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Pharmacokinetic and bioequivalence comparison between ursodeoxycholic acid tablets 500mg: An open label, balanced, randomized-sequence, single-dose, two-period crossover study in healthy male volunteers

Nageswara Rao Pilli[a*], Swetha Savakula[b], S. Sai Satyanaraya Reddy[c] and Ravindra Reedy S.[c]

[a]*Piramal clinical research, Hyderabad, Telangana, India*
[b]*ClinSync clinical Research Pvt Ltd, Hyderabad, Telangana, India*
[c]*Vardhaman College of Engineering, Hyderabad, Telangana, India*

ABSTRACT

This present bioequivalence study was designed to determine the pharmacokinetic, bioavailability and bioequivalence of Ursodeoxycholic Acid 500mg Tablets in comparison with URSO FORTE™ Ursodiol 500mg Tablets after single dose administration under fed conditions in healthy adult male subjects. Therefore the design of an open label, balanced, randomized, two-sequence, single dose, two way crossover study with a wash-out period of at least 7 days was used. An open-labeled, balanced, single-dose with food, two-treatment, two-period, two-sequence, randomized crossover study was conducted in 12 healthy male volunteers. Each volunteer received a 500mg tablet of the reference or test drug respectively. On the day of dosing, blood samples were collected before dosing and at various time points up to 24 hours after dosing. Analysis of Ursodeoxycholic Acid concentrations was performed using a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) method. The pharmacokinetic parameters including C_{max}, AUC_{0-t}, AUC_{0-inf}, T_{max}, $t_{1/2}$ and K_{el} were analyzed using the non-compartmental model. Drug safety and tolerability were assessed. The pharmacokinetic parameters including C_{max}, AUC_{0-t}, AUC_{0-inf}, T_{max}, $t_{1/2}$ and K_{el} were analyzed using the non-compartmental model. Drug safety and tolerability were assessed. The primary pharmacokinetic parameters (C_{max}, AUC_{0-t} and AUC_{0-inf}) 90%CI were within the 80 to 125% interval required for bioequivalence as stipulated in the current regulations of the USFDA acceptance criteria. The geometric mean ratios (Test/Reference) between the two products of 500mg tablets under fed condition were 111.69% (91.86%-119.53%) for C_{max} ratios, 88.84% (85.74%-109.49%) for AUC_{0-t} ratios and 92.36% (90.49%-107.97%) for AUC_{0-inf} ratios of Ursodeoxycholic Acid. 12 volunteers had completed both treatment periods. There was no significant difference of the T_{max} parameter between the two formulations ($p > 0.05$). No serious adverse events related to the study drugs were found. This single dose study found that the test formulation ursodeoxycholic acid 500mg tablets are bioequivalent to the reference formulation URSO FORTE™ Ursodiol 500mg Tablets in terms of extent and rate of absorption, under fed condition in healthy adult male volunteers according to the USFDA regulatory guidance.

Keywords: Ursodeoxycholic Acid, Bioavailability, Bioequivalence, Intrasubject Variability

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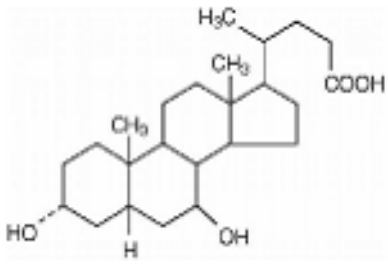
INTRODUCTION

Ursodeoxycholic acid [UDCA] [1] is a naturally occurring bile acid derived from cholesterol, found in small quantities in normal human bile and in larger quantities in the biles of certain species of bears. It is a bitter-tasting white powder consisting of crystalline particles freely soluble in ethanol and glacial acetic acid, slightly soluble in chloroform, sparingly soluble in ether, and practically insoluble in water. The chemical name of ursodeoxycholic acid is 3α, 7β-dihydroxy-5β-cholan-24-oic [$C_{24}H_{40}O_4$]. It has a molecular weight of 392.56 g/mol. Its structure is shown below.



Following oral administration [2-3], the majority of ursodeoxycholic acid is absorbed by passive diffusion and its absorption is incomplete. Once absorbed, Ursodeoxycholic acid undergoes hepatic extraction to the extent of about 50% in the absence of liver disease. As the severity of liver disease increases, the extent of extraction decreases. In the liver, Ursodeoxycholic acid is conjugated with glycine or taurine, and then secreted into bile. These conjugates of ursodeoxycholic acid are absorbed in the small intestine by passive and active mechanisms. The conjugates can also be deconjugated in the ileum by intestinal enzymes, leading to the formation of free Ursodeoxycholic acid that can be reabsorbed and reconjugated in the liver. Nonabsorbed Ursodeoxycholic acid passes into the colon where it is mostly 7-dehydroxylated to lithocholic acid. Some ursodeoxycholic acid is epimerized to chenodiol [CDCA] via a 7-oxo intermediate. Chenodiol also undergoes 7-dehydroxylation to form lithocholic acid. These metabolites are poorly soluble and excreted in the feces. A small portion of lithocholic acid is reabsorbed, conjugated in the liver with glycine, or taurine and sulfated at the 3 position. The resulting sulfated lithocholic acid conjugates are excreted in bile and then lost in feces. In healthy subjects, at least 70% of ursodeoxycholic acid [unconjugated] is bound to plasma protein. No information is available on the binding of conjugated ursodeoxycholic acid to plasma protein in healthy subjects or patients. Its volume of distribution has not been determined, but is expected to be small since the drug is mostly distributed in the bile and small intestine. Ursodeoxycholic acid is excreted primarily in the feces. With treatment, urinary excretion increases, but remains less than 1% except in severe cholestatic liver disease. During chronic administration of urso [ursodiol] diol, it becomes a major biliary and plasma bile acid. At a chronic dose of 13 to 15 mg/kg/day, Ursodeoxycholic acid constitutes 30-50% of biliary and plasma bile acids.

The rationale of this present bioequivalence study for two formulations of 500mg Ursodeoxycholic acid tablets was examined between generic drug Ursodeoxycholic acid 500mg tablets as the test product and URSO FORTE™ (Forest Labs Inc) as the reference product. This bioequivalence study could give assurance when prescribing less expensive generic drugs as alternatives with similar efficacy and safety.

The study objectives of this present study are to assess the single dose bioequivalence of Ursodeoxycholic acid 500mg tablets With URSO FORTE™ (Forest Labs Inc) in healthy, adult, human study participants under fed conditions and to monitor the clinical status, adverse events and laboratory investigations and assess relative safety and tolerance of Ursodeoxycholic Acid formulations under fed conditions.

MATERIALS AND METHODS

According to the USFDA Regulatory individual product recommendations, two studies (Fed and Fasting) to be done with 500mg Ursodeoxycholic Acid tablets to obtain marketing authorization in USA.

USFDA Waiver request of in-vivo testing [4]: 250 mg based on (i) acceptable bioequivalence studies on the 500 mg strength, (ii) proportionally similar across all strengths, and (iii) acceptable in vitro dissolution testing of all strengths.

Study drugs

Ursodeoxycholic acid 500mg tablets and URSO FORTE™ (Ursodiol-500mg) from Forest Labs Inc. were used as the test and the reference products respectively. Both products were prepared as Ursodeoxycholic acid tablets equivalent to Ursodeoxycholic acid 500mg. Both the products were stored at controlled room temperature 25˚C (77 ˚F).

Study population

The study was carried out at ClinSync clinical Research Private Limited, India. The study protocol was approved by the Ethics Committee. In addition, the protocol was performed in accordance with the Declaration of Helsinki Principles [5] as outlined in the ICH-E6 Guidelines for Good Clinical Practice (GCP) [6]. All subjects were given a detailed description of the study and written informed consent was obtained prior to the enrollment.

The sample size was estimated based on, Coefficient of variation (C.V.) of the drug, sufficient statistical power to detect 20% difference with the power of 0.8 in C_{max} and AUC between the test and reference product, Regulatory requirements.

Sample size was based on estimates obtained from reported literature and previous studies. Assuming a formulation ratio (T/R) ranging from 0.95-1.05 a sample of 12 subjects including dropouts would be sufficient to show bioequivalence between the two formulations with a power of at least 80%. Hence sample size of 12 subjects was enrolled in the study.

12 healthy male volunteers between the ages of 18-45 years with a body mass index between 18.5 kg/m^2 and 24.9 kg/m^2, with body weight equal to or not less than 50 kg were assessed to be in good physical condition by a complete medical screening including a medical history, physical examination and laboratory screening test for hematologic and blood biochemistry parameters. Subjects with a history of hypersensitivity to any ingredients in the Ursodeoxycholic acid products and/or related drugs or its constituents or who were taking any medication or alcohol for a 21-day period prior to the study were excluded. Subjects who had a history of cardiovascular, hepatic, renal, gastrointestinal or hematologic disease were excluded from the study.

Study design

The study was an open-labeled, single-dose, study taken with food, two-treatment, two-period, two-sequence randomized two way crossover with at least one week washout period. Subjects were randomly allocated to two groups by the sequence of product administered [Test-Reference (TR) and Reference-Test (RT) group]. In each period, 1X500mg tablet of Ursodeoxycholic acid of the test or reference product was administered 30 minutes after starting a high fat, high calorie breakfast at the same time in the morning before dosing. Subjects were housed 64 hours prior to dosing in the clinical facility from a time adequate to ensure 10 hours supervised fasting before consuming high fat breakfast and were allowed to leave the facility after 24.00 hours post-dose sample in each period.

A standard breakfast was provided to the subjects 30 minutes prior to the -48, -24 and 0 hour sample collection time points. The subjects received a standard meal at about 4.0, 9.0 and 13.0 hours after dosing in each period. During housing, all meal plans were identical for all the periods. Drinking water was not allowed from one hour before dosing till one hour post-dose (except for 240 ± 02 mL of drinking water given for dosing). Before and after that, drinking water was allowed at *ad libitum*. After a minimum of 1 week washout period, the subjects were crossed over to the next treatment following the same procedure as conducted in the 1st period.

Sample collection

During dosing day in each period, 29 blood samples will be collected as per the following schedule: Pre dose samples -48, -42, -36, -30, -24, -18, -12, -6, and 0 hours (within 02 hrs) prior to drug administration and the others at 10min, 20min, 30min, 40min, 50min, 60min, 70min, 80min, 90min, 100min, 110min, 2.00hr, 2.50hr, 3.0hr, 4.00hr, 6.00hr, 8.00hr, 10.00hr, 12.00hr and 24.00hours post dose. The total volume collected per study participant in this study will not exceed approximately 321 mL including up to 9 mL for screening, and 7-9 mL for post clinical assessment of lab parameters and 18 mL for discarded blood sample resulting from use of intravenous cannula for 12 hours and 2-9 mL was collected for repeat/additional lab tests, if required. For separating plasma, all blood samples were centrifuged at 3800 RPM for 10 minutes at 4°C ± 2°C.

Centrifugation of all samples was done as early as possible after each sample draw time point. After centrifugation, plasma samples were aliquoted into two sets in properly labeled polypropylene tubes and immediately stored at about -60°C or colder.

Ursodeoxycholic Acid analysis by LC-MS/MS

The published LC-MS/MS method [7] was validated according to USFDA regulations [8] for quantification of telmisartan from extracted subject plasma samples. Retrieved the frozen CC, QC samples and subject samples from the deep freezer and thawed in water bath maintained at room temperature, vortexed to mix. Removed the caps from the polypropylene tubes. Aliquoted 0.100mL (100µL) of CC, QC samples and subject samples into pre-labelled Polypropylene tubes. Added 30 µL of ISTD dilution (about 1.5µg/mL), and vortexed to mix. Added 1.0 mL of Extraction solvent (Acetonitrile), vortexed for 5min. Centrifuged the polypropylene tubes at 14,000 rpm and 10° C for 5 min, transferred approximately 0.800 mL of supernatant to prelabelled HPLC vials, then to the auto sampler.

The High-performance liquid chromatography (HPLC) SILHTC system (Shimadzu Corporation, Kyoto, Japan) is equipped with LC-20 AD VP binary pump, A DGU 20A3 Degasser, and a SIL-HTC auto sampler equipped with A CTO-10AS VP thermo stated column[9-11]. The chromatography was carried isocratically at room temperature

using a Thermo Bio basic (C4, 5 µm, 150×4.6 mm) column. The mobile phase consisted of 40:40:20; Methanol: Acetonitrile: 10mM Ammonium Acetate Buffer. The flow-rate was 0.4 ml/min. The duration of the analytical time was 5 min. The analytical column effluent is directed through the divert valve to a thermo electron TSQ quantum discovery mass spectrometer [12-14].

Chromatograms were acquired on a TSQ tandem mass spectrometry (Thermo Finnegan, Sanjose, CA, USA) equipped with Electrospray ionization (ESI) and connected to a PC runs with the standard software Xcalibur 2.0.7 and LC Quan 2.5.6 [15-16]. Mass spectroscopic detection was performed on a Triple quadrapole instrument (Thermo, TSQ Quantum Discovery Max). Robotic liquid handling system is operated using the software package. The calibration curve is constructed by weighted $1/x^2$ least-square linear regression analysis of the peak area ratio (drug/ISTD) vs. the concentration of drug [17-18].

Pharmacokinetic and statistical analysis [19-21]

For the purpose of Average Bioequivalence analysis C_{max}, AUC_{0-t} and AUC_{0-inf} were considered as the primary variables and T_{max}, $t_{1/2}$ and K_{el} were considered as the secondary variables. General Linear Model for analysis of variance (ANOVA) for crossover design was performed for log-transformed data and used to assess the effect of formulations, periods, sequences and subjects nested in sequence on these parameters. The difference between two related parameters was considered statistically significant for a *p*-value equal to or less than 0.05. 90% confidence interval (CI) for the ratios of geometric mean Test/Reference (T/R) for C_{max}, AUC_{0-t} and AUC_{0-inf} was calculated based on least squares means from the ANOVA of log-transformed data.

The 90% geometric CI of the ratio (T/R) of least squares means from the ANOVA of the log-transformed C_{max}, AUC_{0-t} and AUC_{0-inf} should be within 80.00% to 125.00%.

Tolerability assessment

Physical examination and measurement of vital signs (Blood Pressure, Pulse Rate and Oral Temperature) were examined at the time of Check-in, prior to administration of the each study drug (i.e. -48, -42, -36, -30, -24, -18, -12, -6, 0.00 hr), 1.00, 3.00, 6.00, 12.00 and 24.00hours post dose and during the entire study period. Adverse events were monitored throughout the study and recorded by physicians.

RESULTS

Study population

12 healthy male adults eligible for the study enrollment were randomly divided into 2 groups [Test-Reference (TR) and Reference-Test (RT)] according to the sequence of drug administration. All the subjects had completed both the periods. Thus, this study was balanced in each sequence and the results from 12 volunteers were used for pharmacokinetic and statistical analysis. Table 1 demonstrates the demographic characteristics of the volunteers.

Bioanalysis and pharmacokinetics

The instrument is operated in the Negative ion mode. The precursor ions at 391.280 m/z and 293.023 m/z for Ursodeoxy Cholic Acid and Diclofenac respectively are selected by the first quadrupole (Q1). After collision-induced fragmentation in Q2, the product ions at 391.280 m/z and 250.032 m/z for Ursodeoxy Cholic Acid and Diclofenac, respectively, are monitored in Q3. A resolution of one unit (at half peak height) is used for both Q1 and Q3. The method was fully validated using these Q1 and Q3 masses for both analyte and IS with satisfactory results. *Linear calibration curves* were obtained with a coefficient of correlation (r^2) usually higher than 0.995 in range of 0.1-3.05 µg/ml**.** For each calibration standard level, the concentration was back calculated from the linear regression curve equation.

No significant difference was observed in any of the analyzed pharmacokinetic parameters for Ursodeoxycholic acid was shown in Table 2. The mean of the pre-dose ursodeoxycholic acid was used for the baseline adjustment of the post-dose levels. Baseline concentrations were determined for each dosing period, and baseline corrections were period specific. If a negative plasma concentration value results after baseline correction, these were changed to 0 prior to calculating the baseline corrected AUC.

Table 1: Demographic characteristics

Category		Treatment		Total
		Test (T)	Reference (R)	
Age (years)	Mean ± SD	23.84 ± 4.10	23.84 ± 4.00	23.84 ± 4.05
	Range	18.0 – 36.0	19.0 – 36.0	18.0 – 36.0
	Median	23.0	23.0	23.0
	N	12	12	24
Age Groups	< 18	00	00	00
	18 – 40	12	12	24
	41 – 64	00	00	00
	65 – 75	00	00	00
	> 75	00	00	00
Gender	Female	00	00	00
	Male	12	12	24
Race	American	00	00	00
	Hispanic	00	00	00
	Caucasian	00	00	00
	Asian	12	12	24
Height (cm)	Mean ± SD	163.52 ± 5.69	164.24 ± 5.67	165.48 ± 5.67
	Range	157.0 – 174.0	159.0 – 177.0	157.0 – 177.0
	N	12	12	24
Weight (kg)	Mean ± SD	58.96 ± 6.24	61.56 ± 6.43	60.26 ± 6.41
	Range	52.0 – 70.0	52.0 – 77.0	52.0 – 77.0
	N	12	12	24
BMI (kg/m^2)	Mean ± SD	21.86 ± 1.46	22.10 ± 1.79	21.98 ± 1.62
	Range	20.1 – 24.8	20.0 – 24.9	20.0 – 24.9
	N	12	12	24

Table 2: Pharmacokinetic Parameters of Ursodeoxycholic Acid for Both Formulations

PK Parameters	**Formulation [UDCA]**	
	Test	**Reference**
C_{max} [ng/mL]	2741.258	2454.362
AUC_{0-t} [ng.h/mL]	15613.320	17575.245
AUC_{0-inf} [ng.h/mL]	17818.380	25788.118
T_{max} [H]	2.138	2.653
K_{el} [H^{-1}]	0.157	0.112
$T_{1/2}$ [H]	6.064	12.453

Bioequivalence analysis

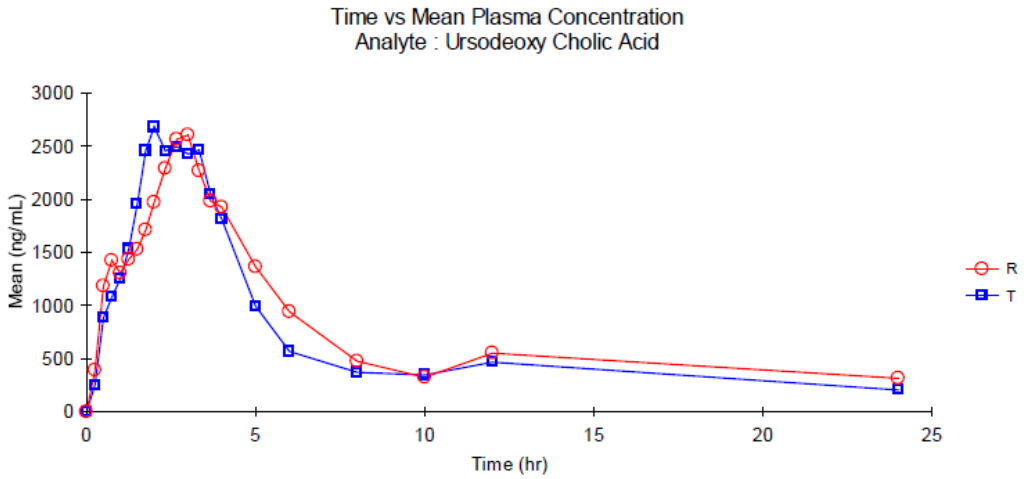
Ninety percent confidence interval of geometric mean ratios of bioavailability parameters between the test and reference formulation are presented in Table 3. The statistical analysis obtained from this study showed that the point estimate (90% CI) of the geometric mean ratio (GMR) (T/R) of C_{max}, AUC_{0-t} and AUC_{0-inf} was entirely within the equivalence criteria (80.00-125.00%) which was 111.69% (91.86%-119.53%) for C_{max} ratios, 88.84% (85.74%-109.49%) for AUC_{0-t} ratios and 92.36% (90.49%-107.97%) for AUC_{0-inf} ratios of Ursodeoxycholic Acid.

Table 3: Bioequivalence Parameters for Ursodeoxycholic Acid

Parameter	**UDCL**		
	C_{max}	AUC_t	AUC_{inf}
90% CI Lower Limit	91.86	85.74	90.49
90% CI Upper Limit	119.53	109.49	107.97
T/R Ratio (%)	111.69	88.84	92.36
Power	0.91	0.92	1
Intra Subject Variability	10.12	3.2	5.1
Inter Subject Variability	28.64	45.11	49.56
ANOVA (p-Value)			
Sequence	0.1	0.2	0.2
Period	0.8	0.4	0.4
Treatment	0.5	0.5	0.6

In addition, no significant difference of the T_{max} parameter between the two studied formulations was observed ($p > 0.05$). Therefore, it was concluded that the two formulations of Ursodeoxycholic Acid were bioequivalent in terms of rate and extent of absorption for the drug. The mean plasma concentration vs time profiles were given in Fig 2.

Fig 2: Time vs. Mean Plasma Concentration Graph of Ursodeoxycholic Acid



Tolerability

Almost all volunteers taking both Ursodeoxycholic acid formulations were noted for mild adverse events. Most common events were drowsiness, nausea and loss of appetite. However, no subject had any severe adverse event or withdrew from the study because of an adverse event.

DISCUSSION

An open-labeled, single-dose with food, two-treatment, two-period, two-sequence randomized two way crossover design in 12 healthy adult volunteers was considered appropriate and standard for bioequivalence evaluation of the generic and the reference products. The study simulates real life conditions including the influence of meals as well as circadian effects on the performance of the product. For a safety reason, co-administration of the drug with food can reduce nausea, a common side effect of Ursodeoxycholic acid.

In general, the pharmacokinetic parameters for both formulations were similar to the pharmacokinetic parameters of Ursodeoxycholic Acid in previous published data. This study demonstrated that 90% CI of the logarithmic transformed of parameters C_{max}, AUC_{0-t} and AUC_{0-inf} were contained in 80.00-125.00%. In addition, no significant differences of the T_{max} values between the two formulations were observed ($p>0.05$). Therefore, the two formulations of Ursodeoxycholic Acid are considered bioequivalent in terms of the rate and extent of absorption. Moreover, both formulations were well tolerated. Hence, the test (Ursodeoxycholic Acid) and reference **(**URSOFORTE**)** formulations of Ursodeoxycholic Acid 500mg are bioequivalent.

CONCLUSION

This single dose study found that the test formulation Ursodeoxycholic Acid tablets is bioequivalent to the reference formulation URSO FORTE^{TM} Ursodiol tablets the extent and the rate of absorption, of 500mg under fed condition in healthy adult male volunteers according to the USFDA regulatory guidance.

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