

Bioavailability of four ursodeoxycholic acid preparations

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SUMMARY

Background: Ursodeoxycholic acid (UDCA) is the drug of choice for treating primary biliary cirrhosis and dissolving cholesterol gallstones.

Objectives: The objective of this study was to compare the bioavailability of four commercially available ursodeoxycholic acid formulations in standardized doses.

Methods: Twenty-four healthy subjects were studied in groups of four, and received each of the different UDCA preparations in random order, with a 1-week washout or more in-between. Serum UDCA levels were determined for a 6-h period. The mean area under the curve (AUC), C_{\max} and T_{\max} were determined for each drug formulation, and the results compared. Dose proportionality was determined using the Canadian Ursofalk tablet using either 250 mg, 500 mg or 750 mg dosing. The intraparticipant variability was assessed by asking each participant to repeat the last drug that they took the second time, 1 week later.

Results: The mean AUC was $68.99 \mu\text{mol}/1.6 \text{ h}^{-1}$ for the USA UDCA tablet, $59.34 \mu\text{mol}/1.6 \text{ h}^{-1}$ for the Canadian UDCA tablet, $55.55 \mu\text{mol}/1.6 \text{ h}^{-1}$ for Ursolvan capsules, and $46.66 \mu\text{mol}/1.6 \text{ h}^{-1}$ for Actigall capsules. The mean C_{\max} values were 24.29, 17.85, 16.63 and 13.32 nmol/mL, respectively. The mean T_{\max} was 1.82, 2.3, 2.79 and 3.39 h, respectively. Linear regression analysis assessing the direct proportionality of AUC on the dose for the Canadian UDCA tablet gave an estimate of $0.063 + 0.0164$ (standard error, P -value = 0.0117), e.g. if the dose increases from 250 mg to 500 mg, the serum ursodeoxycholic acid increases by $250 \times 0.063 = 15.75$. There was excellent reproducibility for the AUC for the North American tablets (0.97, 0.88) compared to the two capsules (0.32, 0.15).

Conclusions: The significantly higher AUC and C_{\max} and shorter T_{\max} for the Canadian Ursofalk tablets compared to the UDCA capsule preparations supports better bioavailability.

INTRODUCTION

The purpose of this study was to determine the bioequivalence of four preparations of ursodeoxycholic acid (UDCA), which is currently the drug of choice for dissolving of cholesterol gallstones, and the probable drug of choice for chronic cholestatic liver disease, especially primary biliary cirrhosis.

METHODS

The following test preparations were used: drug A: two Ursofalk Canadian tablets, 500 mg total dose; drug B: two Actigall capsules, 600 mg total dose; drug C: two Ursofalk American tablets, 500 mg total dose; drug D: three Ursolvan capsules, 600 mg total dose. Twenty-four healthy subjects, 12 men and 12 women, who were not taking medication and had no history of gastrointestinal, liver or renal disease, were asked to undergo a haemogram and SMAC (simultaneous multiple analysis computer) to document normal liver and renal function. Each healthy volunteer was asked to

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give a 5-mL sample of blood, drawn before and hourly for 6 hours after taking the test drug by mouth for an estimation of total serum bile acid and serum ursodeoxycholic acid. The exact timing of the ingestion of the test drug and the blood samples was noted. The drug was taken orally while fasting and with 4 oz. of water. The bioequivalence study was conducted on each of five different days, separated by at least a 1-week period.

Three volunteers were asked to undergo repeat studies using 250 mg, 500 mg and 750 mg of one preparation (Canadian Ursofalk tablets) to assess the effect of different doses on bioavailability, independent of possible formulation effects, and to give a better evaluation of the accuracy of the study design/methods employed.

Bile acid measurement

Ursodeoxycholic acid contains a 7β -hydroxyl group that can be measured using *C. absonum*, 7β -hydroxysteroid-dehydrogenase, originally described and produced in our laboratories.^{1, 2} Serum UDCA was measured every three months for two years in the large, multicentre, prospective, placebo-controlled Canadian trial of UDCA in 224 patients with Primary Biliary Cirrhosis.³ The sensitivity, specificity, accuracy, reproducibility, and linearity of this enzyme assay is documented.³ This enzyme has been supplied to others to measure UDCA in serum.⁴

All bile acids contain a hydroxyl group at the 3 position. This can be readily and sensitively detected by utilizing the standard 3α -hydroxysteroid-dehydrogenase assay, well described in the literature.⁵

Both enzymes have been used to measure bile acids in bile and serum in previous studies.⁶⁻⁸

The reason for a 6 h study is related to the enterohepatic cycling of bile acid when, during this time, a second peak of the serum level may be present. In a volunteer, ursodeoxycholic acid rose from undetectable levels to 9.24 nmol/mL at 1 h, 15.7 at 2 h, 8.4 at 3 h, 4.1 at 4 h, 8.4 at 5 h, and undetectable at 6 h.

Ursodeoxycholic acid is approximately 50% conjugated by the liver after 8 h in patients with an ileostomy. In the intact subject after enterohepatic cycling, this conjugated ursodeoxycholic acid will be reconverted to free acid in the colon, where it will be reabsorbed. Previous papers, specifically those by Bazzoli *et al.*, have shown that serum ursodeoxycholic acid is a very sensitive, specific and convenient means of predicting

the presence of increased levels in bile of ursodeoxycholic acid during the enterohepatic cycle.⁹

Study design

The present study utilized a single dose, five-way crossover design and was conducted in 24 healthy human volunteers. Postadministered serum levels of ursodeoxycholic acid in peripheral blood were used as the major parameter for measurement and the results were correlated with serum total bile acid concentrations. Twenty-four volunteers provided serum samples for this purpose. The study design was of six blocks of four participants. The sequence of taking the four drugs A, B, C and D was the same for the four in each group. The drug order was changed for each of the other groups, i.e. ABCD, ADBC, CADB, BCDA, CABD and BADC. At the end of the study block, each subject in each group repeated the last drug, in order to ascertain reproducibility. There was at least a 1-week washout period between each drug regimen to minimize any carryover effect.

Each participant's age, sex, height and weight was recorded. The 24 subjects, who were of legal age, were within 15% of their normal weight for height, as per the Metropolitan Life Insurance statistics. Subjects were provided with reimbursed for their expenses. Subjects with a history of chronic alcohol or drug abuse, gastrointestinal, renal, hepatic or cardiovascular disease, or any other disease which would interfere with compliance, were ineligible for this study. Similarly, pregnant or lactating women were excluded. No other drug was taken concurrently with this study.

Statistical methods

The time to peak (T_{max}), the maximum serum level (C_{max}) were measured, and the area under the curve (AUC) was calculated by the Trapezoid Rule from 0 to 6 h for all participants. Each of these measures was analysed in a crossover design using analysis of variance methods. The analysis of variance models used terms for subjects, period effects, direct treatment effects and first order carryover effects.¹⁰ The bioavailability of each formulation was determined and compared by calculating the mean AUCs of a standard dose. The dose proportionality of the formulations was determined from the ratio of the mean AUCs obtained with the various doses, on the basis that AUC should be directly proportional to dose.

The analysis measured the intraparticipant variability, as measured by comparing periods four and five, when the drugs were repeated for each participant.

A crossover trial is a clinical trial where each experimental unit receives two or more treatments. In the present study we had four treatments (A,B,C,D) and five time-periods. A washout period of 7 days was allowed between each treatment allocation. We had the following model:

$$Y_{ijk} = \mu + S_{ik} + \pi_j + T_{d[ij]} + \lambda_{d[i,j-1]} + e_{ijk}$$

where Y_{ijk} is the response (AUC, T_{max} or C_{max}) of individual k in period j of group i ; μ is overall mean, S_{ik} is the effect of subject k in group i , π_j the effect of period j , $T_{d[ij]}$ is the direct effect of the treatment administered in period j of group i , $\lambda_{d[i,j-1]}$ is the effect of the carryover of the d th treatment administered in period $j - 1$ of group i , where $\lambda_{[io]} = 0$ and e_{ijk} is the random error for subject k in period j and in group i . Note that we cannot test the presence of carryover effects; all we can test is whether the carryover effects are the same for each drug. Thus, we present two sets of results, one with carryover effects included, and if they are not significantly different, we also present results without the carryover effects.

To determine the significance of the various factors, we fit the models in the following sequential fashion:

Model 1 : $Y_{ijk} = \mu + S_{ik} + e_{ijk}$

Model 2 : $Y_{ijk} = \mu + S_{ik} + \pi_j + e_{ijk}$

Model 3 : $Y_{ijk} = \mu + S_{ik} + \pi_j + T_{d[ij]} + e_{ijk}$

Model 4 : $Y_{ijk} = \mu + S_{ik} + \pi_j + T_{d[ij]} + \lambda_{d[i,j-1]} + e_{ijk}$

Comparing the residual sum of squares of each model enables us to construct type I tests for each term. That is, we must first determine the effect of carryover effects. If there are no carryover effects, we then test for the drug effects.

Power calculations were assessed for small, medium and large sizes.¹¹ With the current sample size we have 17% power to detect a small effect, 66% power for a medium effect and 94% power to detect a large effect. The small, medium and large effects for each measure correspond to the following differences:

Ursodeoxycholic acid

	AUC	C_{max}	T_{max}
Small	3.25	1.49	0.28
Medium	8.13	3.72	0.78
Large	13.00	5.95	1.11

RESULTS

This section describes the results of the crossover models when applied to the serum results. Specifically, we applied the models to AUC, C_{max} and T_{max} for the serum UDCA concentrations.

To ensure comparable doses were assessed and since the dosages of the drug differ, we adjusted the AUC and the C_{max} measures for drugs B and D downward by a factor of (500/600). We performed analysis on both the original and the adjusted scores.

Serum ursodeoxycholic acid

The adjusted results for serum ursodeoxycholic acid are displayed in Table 1. We found highly significant differences among the drugs for both AUC ($P < 0.0001$) and C_{max} ($P < 0.0001$) and T_{max} ($P = 0.0013$), with no evidence of differences among carryover effects. Part C of the table gives the results of Tukey–Kramer *post hoc* comparisons. The drug effect dominated the small carryover effect B followed by C. The AUC for drug C was higher than that for drug B ($P = 0.0001$) and drug D ($P = 0.0127$) and drug A is higher than drug B ($P = 0.0365$). There was no difference between drug A and drug C. The C_{max} for drug C was higher than drug B ($P = 0.0001$), drug D ($P = 0.0011$) and drug A ($P = 0.0155$).

The T_{max} for drug C was shorter than drug B ($P = 0.0009$) and drug D ($P = 0.047$), and drug A was marginally shorter than drug B ($P = 0.06$).

Drugs A and C were both compressed tablets of the same formulation, made commercially in Canada and the USA by different companies. It would be expected that these drugs would give identical test results. Indeed, there were no significant differences for AUC and for T_{max} between drugs A and C. However, there was a difference in C_{max} between drugs A and C. It was interesting to look at the combined average effect of drugs A and C and compare the results with drug B and drug D, both of which were different capsule formulations made in different European countries. As shown in

Table 1. Comparison of drugs A,B,C,D; serum ursodeoxycholic acid

Source	d.f.	Area under curve		C_{\max}		T_{\max}	
		F	P-value	F	P-value	F	P-value
(A)							
Subjects	23	3.87	< 0.0001	3.20	< 0.0001	0.77	0.75
Period	4	0.86	0.4929	1.31	0.2711	0.50	0.74
Drug	3	9.17	< 0.0001	11.41	< 0.0001	5.72	0.0013
Carryover	3	1.46	0.2321	2.82	0.0437	0.81	0.49
(B) Without the carryover effect							
Subjects	23	3.81	< 0.0001	3.01	< 0.0001	0.77	0.75
Period	4	0.84	0.5008	1.24	0.3007	0.50	0.73
Drug	3	9.03	< 0.0001	10.74	< 0.0001	5.75	0.0012
(C.1) Adjusted mean							
Drug		Area under curve P-values					
		B	C	D			
A	59.34	0.0365	0.1635	0.8558			
B	46.66	—	0.0001	0.1918			
C	68.99	—	—	0.0127			
D	55.55						
(C.2) Adjusted mean							
Drug		C_{\max} P-values					
		B	C	D			
A	17.85	0.1493	0.0155	0.9437			
B	13.32	—	0.0001	0.3653			
C	24.29	—	—	0.0011			
D	16.63	—	—	—			
(C.3) Adjusted mean							
Drug		T_{\max} P-values					
		B	C	D			
A	2.30	0.06	0.61	0.63			
B	3.39	—	0.0009	0.55			
C	1.82	—	—	0.047			
D	2.79	—	—	—			

Table 2, there were highly significant differences between drugs A, C and drug B for serum UDCA, AUC, C_{\max} and T_{\max} ($P < 0.0001$, $P < 0.0001$ and $P < 0.0003$, respectively). Similarly, there were significant differences between serum UDCA, AUC, C_{\max} and T_{\max} for drugs A, C vs. drug D ($P = 0.0299$, $P = 0.0148$ and $P = 0.0316$, respectively).

Dose proportionality

This section reports on the dose proportionality of the Canadian Ursofalk tablets. We used a linear regression analysis to assess the direct proportionality of AUC on dose (250 mg, 500 mg and 750 mg), for serum ursodeoxycholic acid. The regression model also included

Table 2. Comparison of drugs A,C versus drug B and drug D

	Serum UDCA											
	AUC				C_{\max}				T_{\max}			
	Diff.	s.e.	T	Sig.	Diff.	s.e.	T	Sig.	Diff.	s.e.	T	Sig.
A,C vs. B	17.50	3.86	4.54	< 0.0001	7.75	1.77	4.39	< 0.0001	-1.23	0.33	-3.73	0.0003
A,C vs. D	8.62	3.90	2.21	0.0299	4.44	1.79	2.49	0.0148	-0.73	0.33	-2.18	0.0316

subject effects, however, we do not present the results for the subject effects.

The following gives the results for serum UDCA:

Parameter	Estimate	Std error	Significance
Serum UDCA, AUC	0.063	0.0164	0.0117

The parameter gives the proportionality estimate. That is, if the dose increased from 250 mg to 500 mg, the ursodeoxycholic acid increased by $250 \times 0.063 = 15.75$.

Intraparticipant variability

This section assesses the intraparticipant variability (reproducibility); that is, for time periods 4 and 5 when the same formulation was repeated. To assess this we took the difference between the patient responses on the drug for period 5 minus period 4, as shown in Table 3.

The intraclass correlations coefficients were calculated. There was an excellent reproducibility for the AUC for drug C compared to either drug B or drug D. Two subjects repeating drug A and two subjects repeating drug C did not complete their studies.

DISCUSSION

UDCA has a low aqueous solubility and is incompletely absorbed by nonsaturated passive absorption in the

upper gastrointestinal tract, and actively in the distal ileum. It is partially metabolized by intestinal bacteria to lithocholic acid, which, if enterocycled to the liver, undergoes sulphation and conjugation and is mainly excreted via the kidneys.¹² Absorbed UDCA undergoes hepatic conjugation with glycine and taurine, and then undergoes enterohepatic cycling.

There was a very high efficient and constant extraction by the hepatocyte of enterohepatic cycling bile acids. Hence, if bile acid absorption varies between different pharmaceutical preparations of UDCA, there would be varying amounts presented to the liver cell and varying amounts that gain access to the peripheral blood. A higher bile acid absorption results in increased load to the liver, and a higher amount seen in the peripheral blood. Using, for example, 40% for intestinal absorption and 95% liver extraction, there would be 200 mg absorbed from a 500 mg capsule, 190 mg retained in the liver and 10 mg 'seen' in the peripheral blood. Similarly, with, for example, 80% absorption, 400 mg would be absorbed from a 500 mg tablet and 380 mg retained by the liver, leaving 20 mg to pass to the peripheral blood. If the hepatic extraction stays constant, then doubling of the intestinal absorption may result in doubling the amount in the peripheral blood. The measurable amount in the blood; i.e. AUC should reflect the spillover through the liver; i.e. after hepatic extraction. If the proportion of hepatic extraction was constant, then the AUC should reflect the proportion absorption through the intestine. A higher absorption should be reflected in a higher AUC. If the different preparations of UDCA are extracted by the liver in the same proportions, then the AUC should be proportionate to intestinal absorption and reflects bioavailability. Higher absorption should also be reflected in tighter reproducibility compared to lower absorption. We documented higher intraclass correlation coefficients for the ursodeoxycholate tablets than for the capsules. Consequently, we propose that the significantly different AUC found for the UDCA tablets compared to the capsules reflects better absorption of the tablets in these same healthy individuals who each ingested all four UDCA compounds on different occasions.

We have compared the bioavailability of four formulations of ursodeoxycholic acid: two tablet preparations of Ursofalk (drug A and drug C), made separately in Canada and the USA, respectively, for the same company; two capsule preparations, Actigall (drug B), and Ursolvan (drug D), made in Europe.

Table 3. Intraparticipant variability

	N	Serum UDCA				ICC
		Mean	s.d.	Min	Max	
Drug A						
AUC	2	-10.85	17.56	-23.27	1.56	-0.88
C _{max}	2	-2.00	3.82	-4.70	0.70	0.53
T _{max}	2	-0.50	0.71	-1	0	0
Drug B						
AUC	4	-13.12	21.58	-39.33	7.56	0.32
C _{max}	4	-3.93	5.93	-11.60	1.15	0.54
T _{max}	4	1.50	4.04	-4	5	-0.66
Drug C						
AUC	6	-0.29	4.61	-6.81	4.46	0.97
C _{max}	6	7.30	6.03	-0.23	13.71	0.34
T _{max}	6	-1.17	1.47	-4	0	-0.19
Drug D						
AUC	8	2.15	37.66	-76.04	42.96	0.15
C _{max}	8	1.21	9.85	-19.49	15.18	0.27
T _{max}	8	-0.38	1.19	-2	1	0.37

ICC is the intraclass correlation coefficient.

We have describe a better absorption reflected by significantly higher AUCs and C_{\max} and shorter T_{\max} for the North American tablets compared to the UDCA capsules for the standardized 500 mg dose.

There was no difference between the AUCs for the two North American tablets. The AUC was $68.99 \mu\text{mol/L.6 h}^{-1}$ for the USA UDCA tablet, $59.34 \mu\text{mol/L.6 h}^{-1}$ for the Canadian UDCA tablet, $55.55 \mu\text{mol/L.6 h}^{-1}$ Ursolvan, and $46.66 \mu\text{mol/L.6 h}^{-1}$ for Actigall. The C_{\max} values for the USA UDCA tablet was 24.29 nmol/mL and 17.85 nmol/mL for the Canadian UDCA tablet. The C_{\max} was 16.63 nmol/mL for Ursolvan and 13.32 nmol/mL for Actigall.

The T_{\max} was 1.82 h for the USA Ursolvan tablet, 2.30 h for the Canadian Ursolvan tablet, 2.79 h for Ursolvan and 3.39 h for Actigall.

There are very few bioavailability studies available, and none for UDCA (Ursolvan) tablets. Simoni and co-workers have described a new enteric-coated UDCA formulation which sinks in the stomach and releases the drug at a $\text{pH} = 6.5$.¹² This formulation has a barrier-coating of copolymers of metacrylic acids (Erregierre SpA, Bergamo, Italy). The AUC was $39.0 \mu\text{mol/L}^{-1}$ (8 h) for the new compound vs. 30.5 for the conventional gelatin UDCA capsule.

Parquet *et al.* studied the bioavailability of ursodeoxycholic acid (500 mg capsule) in seven healthy volunteers.¹⁴ They reported a double peak serum profile over a 240-minute period. The second peak was shown, by both radioassay and gas liquid chromatography, to be due to enterohepatic cycling; C_{\max} was $11.3 \mu\text{mol/L}^{-1}$ and $11.4 \mu\text{mol/L}^{-1}$, respectively.

Serum UDCA was also measured by Colombo¹⁵ after an oral loading dose in children with cystic fibrosis and normal controls ($n = 8$). The serum levels were measured by radioimmunoassay, and double peaks were also seen in the control subjects, with $C_{\max} = 7 \mu\text{mol/L}$. The AUC was $52.98 \pm 5.87\%$ dose/ $\text{L} \times \text{h} \times \text{kg body wt}$.

A micellar preparation of UDCA buffered in sodium bicarbonate (Giuliani, Milano, Italy) was studied in 10 healthy subjects and patients with liver disease.¹⁶ A comparison between the same UDCA dose given orally and intravenously revealed a 50% systemic availability in the normal subjects. Whereas, in liver disease the systemic availability is increased due to portal-systemic spillover and a likely diminished hepatic first-pass extraction. T_{\max} was $18 \pm 6 \text{ min}$, C_{\max} $9.3 \mu\text{mol/L}$ and AUC (0– ∞) $331 \pm 59 \mu\text{mol/L}$.

Efforts to improve the absorption of UDCA have led to new compounds. Panini *et al.* reported improved UDCA bioavailability using 2-hydroxypropyl- β -cyclodextrin complexed UDCA in healthy volunteers.¹⁷ The control UDCA compound is Ursacol, Zambon Group, Bresso, Italy, 150 mg tablets. Six volunteers were given three tablets of the new compound or Ursacol at 3-day intervals. Serum was collected for 4 h. The C_{\max} was $6.1 \mu\text{g/mL}$ and $2.9 \mu\text{g/mL}$, T_{\max} 63 min and 83 min and the AUCs 8.1 and $3.8 \mu\text{gh/mL}$, respectively. This review of the UDCA bioavailability literature documents different UDCA formulations, different doses and different serum UDCA methodologies, making comparisons difficult. We have reported bioavailability data from four commercially available preparations tested in the same laboratory by the same methodology and reported our results. The UDCA North American tablets have higher AUCs and shorter time to maximal concentrations, implying better absorption than the two UDCA capsule formulations. UDCA tablets are widely used in North American primarily in patients with chronic cholestatic liver disease (primary biliary cirrhosis) and other chronic liver disorders, and in selected patients with cholesterol gallstone disease.

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