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REVIEW ARTICLE

BIOEQUIVALENCE AND PHARMACOKINETIC COMPARISON BETWEEN CLONIDINE HYDROCHLORIDE TABLETS 0.3MG: AN OPEN LABEL, BALANCED, RANDOMIZED-SEQUENCE, SINGLE-DOSE, TWO-PERIOD CROSSOVER STUDY IN HEALTHY MALE VOLUNTEERS

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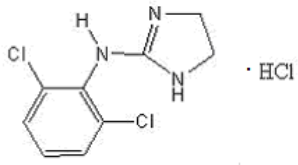
Abstract: **Background:** This present bioequivalence study was designed to determine the pharmacokinetic, bioavailability and bioequivalence of Clonidine Hydrochloride 0.3mg Tablets In Comparison With Catapres™ Clonidine Hydrochloride 0.3mg Tablets after single dose administration under fasting conditions 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. **Methods:** 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 0.3mg 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 96 hours after dosing. Analysis of clonidine 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.

Results: 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 0.3mg tablets under fasting condition were 95.49% (89.92%-116.23%) for C_{max} ratios, 82.51% (91.41%-110.5%) for AUC_{0-t} ratios and 94.93% (93.64%-108.96%) for AUC_{0-inf} ratios of Clonidine. 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. **Conclusion:** This single dose study found that the test formulation Clonidine Hydrochloride 0.3mg Tablets Are Bioequivalent to the Reference Formulation Catapres™ Clonidine Hydrochloride 0.3mg Tablets In terms of extent and rate of absorption, under fasting condition in healthy adult male volunteers according to the USFDA regulatory guidance.

Keywords: Clonidine Hydrochloride, Bioavailability, Bioequivalence, Intrasubject Variability

INTRODUCTION:

Clonidine hydrochloride is a centrally acting alpha-agonist hypotensive agent available as tablets for oral administration in three dosage strengths: 0.1 mg, 0.2 mg and 0.3 mg. The 0.1 mg tablet is equivalent to 0.087 mg of the free base [1]. Clonidine hydrochloride is an imidazoline derivative and exists as a mesomeric compound. The chemical name is 2-[2, 6-dichlorophenylamino]-2-imidazoline hydrochloride. The following is the structural formula: $C_9H_9Cl_2N_3.HCl$ with molecular weight 266.56 g/mol and the following structure:



Clonidine hydrochloride is an odorless, bitter, white, crystalline substance soluble in water and alcohol. Clonidine acutely stimulates growth hormone release in both children199-202 and adults, but does not produce a chronic elevation of growth hormone with long-term use. The plasma level of Clonidine peaks in approximately 3 to 5 hrs and the plasma half-life ranges from 12 to 16 hrs. The half-life increases up to 41 hrs in patients with severe impairment of renal function. Following oral administration about 40-60% of the absorbed dose is recovered in the urine as unchanged drug in 24 hrs. About 50% of the absorbed dose is metabolized in the liver. Neither food nor the race of the patient influences the pharmacokinetics of Clonidine.

The rationale of this present bioequivalence study for two formulations of 0.3mg Clonidine Hydrochloride Tablets was examined between generic drug Clonidine Hydrochloride 0.3mg Tablets as the test product and Catapres™ (Boehringer Ingelheim) 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 Clonidine Hydrochloride 0.3mg Tablets With Catapres™ (Boehringer Ingelheim) in healthy, adult, human study participants under fasting conditions and to monitor the clinical status, adverse events, laboratory investigations and assess relative safety and tolerance of Clonidine Hydrochloride formulations under fasting conditions.

MATERIALS AND METHODS

According to the USFDA Regulatory individual product recommendations, One study (Fasting) to be done with 0.3mg Clonidine Hydrochloride Tablets to obtain marketing authorization in USA. USFDA Waiver request of in-vivo testing [2]: 0.1 mg and 0.2 mg based on (i) acceptable bioequivalence study on the 0.3 mg strength, (ii) proportional similarity of the formulations across all strengths, and (iii) acceptable in vitro dissolution testing of all strengths.

Study drugs

Clonidine Hydrochloride 0.3mg Tablets and Catapres™ from Boehringer Ingelheim were used as the test and the reference products respectively. Both products were prepared as Clonidine Hydrochloride Tablets Equivalent to Clonidine Hydrochloride 0.3mg. 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 [3] as outlined in the ICH-E6 Guidelines for Good Clinical Practice (GCP) [4]. 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 Clonidine 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, 1X0.3mg tablet of clonidine Hydrochloride 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 12 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 postdose sample in each period. 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, 21 blood samples (6 mL each) will be collected as per the following schedule: Pre dose sample(0.00 hr) within 02 hrs prior to drug administration and the others at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 3.00, 4.00, 6.00, 8.00, 10.00, 12.00, 16.00, 20.00, 24.00, 48.00, 72.00 and 96 hours 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.

Clonidine analysis by LC-MS/MS

The published LC-MS/MS method [5] was validated according to USFDA regulations [6] for quantification of clonidine from extracted subject plasma samples. Plasma samples (0.500 mL) were pipetted into a 5-mL Ria vials, 50 µL of IS working solution (500 ng/mL) and 100µL 10mM Ammonium Acetate pH 6.8 were added. After vortex mixing for 1 min's, a 2.0-mL 80:20 Diethyl ether: Dichloromethane was added and the samples were vortex-mixed for 10 min's. Centrifuged the Ria vials at 4000 rpm at 10°C for 10 min, transferred approximately 1.6mL of supernatant to prelabelled glass vials and evaporated to dryness using nitrogen evaporator maintained at 37°C. After completion of evaporation reconstituted the Ria vials containing drug using 150µL of 80:20 (50:50 Methanol: Acetonitrile):10mM Ammonium Acetate (pH 6.8), vortexed, transferred to HPLC vials and a 10-µL aliquot was injected into the chromatographic system.

The high-performance liquid chromatography (HPLC) SILHTC system (Shimadzu Corporation, Kyoto, Japan)[7-10] is equipped with LC-20 AD VP binary pump, a DGU20A3 Degasser, and a SIL-HTC auto sampler equipped with a CTO-10AS VP thermostated column. The chromatography[7-8] was on Cohesive PropelC18, (5 µm, 3.0 x 50mm) at a temperature of 20°C. The isocratic mobile phase composition was a mixture of 80:20 (50:50 Methanol: Acetonitrile):10mM ammonium acetate (pH 6.8), which was pumped at a flow rate of 0.35 mL/min[11-14]. Mass spectrometric detection was performed on a TSQ Quantum Discovery MAX triple quadrapole instrument (Thermo Finnigan, USA) using the Selective reaction monitoring (SRM) mode. A turbo electrospray ionization (ESI) interface in positive mode was used. Data processing was performed on LC Quan 2.5.6. Software package (Thermo)[15-17].

Pharmacokinetic and statistical analysis [18-19]

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 (0.00 hr), 1.00, 3.00, 6.00, 12.00, 24.00, 36.00, 48.00, 72.00 and 96 hours 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.

Table 1: Demographic characteristics

Category		Treatment		Total
		Test (T)	Reference (R)	
Age (years)	*Mean ± SD*	25.18 ± 3.10	25.23 ± 3.59	23.21 ± 3.34
	Range	19.0 – 38.0	19.0 – 38.0	19.0 – 38.0
	Median	28.0	28.0	28.0
	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	168. 23± 6.79	168.25 ± 6.67	168.2 ± 6.73
	Range	156.0 – 174.0	156.0 – 175.0	156.0 – 175.0
	N	12	12	24
Weight (kg)	Mean ± SD	56.90 ± 5.26	60.54 ± 5.34	58.73 ± 5.3
	Range	52.0 – 70.0	52.0 – 73.0	52.0 – 73.0
	N	12	12	24
BMI (kg/m^2)	Mean ± SD	20.68 ± 1.27	20.80 ± 1.79	20.70 ± 1.53
	Range	20.1 – 24.6	20.0 – 24.8	20.0 – 24.8
	N	12	12	24

Bioanalysis and pharmacokinetics

The Mass instrument is operated in the positive ion mode. The precursor ions at m/z 230.200 for Clonidine and m/z 531.200 for Ketoconazole are selected by the first quadrupole (Q1). After collision-induced fragmentation in Q2, the product ions at m/z 213.900 for Clonidine and m/z m/z 243.400 Ketoconazole 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 compounds with satisfactory results. *Linear calibration curves* were obtained with a coefficient of correlation (r^2) usually higher than 0.996 in range of 50 pg/mL–500 ng/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 Clonidine was shown in Table 2.

Table 2: Pharmacokinetic Parameters of Clonidine for Both Formulations

PK Parameters	Formulation [Clonidine]	
	Test	Reference
C_{max} [ng/mL]	187.018	195.850
AUC_{0-t} [ng.h/mL]	3201.390	3880.033
AUC_{0-inf} [ng.h/mL]	5200.511	5478.147
T_{max} [H]	8.376	4.175
K_{el} [H^{-1}]	0.069	0.035
$T_{1/2}$ [H]	4.600	2.600

Bioequivalence analysis

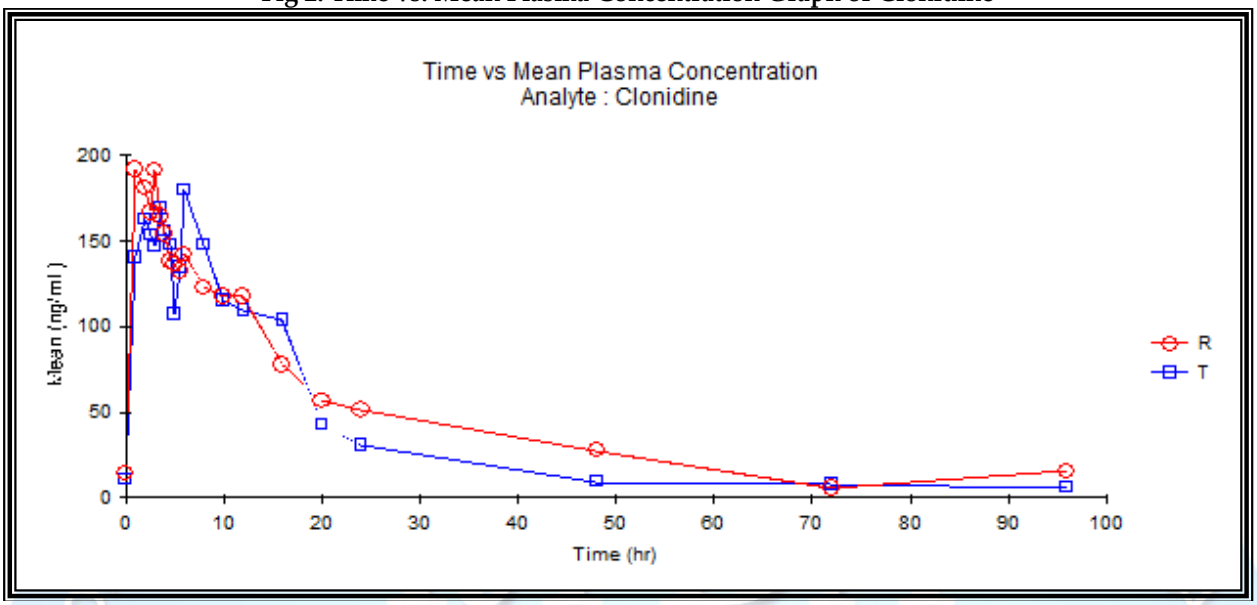
Ninety percent confidence interval of geometric mean ratios of bioavailability parameters between the test and reference formulation are presented in Table 3. The statistical analysis obtained from this study showed that the point estimate (90% CI) of the geometric mean ratio (GMR) (T/R) of C_{max}, AUC_{0-t} and AUC_{0-inf} was entirely within the equivalence criteria (80.00-125.00%) which was 95.49% (89.92%-116.23%) for C_{max} ratios, 82.51% (91.41%-110.5%) for AUC_{0-t} ratios and 94.93% (93.64%-108.96%) for AUC_{0-inf} ratios of Clonidine.

Table 3: Bioequivalence Parameters for Clonidine

Parameter	Clonidine		
	C_{max}	AUC_t	AUC_{inf}
90% CI Lower Limit	89.92	91.41	93.64
90% CI Upper Limit	116.23	110.5	108.96
T/R Ratio (%)	95.49	82.51	94.93
Power	1	0.9	1
Intra Subject Variability	13.67	4.5	4.1
Inter Subject Variability	28.67	50.6	51.44
ANOVA (p-Value)			
Sequence	0.1	0.1642	0.1759
Period	0.9	0.5	0.4
Treatment	0.543	0.514	0.697

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 Clonidine Hydrochloride 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 Clonidine



Tolerability

Almost all volunteers taking both Clonidine Hydrochloride 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 Clonidine Hydrochloride.

In general, the pharmacokinetic parameters for both formulations were similar to the pharmacokinetic parameters of Clonidine Hydrochloride 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 Clonidine Hydrochloride are considered bioequivalent in terms of the rate and extent of absorption. Moreover, both formulations were well tolerated. Hence, the test (Clonidine Hydrochloride) and reference (Catapres™) formulations of Clonidine Hydrochloride 0.3mg are bioequivalent.

CONCLUSION

This single dose study found that the test formulation Clonidine Hydrochloride Tablets is bioequivalent to the reference formulation Catapres™ Clonidine Hydrochloride tablets the extent and the rate of absorption, of 0.3mg under fasting condition in healthy adult male volunteers according to the USFDA regulatory guidance.

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