such as primary biliary cirrhosis [4, 5]. When used as a drug for cholesterol gallstone dissolution, a reduction in the amount of deoxycholic acid (DCA) and a 50% enrichment of UDCA in bile

leads to selective reduction of cholesterol secretion and bile becomes undersaturated in cholesterol. Moreover, a higher percentage of UDCA in bile facilitates cholesterol dissolution via the formation of a vesicular phase [6]. If the drug is used for the treatment of cholestatic liver disease, the displacement of endogenous cytotoxic BA like DCA or chenodeoxycholic acid (CDCA) leads to an improvement of cholestasis.

The clinical efficacy of the drug is limited when used for cholesterol gallstone dissolution because, when it is given chronically at a dose of $15 \text{ mg kg}^{-1} \text{ day}^{-1}$, only 50% of selected patients effectively have complete dissolution.

During chronic administration one of the reasons why UDCA has a limited efficacy is that its

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accumulation in bile reaches only 40–50% of total BA pool. This is due to poor intestinal absorption of UDCA that reaches at most 30–40% of the administered dose [7–10].

UDCA in its protonated form is poorly soluble in water (5 mg l⁻¹) and precipitates at the low pH of the stomach. When the chyme reaches the duodenum it finds an alkaline pH, caused by biliary and pancreatic secretion, that is ideal for UDCA solubilization.

One of the reasons for the poor absorption could be an incomplete solubilization in the intestinal lumen because the pH does not reach the desired alkalinity required by UDCA (8.4) [6]. UDCA behaves differently from other dihydroxy BA such as CDCA that is also commercially available as drug for cholesterol gallstone dissolution. The latter is more lipophilic and requires a lower pH to be solubilized (7.6). In fact CDCA with respect to UDCA has a lower critical micellar pH (CMpH), as a result of a lower critical micellar concentration (CMC) and a similar solubility of the acid form.

The major objectives therefore in clinical pharmacology regarding this drug are to improve its intestinal solubilization and absorption and hence to increase its accumulation in the total BA pool. UDCA is currently formulated as acid form in conventional gelatin capsules or tablets; few attempts have been made so far to optimize its formulation for enhanced absorption.

The aim of the present paper was to develop a new formulation of UDCA that has a pH-dependent enteric coat, resistant at low pH, and that is weighted so it would probably sink in the stomach. Gastric emptying should expel the tablet as such into the duodenum with the indigestible solids at the end of the gastric phase of digestion during which there is sustained biliary and pancreatic secretion. In the duodenal lumen, in the presence of an alkaline pH, the coat dissolves and the drug is released all at once and readily solubilized. This has at least two advantages: one is that it creates a high concentration gradient that is the driving force behind the UDCA passive absorption process (Igimi & Carey, 1980; Ota *et al.*, 1985; Aldini *et al.*, 1989), the other is that the drug is released late in the duodenal lumen when the pH is at its highest. In order to verify the UDCA intestinal absorption, after a single oral dose (450 mg), serum UDCA levels were measured using a specific solid-phase enzyme immunoassay. We compared the new formulation with commercial UDCA in a gelatin capsule and a floating enteric-coated hard-gelatin capsule which is released by the stomach in the early phase of the digestive process, i.e. together with the acidic gastric juice. Even if the coat dissolves and the capsule disintegrates, UDCA may not find a sufficiently high pH (8.5) for its solubilization and absorption.

MATERIALS AND METHODS

UDCA formulations

UDCA was kindly supplied by Erregierre SpA, Bergamo, Italy and was more than 99.9% pure, as assessed by TLC and HPLC [11]. The enteric coated formulations (sinking and floating) were barrier coating depot forms. The polymeric film was constituted by copolymers of metacrylic acids. It had a pH-dependent solubility that was stable at pH 3.5 and dissolved at pH 6.5. The eccipients in the sinking tablet were formulated to assure a density greater than 1 so that it would sink in the stomach (Italian patent application, MI92A000903).

The composition of the two formulations developed and of the conventional formulation are reported in Table I.

The UDCA released from the formulations was studied *in vitro* according to conventional USP XXII/NF XVII specifications. In particular the pH of the dissolution medium was kept at pH 8.5 to ensure dissolution of UDCA, which is soluble only at this pH [6].

The stability of the formulations was studied at room temperature and 40°C for 6 months.

Subjects

We studied 12 healthy volunteers (seven males and five females; mean age 40 years, range 26–47; BMI 23–27). Upper abdominal ultrasonic examination and biochemical blood tests were carried out to exclude any pathology. The dosage forms were administered orally to each subject in three separate experiments performed at 10-day intervals and in randomized

Table I
Composition of the three formulations studied (mg)

<i>Floating hard-gelatin capsules</i>	
<i>Filling</i>	
UDCA	450
sodium-starch glycolate	45
magnesium stearate (USP/NP)	5
<i>Gelatin coating</i>	
methacrylic acid copolymer (USP/NP)	35
ethyl phthalate (USP/NP)	0.5
<i>Sinking tablets</i>	
UDCA	450
sodium starch glycolate	100
microcrystalline cellulose (AVICEL) (USP/NP)	335
silicon dioxide (USP/NP)	5
magnesium stearate (USP/NP)	10
methacrylic acid copolymer (USP/NP)	35.8
ethyl phthalate (USP/NP)	0.5
<i>Conventional gelatin capsule</i>	
UDCA	450
sodium-starch glycolate	65
magnesium stearate (USP/NP)	10

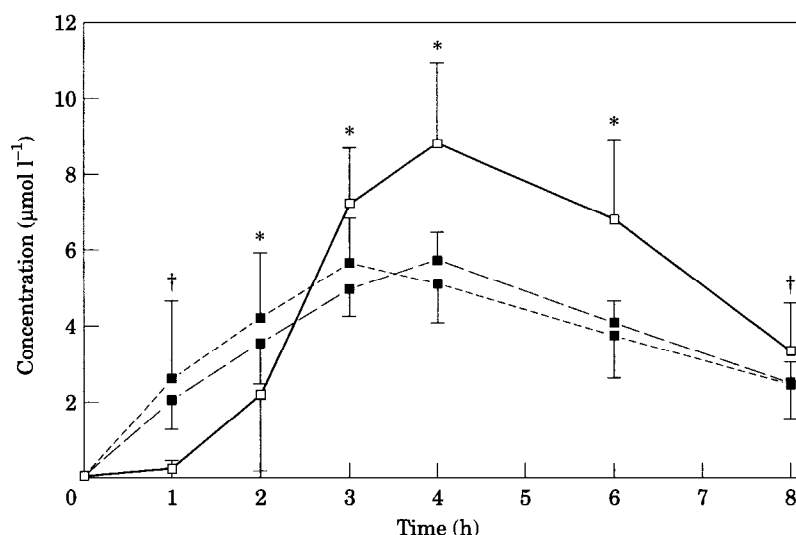


Fig. 1. 1) Serum UDCA levels after a 450-mg single-dose oral administration of the three UDCA formulations studied. (mean value \pm SD). Sinking tablet (A) (—), conventional capsule (B) (···), floating capsule (C) (- - -). *A vs B, A vs C $p < 0.001$; †A vs B, A vs C $p < 0.05$.

order. The dosage form was administered at 12:00 hrs after a standard meal that consisted of 40 g of boiled rice, seasoned with butter and cheese, 120 g of chicken, 50 g of bread, 1 stewed apple and 200 ml of water.

Blood samples were taken before, and at 1, 2, 3, 4, 6, and 8 h after ingestion of the dosage form.

The study was approved by a local Ethics Committee.

Analytical methods

Serum levels of UDCA were evaluated by quantitative solid-phase competitive enzyme immunoassay [12]. Polyclonal antibodies for UDCA were raised in rabbit using C-24 UDCA bovine serum albumin conjugate [13]. The antibodies for UDCA were evaluated for their specificity, titre and affinity. They were then purified and immobilized on polystyrene microtitre plates. A suitable UDCA-horseradish-peroxidase (HRP) derivative was synthesized from UDCA using a mixed anhydride method, purified and used as an enzymatic tracer. The developed method is of competitive type and allows direct analysis of total UDCA in less than 10 μ l of serum.

The assay was preceded by a BA class separation, since it does not discriminate UDCA from its taurine and glycine conjugates that are formed during each enterohepatic cycle. Total BA were isolated from serum using a reverse-phase C-18 column, then the free BA fraction was separated by solid-phase extraction on ion exchange BE-SAX cartridges (Analytichem Int, Harbor City, CA, U.S.A.) [14]. Two-hundred microlitres of serum was diluted with 5 ml of NaOH 0.1 N, incubated at 70°C for 30 min, cooled and then passed through the previously activated C-18 cartridge. The column was washed with 10 ml of water and the BA eluted with methanol

directly in the previously conditioned BE-SAX column. The free BA fraction was eluted with 5.5 ml of methanol and then dried under vacuum and reconstituted with an appropriate amount (1–10 ml) of phosphate buffer 0.1 M pH 7.2 to determine an adequate concentration in the sample and stay within the limits of the standard curve. To 100 μ l of the extract, 100 μ l of the UDCA-HRP enzymatic tracer was added. A standard curve ranging from 0.001 to 1 μ mol l⁻¹ was also prepared. The microtitre plates were left to incubate at 37°C for 1 h and then washed three times with phosphate buffer 0.1 M pH 7.4. One-hundred microlitres of chromogenic substrate (H₂O₂-o-phenyldiamine) in 0.1 M citrate-borate buffer pH 6 was added and after 30 min the enzymatic reaction was stopped with 100 μ l of H₂SO₄ 4 N. The absorbance was measured with a microtitre reader at 490 nm. The concentration of the sample was calculated by the calibration curve and expressed as micromoles per litre.

The precision of the assay was assessed using serum pools at high (50 μ mol l⁻¹), medium (5 μ mol l⁻¹) and low (0.5 μ mol l⁻¹) UDCA concentration. The inter- and intraassay variance was calculated by performing the analysis in 10 consecutive assays and results expressed as mean values \pm SD.

Data presentation

The serum concentration (μ mol l⁻¹) at each time interval after the administration of the three formulations was used to calculate the 8 h area under the serum-UDCA-concentration-time curve [AUC, μ mol l⁻¹ (8 h)], the maximum concentration (C_{max} , μ mol l⁻¹) and the time at which it occurred (t_{max} , h). Repeated measurement of variance (ANOVA) was used when interaction terms were significant, Fisher's least significant difference was used to test simultaneously valid simple effects. Statistical

analysis was performed using the Statistical Analysis System (SAS) version 6.04.

RESULTS

Both the new formulations were stable at room temperature and at 40°C for at least 6 months. The *in vitro* studies showed that UDCA released from the formulations into the dissolution medium was >85% after 8 h.

The development UDCA-specific solid-phase enzyme immunoassay made possible analysis of the administered drug in less than 10 µl of serum with a detection limit of 0.1 pmol. The assay is specific for total UDCA and other endogenous dihydroxy BA such as CDCA and DCA do not cross react with the antibody. The imprecision of the method both in the intra- and interassay study was below 8%, showing the validity of the data reported.

Despite high intra-subject variability, the mean serum UDCA concentrations *vs* time curves for the normal and floating formulations were very similar whereas the sinking tablet showing a maximum peak that was much higher and a more rapid return to low values (Figure 1). The mean AUC, t_{\max} and C_{\max} values of the twelve studied subjects are shown in Table II. The sinking formulation gave a mean AUC of $39.0 \pm 8.5 \mu\text{mol l}^{-1}$ (8 hr) that is significantly higher ($p < 0.01$) than those of the other two preparations. The sinking tablet also has a C_{\max} that is significantly ($p < 0.01$) higher and t_{\max} significantly later ($p < 0.001$) *vs* conventional and $p < 0.05$ *vs* floating). No significant differences in AUC, t_{\max} and C_{\max} values were found between the conventional and the floating UDCA formulations.

DISCUSSION

The evaluation of intestinal absorption for an enterohepatic drug, such as UDCA, using serum levels is limited by its high hepatic first pass clearance

(50%) that greatly reduces serum concentrations in systemic blood [15–17]. Only with the use of the developed enzyme immunoassay was it possible to analyze serum UDCA with adequate sensitivity and precision in a high number of samples. The conventional physico-chemical methods such as HPLC or GC are time consuming and require a complex preanalytical procedure.

If hepatic uptake and portal blood flow are constant, serum BA levels are closely related to portal blood concentrations and thus to intestinal absorption [18, 19]. In order to reduce the variability related to differences in gastric emptying and intestinal transit time between subjects, we carried out a cross-over study and administered the three formulations, in randomized order, in three separate experiments to the same subject. A 10-day wash-out period was observed between each trial. The results show that the UDCA sinking formulation increases the mean AUC when compared to the conventional formulation and to the floating, enteric-coated formulation. Intrasubject variability was observed in terms of C_{\max} , t_{\max} and AUC as a result of differences in gastric emptying, intestinal transit time and pH, biliary and pancreatic secretion. However, mean AUC, C_{\max} and t_{\max} were significantly different in the sinking formulation with respect to both the other two formulations (Table II.)

This study adds weight to the *in vitro* experiments that demonstrate the importance of pH in the intestinal absorption of unconjugated BA [6]. Slowing the arrival of UDCA in the intestinal lumen, when pH is at its highest, gives rise to an increase in the bioavailability of UDCA. Only at pH 8.4, which represents the critical micellar pH, is UDCA solubilized to form the ionized species in micellar solution. The fact that the floating, enteric-coated tablet did not increase the AUC with respect to the conventional gelatin capsule leads us to believe that the innovation regards the weighting and not the gastric protection. The stomach-floating formulation reaches the intestine early, together with the liquid phase and in the presence of acidic gastric juice. Consequently, the pH of the intestine is still too low and UDCA is poorly solubilized. Moreover, the new enteric-coated, sinking tablet prevents dispersion of UDCA in the stomach and, once released, leads to an elevated concentration gradient in the intestine. This facilitates the absorption of unconjugated UDCA which undergoes only passive transport in the intestine [20].

We conclude that this new formulation has many advantages over the conventional capsule and when compared to a similar formulation which floats in the stomach. The first advantage derives from the optimal time period of its disintegration, i.e. during sustained biliary and pancreatic secretion that alkalizes the intestinal lumen thus facilitating the solubilization of UDCA. Physiologically after a meal the gallbladder contracts and bile is released in the intestine together

Table II
Pharmacokinetic parameters of serum UDCA levels after single oral dose (450 mg) of the three studied formulations in 12 healthy volunteers. (mean \pm SD).

	C_{\max} ($\mu\text{mol h}^{-1}$)	t_{\max} (h)	AUC ($\mu\text{mol h}^{-1}$ (8 h))
Sinking (A)	8.67 ± 2.07	4.17 ± 1.35	39.5 ± 8.5
Conventional (B)	5.53 ± 1.16	3.00 ± 0.86	30.5 ± 4.9
Floating (C)	5.65 ± 0.65	3.67 ± 0.99	29.3 ± 3.4

C_{\max} : A *vs* B, A *vs* C $p < 0.001$.

t_{\max} : A *vs* B $p < 0.001$, A *vs* C $p < 0.05$.

AUC: A *vs* B, A *vs* C $p < 0.01$.

with gastric juices. The stomach takes 1–2 h to empty completely and it is after this time that pH starts to increase. During the later phase of digestion sustained biliary secretion is maintained by the BA returning to the liver once reabsorbed by the intestine.

The second advantage is that UDCA is released in bulk and thus its passive diffusion is facilitated. This is shown by its serum level profile in which serum UDCA suddenly increases reaching higher C_{\max} values and after 8 h its concentration returns to low levels.

In conclusion, the new sinking enteric coated formulation increases the bioavailability of conventional commercially available formulations, in which UDCA is only 30–40% absorbed. The increased absorption should lead to increased accumulation of the drug in the enterohepatic circulation and to displacement of potentially toxic endogenous dihydroxy BA.

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