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A comparative, Bioequivalence study to evaluate the safety and pharmacokinetic profile of single dose Cinacalcet Hydrochloride Tablets in healthy, adult, human subjects under fed conditions

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ABSTRACT

This study compares the safety and pharmacokinetics parameters of Cinacalcet Hydrochloride Tablets 90mg with the innovator (reference) product, on the basis that if two formulations exhibit similar drug concentration-time profiles in the blood/plasma, they should exhibit similar therapeutic effects. An open label, balanced, analyst blind, randomized, two-treatment, two-period, two sequence, single dose, crossover oral bioequivalence study of Cinacalcet Hydrochloride Tablets 90mg manufactured by Macleods Pharmaceuticals Ltd., India comparing with Sensipar™ (Cinacalcet Hydrochloride) Tablets 90mg manufactured by Amgen USA Inc. on 24 + 4 (standby) healthy, adult, human subjects under fed conditions. To monitor the safety and tolerability of a single dose of the test product as compared to the reference product in healthy adult male human subjects under fed condition. In the following sections, requirements for the design and conduct of comparative bioavailability studies are formulated. Investigator(s) should have appropriate expertise, qualifications and competence to undertake a proposed study and is familiar with pharmacokinetic theories underlying bioavailability studies. The design should be based on a reasonable knowledge of the pharmacodynamics and/or the pharmacokinetics of the active substance. The aim of a bioequivalence study is to demonstrate equivalence within the acceptance range regarded as clinically relevant. In conclusion, the test formulation is bioequivalent to the reference in terms of both the rate and extent of absorption. Both the formulations are well tolerated following a single dose administration of the investigational product. No serious clinical adverse events causing death, disability, hospitalization, or dropouts of the subjects were encountered.

Keywords: Bioequivalence study, Cinacalcet Hydrochloride, Bioavailability.

INTRODUCTION

Secondary hyperparathyroidism commonly occurs in patients with chronic kidney disease and is characterized by elevated levels of serum parathyroid hormone (PTH) and abnormalities in bone

and mineral metabolism. The changes in bone and mineral metabolism result from abnormalities in the regulation of the intracellular and extracellular levels of PTH, calcium, phosphorus, and vitamin D.

Treatment is targeted at controlling abnormal PTH, calcium, and phosphorus levels and preventing further complications (eg, symptomatic bone disease, extraskeletal calcifications, death). Traditional forms of therapy for secondary hyperparathyroidism have included calcium supplementation, dietary phosphorus restriction, phosphate-binding agents, vitamin D or its analogs, and surgery. These forms of therapy are mainly focused at altering PTH, calcium, and phosphorus concentrations. None of these forms of therapy have worked directly on the parathyroid or the calcium-sensing receptors within the parathyroid gland. The secretion of parathyroid hormone, whose primary role is to maintain calcium levels by the parathyroid, is regulated by changes in extracellular calcium concentrations. When the concentration of extracellular calcium is increased, the amount of parathyroid hormone secreted by the parathyroid is reduced. When the concentration of extracellular calcium is decreased, the amount of parathyroid hormone secreted is increased. This autoregulation is controlled by calcium-sensing receptors within the parathyroid gland. Elevation of parathyroid hormone and hyperphosphatemia, such as that observed in patientswith chronic kidney disease, results in alterations in bone production, decreased renal tubular resorption of phosphorus, increased renal tubular reabsorption of calcium, and alterations in the gastrointestinal absorption of calcium and phosphorus. These changes increase the concentration of the extracellular calcium and decrease the concentration of the phosphorus.Cinacalcet hydrochloride is an oral calcimimetic agent. Calcimimetic compounds bind to and modulate the calcium-sensing receptors on the parathyroid gland. Their net effect is to increase the sensitivity of the receptors to calcium levels in the blood, resulting in a reduction in PTH secretion from the parathyroid gland. The activity of cinacalcet depends on both the presence of calcium and the dose of drug. In animal studies, cinacalcet hydrochloride produced a dose dependent reduction in ionized calcium levels. In humans, it produces dose-dependent reductions in serum parathyroid hormone and ionized calcium levels and increases serum calcitonin levels. The mobilization of intracellular calcium depends on the presence of extracellular calcium. Cinacalcet hydrochloride is about 30-fold more potent at lowering serum parathyroid hormone levels than it is at increasing serum calcitonin levels. The R-cinacalcet enantiomer is at least 75-fold more active than the S-enantiomer.

Pharmacokinetics

Maximum cinacalcet plasma concentrations are reached within approximately 2 to 6 hours following oral administration. The peak concentration and area under the curve (AUC) were increased 82% and 68%, respectively, when cinacalcet hydrochloride was administered with a high-fat meal compared with fasting. The peak concentration and AUC were increased 65% and 50%, respectively, when cinacalcet hydrochloride was administered with a lowfat meal compared with fasting. Cinacalcet concentrations decline in a biphasic fashion with a terminal half-life of 30 to 40 hours. Steady-state levels are reached within 7 days. The mean accumulation ratio is approximately 2 with once-daily oral administration. The median accumulation ratio is approximately 2 to 5 with twice-daily oral administration. The AUC and peak concentration increase proportionately over the dose range of 30 to 180 mg once daily. The volume of distribution is about 1,000 L, representing extensive distribution. Cinacalcet is approximately 93% to 97% bound to plasma proteins.

Cinacalcet undergoes metabolism by CYP3A4, CYP2D6, and CYP1A2. Plasma concentrations of the major metabolites greatly exceed cinacalcet concentrations; however, the major metabolites are inactive or have very little activity compared with cinacalcet. The metabolites are primarily excreted renally. Following administration of a radiolabeled dose, 80% of the dose was recovered in the urine and 15% in the feces.

Indications and Uses

Cinacalcet is indicated for the treatment of secondary hyperparathyroidism in patients with Chronic Kidney Disease on dialysis. Cinacalcet is indicated for the treatment of hypercalcemia in patients with parathyroid carcinoma.

Adverse Reactions

The most commonly reported side effects with Cinacalcet were nausea and vomiting. Other side effects include: diarrhea, muscle pain, dizziness, high blood pressure, weakness and tiredness, loss of appetite.

Drug Interactions

Cinacalcet is metabolized by multiple cytochrome P-450 isozymes, primarily CYP3A4, CYP2D6, and CYP1A2. Cinacalcet is also a potent inhibitor of CYP2D6. Therefore, dose adjustments may be required of concomitant medications that are predominantly metabolized by CYP2D6 and have a narrow therapeutic index, including flecainide, vinblastine, thioridazine, and most tricyclic antidepressants. For example, administration of cinacalcet with amitriptyline reportedly increases amitriptyline and nortriptyline (active metabolite formed from amitriptyline) exposure by approximately 20% in CYP2D6 extensive metabolizers. Also, coadministration with ketoconazole, a strong inhibitor of CYP3A4, increases cinacalcet exposure by more than twofold. Therefore, if a patient taking cinacalcet initiates or discontinues therapy with a strong CYP3A4 inhibitor (e.g., ketoconazole, erythromycin, and itraconazole), dose adjustments of cinacalcet may be required, and PTH and calcium concentrations should be monitored closely. The pharmacokinetics of warfarin reported are not affected in patients using cinacalcet along with this drug. The lack of effect of cinacalcet on the pharmacokinetics of r- and s-warfarin and the absence of autoinduction upon multiple dosing in patients indicates that cinacalcet is not an inducer of CYP2C9 in humans. No significant pharmacokinetic interaction was observed when calcium carbonate (1,500 mg), pantoprazole (80 mg), or sevelamer HCl (2,400 mg) was coadministered with 100 mg of cinacalcet.

Precautions and Contraindications

Hypocalcemia

Cinacalcet lowers serum calcium, and therefore patients should be carefully monitored for the occurrence of hypocalcemia. Potential manifestations of hypocalcemia include paresthesias, myalgias, cramping, tetany, and convulsions. Since the seizure threshold can be lowered by significant reductions in serum calcium levels, these levels should be checked closely in patients receiving cinacalcet, particularly in those patients with a history of a seizure disorder.

Cinacalcet treatment should not be initiated if serum calcium is less than the lower limit of the normal range (8.4 mg/dL). Serum calcium should be measured within 1 week after initiation or

dose adjustment of Cinacalcet Once the maintenance dose has been established, serum calcium should be measured approximately monthly.

If serum calcium falls below 8.4 mg/dL but remains above 7.5 mg/dL, or if symptoms of hypocalcemia occur, calcium-containing phosphate binders and/or vitamin D sterols can be used to raise serum calcium. If serum calcium falls below 7.5 mg/dL, or if symptoms of hypocalcemia persist and the dose of vitamin D cannot be increased, withhold administration of Cinacalcet until serum calcium levels reach 8.0 mg/dL, and/or symptoms of hypocalcemia have resolved. Treatment should be re-initiated using the next lowest dose of Cinacalcet.

Adynamic Bone Disease
Adynamic bone disease may develop if iPTH levels are suppressed below 100 pg/mL. The dose of Cinacalcet should be reduced or therapy discontinued.

Hepatic Insufficiency
Cinacalcet exposure as assessed by AUC $_{(0\text{-inf})}$ in patients with moderate and severe hepatic impairment (as indicated by the Child-Pugh method) were 2.4 and 4.2 times higher, respectively, than that in normals. Patients with moderate and severe hepatic impairment should be monitored throughout treatment with Cinacalcet.

Study Rationale
The Macleods Pharmaceuticals Ltd. has developed generic alternative to the reference-listed brand of Cinacalcet Hydrochloride tablets therefore, its bioequivalence to the reference brand must be evaluated. The single dose of test product Cinacalcet Hydrochloride 90 mg Tablets (each tablet contains Cinacalcet 90 mg) manufactured by Macleods Pharmaceuticals Ltd., India was compared with Sensipar™ (Cinacalcet Hydrochloride) Tablets 90mg (each tablet contains Cinacalcet 90 mg) of Manufactured by Amgen USA Inc.

Justification of Choice of Reference Product
Sensipar™ 90 mg tablet is qualified as acceptable reference product.

MATERIALS AND METHODS

Study Design
An open label, balanced, analyst blind, randomized, two-treatment, two-period, two sequence, single dose, crossover bioequivalence study on 24 + 4 (standby) healthy, adult, human subjects under fed conditions.

Objectives
i) *Pharmacokinetic*: To evaluate the comparative oral bioavailability of single dose Cinacalcet Hydrochloride Tablets 90mg (each tablet contains Cinacalcet 90 mg) manufactured by Macleods Pharmaceuticals Ltd., India with Sensipar™ (Cinacalcet Hydrochloride) Tablets 90mg (each tablet containing Cinacalcet 90mg) Manufactured by Amgen USA Inc. in healthy, adult, human subjects under fed conditions.

ii) *Safety*: To monitor the safety and tolerability of a single dose of Cinacalcet Hydrochloride Tablets 90 mg when administered in healthy adult, human subjects.

Investigational products

Test Formulation (T): Cinacalcet Hydrochloride Tablet 90mg (each tablet contains Cinacalcet 90 mg), Manufactured by Macleods Pharmaceuticals Ltd., India. Dose-90mg (1 tablet), Mode of administration: Administered orally with 240 ml of drinking water.

Reference Formulation (R): Sensipar™ (Cinacalcet Hydrochloride) Tablets 90mg (each tablet containing Cinacalcet 90mg), Manufactured by: Amgen USA Inc.Dose-90mg (1 tablet), Mode of administration: Administered orally with 240 ml of drinking water.

Number of Subjects (planned and analysed)

A total of 24 + 4 (stand by) subjects were planned but only 24 subjects were enrolled. Out of which, 17 subjects completed the study. The data of 17 completing subjects were taken for pharmacokinetic and statistical evaluations.

Diagnosis and main criteria for inclusion

Healthy human subjects within the age range of 18 to 45 years with body-mass index (BMI) of ≥ 18.70kg/m^2 and ≤ 25.30 kg/m^2 having absence of significant disease or clinically significant laboratory values or laboratory evaluation, medical history or physical examination during the screening and complying with inclusion and exclusion criteria were included.

Criteria for Evaluation

Efficacy: The 90 % confidence interval for C_{max}, AUC_{0-t} & $AUC_{0-\infty}$ of Cinacalcet will form the basis for concluding the equivalence of Cinacalcet hydrochloride in product Reference and Test. If the confidence intervals are entirely included in the range of 80% – 125 % for C_{max}, AUC_{0-t} & $AUC_{0-\infty}$ log-transformed then the treatments will claimed to be bioequivalent.

***Safety*:** To monitor the safety and tolerability of Cinacalcet Hydrochloride 90mg when administered in healthy adult, human subjects.

***Statistical methods*:** The log-transformed pharmacokinetic parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) are analysed using an ANOVA model. Calculated 90% confidence interval for the ratio of both the products averages (geometric means) of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$. Ratios of mean AUC_{0-t} to mean $AUC_{0-\infty}$ for test and reference are expressed in percentage and power test is performed using SAS® version 9.1.3.

Ethical Conduct of the Study

Independent Ethics Committee

This protocol and corresponding informed consent form (ICF) (containing information about the study to be given to the subjects) to be used to obtain written informed consent of study subjects were reviewed by the IEC and subjects were not be enrolled into the study until the IEC approved the protocol and the ICF.

This study was conducted in accordance with the principles of the Declaration of Helsinki, 'ICH GCP', National Regulations (ICMR Guidelines), 'Indian GCP', and "Schedule Y" of Indian Drugs and Cosmetics Act.

Written Informed Consent

The Principal Investigator or designated study personnel informed the subjects (in English and/or Marathi language understandable by the subject) before initiation of the study through an oral presentation regarding the purpose, procedures to be carried out, investigational products, potential hazards and rights of the study subjects. The subjects were required to understand and sign the ICF prior to check-in for the study in the first period and the signed ICF was filed in the respective study file.

Overall Study Design and Plan – Description

This was an open label, balanced, analyst blind, randomized, two-treatment, two-period, two sequence, single dose, crossover bioequivalence study on 24 + 4 (standby) healthy, adult, human subjects under fed conditions.

Selection of Study Population

The general screening was carried out after obtaining the written consent on IEC approved 'Informed Consent for Screening' from the volunteers. The screening procedure included Demographic data including sex, completed age, height and weight, Body Mass Index (BMI), diet, history of tobacco use, intake of abusive/recreational drugs, alcohol intake, history of blood donation and history of participation in a drug research study. Medical history, including relevant past medical / surgical history, family history, history of allergies (food / drug / any other), past medication history in the last 90 days. Medical examination including recording of vital signs (Blood Pressure (BP), Pulse, Temperature and Respiration), general examination, physical and systemic examination. 12-lead ECG for heart rate, rhythm and specific finding (if any). Chest X-ray (PA view); Laboratory parameter investigation including Complete blood count – erythrocyte count, platelet count, haemoglobin, Hematocrit, leucocyte count, ESR and differential leucocyte count (DLC); Blood grouping (if previously not performed by Bioequivalence department of Macleods Pharmaceuticals Ltd.), Biochemistry – blood sugar (fasting), triglycerides and cholesterol, Hepatic profile – SGOT, SGPT, GGT, Alk. Phosphatase and Bilirubin (Total, Direct, Indirect), Renal profile –creatinine, BUN, calcium, electrolytes (sodium, potassium. chlorides) and Infectious diseases – HIV, HbsAg and HCV and routine urine examination.

No clinically significant abnormalities in ECGs, chest X-ray, (PA view) were reported in subjects who were included in the study. Additionally, serological tests (HIV, Hepatitis B and C, HCV) were negative. The volunteers with laboratory values within normal limits or with clinically non-significant values were called one day prior to the study for study informed consent form presentation.

Only those volunteers who signed the study informed consent form were checked in for the study on the day of check-in (one day prior to dosing).

All the volunteers were found negative for breath alcohol test and urine test for drugs of abuse test [Cocaine (COC), Amphetamines (AMP), Marijuana (TMC), Morphine (MOP), Barbiturates (BAR), and Benzodiazepine (BZO)]. Twenty four fit and consenting volunteers fulfilling inclusion/exclusion criteria were enrolled in the study.

Volunteers were given the rank orders based on their reporting time to the facility on pre-study day. Based on their rank orders and depending on the compliance to the requirements of the protocol, subject numbers were allotted serially.

Inclusion Criteria

Subjects had to fulfill all of the following criteria to be considered for inclusion into this study:

1. Healthy volunteers within the age range of 18 to 45 years.
2. Presently non-tobacco users.
3. Willingness to provide written informed consent to participate in the study.
4. Body mass index of ≥ 18.70kg/m^2 and ≤ 25.30 kg/m^2, with body weight not less than 50 kg (for males).
5. Absence of significant disease or clinically significant abnormal laboratory values or laboratory evaluation, medical history or physical examination during the screening.
6. Have a normal 12-lead ECG or one with abnormality considered to be clinically insignificant.
7. Have a normal chest X-ray PA view.
8. Comprehension of the nature and purpose of the study and compliance with the requirement of the distributed ICF.
9. Body mass index of ≥ 17.70 kg/m^2 and ≤ 23.92 kg/m^2, with body weight not less than 45 kg (for females).
10. Volunteer is regularly menstruating / Volunteer is in postmenopausal phase for at least 1 year / is surgically sterile (for females).
11. Volunteer of child bearing potential practicing an acceptable method of birth control for the duration of the study as judged by the investigator(s) such as condoms, foams, jellies, diaphragm, and intrauterine device (IUD) or abstinence etc. except hormonal contraceptives (for females).

Exclusion Criteria

The subjects were excluded based on the following criteria:

1. Personal history of allergy or hypersensitivity to Cinacalcet Hydrochloride drug or allied drugs.
2. Any major illness in the past 90 days or any clinically significant ongoing chronic medical illness e.g. Congestive Cardiac Failure (Heart failure), Hepatitis, Hypotensive episodes, Hyperglycemia etc.
3. Presence of any clinically significant abnormal values during screening e.g. significant abnormality of liver function test, renal (kidney) function test etc.
4. Severe cardiac, renal or liver impairment, gastro-intestinal disease or other conditions, any other organ or system impairment.
5. History of seizures, epilepsy or any kind of Neurological disorders.
6. Past history of Anaphylaxis or angioedema.
7. Presence of disease markers of HIV and hepatitis B, C virus.

8. History of consumption of alcohol for more than 2 years or having consumed alcohol within 48 hours prior to dosing.
9. Consumption of Xanthine containing derivatives (coffee, tea, cola – drinks, chocolate) or tobacco products within 48 hours prior to dosing.
10. Use of any recreational drug or a history of drug addiction.
11. Participation in any clinical trial within the past 90 days.
12. History of difficulty with donating blood or difficulty in accessibility of veins in left or right arm.
13. Donation of blood (one unit or 350 mL) within 90 days prior to receiving the first dose of study medication.
14. Receipt of any other prescription drug or over the counter (OTC) drugs within two weeks prior to receiving the first dose of study medication or repeated use of drugs within the last four weeks.
15. An unusual diet for whatever reason e.g. low sodium diet, for two weeks prior to receiving any medication and throughout subject's participation in the study.
16. Recent history of dehydration from diarrhoea, vomiting or any other reason within a period of 24 hours prior to the study.
17. Known hypersensitivity to heparin.
18. Use of oral contraceptive for at least 90 days (for females).
19. Pregnant / lactating volunteer (for females).

Treatments Administered

An oral dose of Reference Product (R) Sensipar™ 90mg or Test product (T) Cinacalcet Hydrochloride Tablet were administered at 0.00 hours during each period with 240 mL (about 8 oz) of water at room temperature as per the randomization schedule under the supervision of the Medical Officer where end time of the dosing was recorded in raw data forms. Subjects received the alternate 'treatment' in the subsequent periods, in such a way that each subject would have received the two 'treatments' test and reference each, at the end of the study.

Method of Assigning Subjects to Treatment Groups

The subjects were assigned to the sequence either test or reference product, according to the randomization schedule.

The order of receiving test or reference product for each subject during the study was determined according to randomization schedule (generated using SAS® version 9.1.3).

Subject number was allocated as per the rank order of the reporting time of the subject to the clinical facility.

Drug Concentration Measurements

Concentration of Cinacalcet was measured in plasma samples of the subjects. The blood samples were collected during the study at sampling hours at pre-dose and at 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 24.00, 48.00, 72.00, 96.00, 120.00, 168.00 and 192.00 hours post dose (Time points being relative to the formulation dosing). Samples were collected through an indwelling cannula placed in a forearm vein. The pre-dose samples were collected within one hour prior to drug dosing. The post-dose samples

upto 24 hours in house stay were collected within 2 minutes of the scheduled time where the end time of collection to the nearest minute was recorded. Any deviations above two minutes recorded as protocol deviation. Similarly the post-dose samples during each ambulatory visit collected within one hour of the scheduled time where the end time of collection to the nearest minute was recorded. Any deviations above one hour were recorded as protocol deviation.

The blood sample was collected up to two hours of specified schedule time of blood collection during each ambulatory visit. No ambulatory blood sample collection w made after 2 hours of specified schedule time of blood collection for any subject.

Intravenous indwelling cannula was kept in place as long as required by injecting not more than 0.5 mL of 5 IU/mL of heparin in normal saline solution during the collection of multiple samples. In such a case, the blood sample was collected after discarding the first 0.5 mL of heparinised blood from the tubing. Blood was also withdrawn from vein by using disposable syringe and needle if the cannula was blocked or the cannula is removed for other reasons.

Each blood sample was collected in 6 ml vacutainer tube containing 5% NA_2EDTA solution as anticoagulant during each period. The samples collected at each time point were centrifuged at 4°C and 4000 rpm for 15 minutes to separate plasma, after receiving the blood samples from all the subjects. Plasma samples were centrifuged within 30 minutes after collection of samples; if there was any delay in centrifugation then sample was kept cool in refrigerator. The separated plasma was aliquoted in single aliquot in prelabelled polypropylene tubes during each period. These tubes were labelled with Study Number, Period Number, Subject Number, Sample Number, Time Point (hrs), and Aliquot Number. These tubes were then transferred to a deep freezer maintained at –20°C for temporary storage and finally to a deep freezer maintained below –50°C or colder for storage at the end of the day or as and when required.

968 samples (Aliquot I) of Period-I and Period-II were transferred to Bio analytical section at 1400 hrs. The investigational products were administered in fasting conditions and no food was served till four hours post dose. No fluid, except 240 mL drinking water administered with the investigational products was allowed from 1 hour pre-dose and 2 hours post dose. The investigational products were administered to the subjects while in sitting posture. Subjects were instructed to remain seated or be ambulatory (avoiding any strenuous activity) for first two hours following the drug administration (except during recording of vitals). During this interval, under supervision, subjects were permitted to leave the bed for brief periods, e.g. to use the washroom facilities. Thereafter the subjects were allowed to engage only in normal activities while avoiding severe physical exertion.

Restrictions

All subjects were instructed to abstain from alcohol and xanthine containing food and beverages (chocolates, tea, coffee or cola drinks), cigarettes and tobacco products, for at least 48 hours, prior to dosing and during their participation in the study. Subjects were also instructed to abstain from an unusual diet for whatever reason e.g. low sodium diet, for two weeks prior to receiving any medication and throughout their participation in the study.

Prior and Concomitant Therapy
Receipt of any other prescription drug or over the counter (OTC) drugs within two weeks prior to receiving the first dose of study medication or repeated use of drugs within the last four weeks was an exclusion criterion. Further, the subjects were not supposed to consume any medication during the conduct of the study. Twenty four subjects, who checked-in study, confirmed that they did not consume any medication within the 2 weeks of the start of first period or during the study.

Bioanalytics and data processing
Validated LC-MS/MS method was employed for the estimation of Cinacalcet in plasma. During estimation of Cinacalcet in plasma quality control samples were distributed throughout each batch of study samples.

Whenever possible, samples from each subject were analyzed on the same standard curve. Samples with drug concentration greater than upper limit of the validated range of the analysis would be diluted with the appropriate drug free biological matrix and reanalyzed as per the method validation report.

The analysts concerned were blinded with respect to the randomization code, and as a result to the order of administration of the study medication.

Appropriateness of Measurements
The plasma samples of subjects were analyzed by a validated LC-MS/MS method. The limit of quantification of 0.52 ng /mL for Cinacalcet was enough to quantify the analyte from the plasma samples collected up to 24.00 hours after drug administration. The linearity range of 0.52 ng/mL to 50.95 µg/mL for Cinacalcet was enough to quantify the expected concentration range of Cinacalcet from subject plasma with the proposed dose of 90 mg of Cinacalcet.

Insurance policy
Macleods Pharmaceuticals. Ltd. had an insurance policy to cover the risks to the subjects and/or any other eventualities pertaining to the study.

Confidentiality of data
The data identifying each subject by name was kept confidential and was accessible only to the study personnel and if necessary, to the QA auditors, IEC, Sponsor representative and Regulatory agency.

Statistical and Analytical Plans
Following were the plans for statistical analysis:

- Use SAS® system version 9.1.3 for estimation of pharmacokinetic parameters and its Statistical analysis for Cinacalcet at from their plasma concentration data.
- Report the summary statistics for all pharmacokinetic parameters for both the test and reference products. The reported parameters are the minimum, maximum, arithmetic means, standard deviation and the coefficient of variation for untransformed data and relevant pharmacokinetic parameters are the arithmetic means and the standard deviation for the log-transformed (natural) data.

- Analyze the log-transformed pharmacokinetic parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) using an ANOVA model with main effects of sequence, subject nested within sequence, period, and treatment.
- Use a separate ANOVA model to analyze each of the parameters. Use a 5% level of significance to test significance of all effects.
- Include calculation of mean square error, coefficient of variance and the associated degree of freedom for each analysis of variance.
- Use SAS procedure 'PROC GLM' to perform analysis of variance.
- Calculate and report ratio of geometric means using the LSM for log transformed C_{max}, AUC_{0-t} and $AUC_{0-\infty}$.
- Report the geometric means of the test and reference product. And express ratios of mean AUC_{0-t} to mean $AUC_{0-\infty}$ for test and reference in percentage.
- Calculate the power of the ANOVA model to detect the ratio of the two products averages (geometric means) being equal to 125% (or 80%) at the 5 % significance level for analyses using the log-transformed data.
- Calculate the coefficient of variation using $\sqrt{e^{MSE}-1}$ with help of SAS® version 9.1.3. Where MSE is mean squared error obtained from Analysis of Variance model.
- Calculate a 90% confidence interval for the ratio of both the products averages (geometric means) by first calculating the 90% confidence interval for the differences in the averages (least square means) of the log-transformed data and then taking the antilogarithms of the obtained confidence limits.
- The confidence intervals are entirely included in the range of 80% – 125 % for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} log-transformed then the treatments were claimed to be bioequivalent.

RESULTS

Demographic and Other Baseline Characteristics

The mean weight, height, age and BMI ± SD values for normal healthy human subjects recruited in the study and those analyzed were 58.83 ± 5.477 kg, 1.646 ± 0.0545 meters, 26.7 ± 4.85 years, 21.7239 ± 1.88528 kg/m2 respectively. The demographic data is summarized in table-1.

Table-1. Demographic Data

	Age (Yrs)	Height (m)	Weight (kg)	BMI (kg/m^2)
Number	24	24	24	24
Median	26	1.63	57.7	21.215
Mean	26.7	1.646	58.83	21.7239
Standard Deviation	4.85	0.0545	5.477	1.88528
Minimum	20	1.55	51.3	19.67
Maximum	36	1.75	68.8	25.19

Analysis of Efficacy

The various un-transformed mean pharmacokinetic parameters estimated for both the reference and test formulations of Cinacalcet under fed conditions are mentioned in Table-2.

Table-2. The un-transformed mean pharmacokinetic parameters for both the reference and test formulations

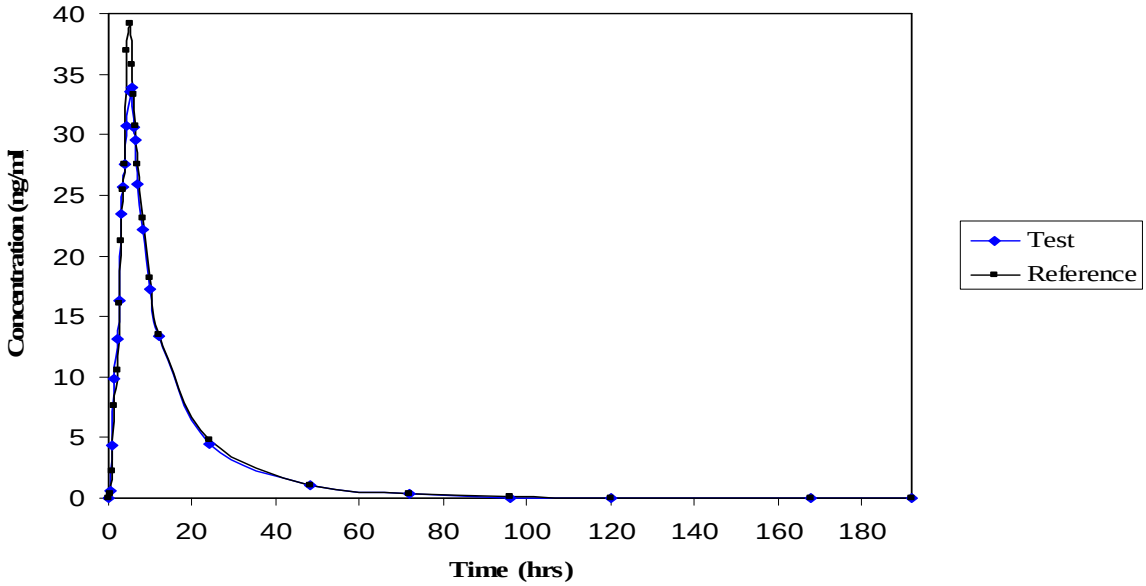
Pharmacokinetic Parameters	N	Test Product			N	Reference Product		
		Arithmetic Mean	S.D.	C.V. (%)		Arithmetic Mean	S.D.	C.V. (%)
C_{max} (ng /mL)	17	43.952	13.5536	30.84	17	41.201	13.2564	32.18
AUC_{0-t} (ng *hr/mL)	17	435.877	181.1029	41.55	17	421.067	152.9870	36.33
$AUC_{0-\infty}$ (ng *hr/mL)	17	452.538	182.1546	40.25	17	436.313	153.3125	35.14
T_{max} (hrs.)	17	4.765	1.3124	27.54	17	4.441	1.4988	33.75
$T_{1/2}$ (hrs)	17	12.462	7.8787	63.22	17	11.176	3.1608	28.28
K_{el} (hr^{-1})	17	0.068	0.0246	36.09	17	0.066	0.0171	25.72

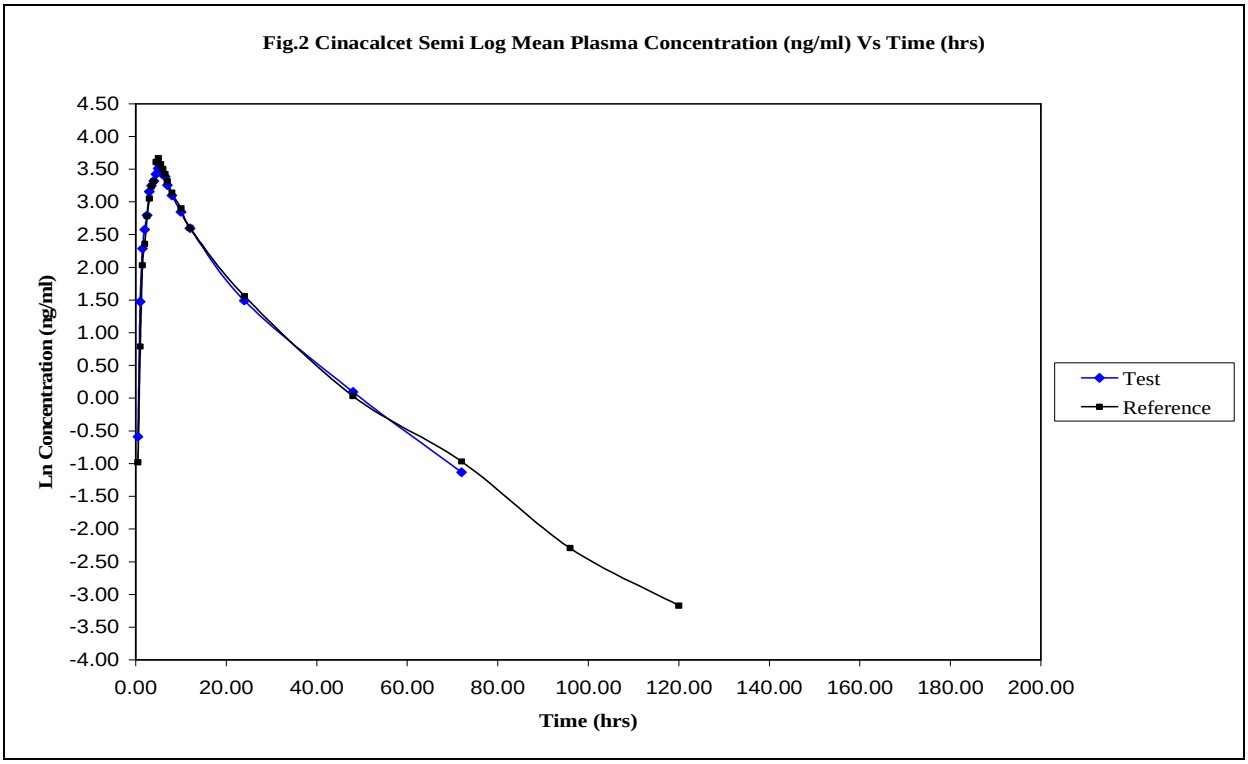
The ln-transformed least square mean and 90% Confidence interval based on least square mean obtained from ANOVA and ratio of geometric mean for the pharmacokinetic parameters Cmax, AUC_{0-t} and $AUC_{0-\infty}$ for Cinacalcet: under fed conditions (i.e. The 90 % confidence intervals of ln-transformed parameters for Cinacalcet) are summarized in the table-3.

Table-3. The 90 % confidence intervals of ln-transformed parameters for Cinacalcet

Geometric mean, ratio and 90 % confidence interval for Cinacalcet				
Pharmacokinetic Parameters	Geometric mean		Ratio (T/R) (%)	90 % Confidence Interval for ln-transformed data (%)
	Test (T)	Reference (R)		
C_{max} (ng /mL)	41.373	38.795	106.64	94.55 - 120.29
AUC_{0-t} (ng *hr/mL)	397.063	391.590	101.40	90.89 - 113.12
$AUC_{0-\infty}$ (ng*hr/mL)	415.746	408.409	101.80	92.75 - 111.72

Fig.1 Cinacalcet Mean Plasma Concentration (ng/ml) Vs Time (hrs)





Ratio And 90% Confidence Interval

The ratio of geometric mean and 90% confidence interval for the ln-transformed pharmacokinetic parameters C_{max}, AUC_{0-t} & $AUC_{0-\infty}$ were found to be respectively 106.64% & 94.55% – 120.29%; 101.40% & 90.89% – 113.12% and 101.80% & 92.75% – 111.72%.

Power and Intra Subject Variability

The power and intra subject variability for ln-transformed pharmacokinetic parameters C_{max}, AUC_{0-t} & $AUC_{0-\infty}$ was found to be respectively 85.95% & 20.19%; 91.54% & 18.31% and 97.21% & 15.54%.

Efficacy Conclusions The 90% confidence intervals for the ratio of both the products averages (geometric mean) of the primary efficacy variables Cmax, AUC0-t and AUC0–∞ lie between the acceptance ranges of 80%-125% for all the parameters.

Thus it is concluded that the test formulation of Cinacalcet Hydrochloride Tablets 90mg (each tablet containing Cinacalcet 90mg) manufactured by Macleods Pharmaceuticals Ltd., India is bioequivalent to Sensipar™ (Cinacalcet Hydrochloride) Tablets 90mg (each tablet containing Cinacalcet Hydrochloride 90mg) manufactured by Amgen USA Inc. in healthy, adult, human subjects under fed conditions.

Safety Conclusions

There were 4 adverse events reported in period I by subject No.05, 06, 07 and 14 and 3 adverse events in period II by subject No.09, 10, and 15. the adverse events observed in this study were

Vomiting, elevation in SGPT, Leucocyte count and Fasting blood sugar, slight decrease in Haemoglobin. All the adverse events were resolved successfully.

On post study safety assessment, adverse event was reported for Subject No.04, 19, 21and 22 No serious adverse event occurred during the conduct of the study.

Table-4. Summary Statistics of Pharmacokinetic Parameters for Cinacalcet (under fed conditions) in 17 healthy, adult human subjects under fed conditions

Product/Statistics	C_{max} (ng /mL)	AUC_{0-t} (ng *hr/mL)	$AUC_{0-\infty}$ (ng *hr/mL)
Untransformed Reference Product (R)			
Arithmetic Mean	41.201	421.067	436.313
S.D.	13.2564	152.9870	153.3125
C.V. %	32.18	36.33	35.14
N	17	17	17
Test Product (T)			
Arithmetic Mean	43.952	435.877	452.538
S.D.	13.5536	181.1029	182.1546
C.V. %	30.84	41.55	40.25
N	17	17	17
Ratio of Arithmetic Mean (% Bioavailability)			
T/R (%)	106.68	103.52	103.72
Ratio (%) for Mean AUC_{0-t} to Mean $AUC_{0-\infty}$			
Reference (R)	96.51		
Test (T)	96.32		
Log Transformed (Natural Log) Least Square Mean			
Reference	3.658	5.970	6.012
Test	3.723	5.984	6.030
Geometric Mean			
Reference	38.795	391.590	408.409
Test	41.373	397.063	415.746
Ratio of geometric Mean			
T/R (%)	106.64	101.40	101.80
90 % Confidence Interval (T/R)			
Lower limit (%)	94.55	90.89	92.75
Upper limit (%)	120.29	113.12	111.72
Power (%)	85.95	91.54	97.21
D.F.	15	15	15
Intra subject C.V. (%)	20.19	18.31	15.54
P-value (ANOVA) for ln-transformed data			
Treatment	0.3637	0.8270	0.7419
Period	0.4849	0.7611	0.9710
Sequence	0.3740	0.8991	0.9062

The summary results and mean pharmacokinetic parameters for both the test and reference formulations have been tabulated in table-4. The statistical output and pharmacokinetic from SAS® version 9.1.3 was calculated.

DISCUSSION

It was an open labelled study because it was not possible to blind the appearance of the products. The analysts concerned, however, were blinded to the sequence of administration of test and reference product to the individual subjects.

The order of receiving treatment was randomized to avoid bias in allocation of sequence to the subjects. There were two treatments: the sponsor's product was the test product while the innovator product was the reference product. The subjects served as their own control, the study being crossover. Since there were two treatments, the trial design was two periods, two sequences. The effect of period and sequence on primary efficacy criteria was analyzed by ANOVA (Analysis of variance).

To reduce variability in the biomedical experimentation and to control factors, which may affect the evaluation and comparison of primary efficacy factors, healthy, adult, male, human subjects were selected.

The number of subjects to be included in the study was derived based on variability of the pharmacokinetic data available in the literature and was estimated to be sufficient to differentiate the bioavailability patterns of products under study. A total of 24 subjects were enrolled out of which 17 subjects completed the study. The plasma samples of 17 completed subjects were analyzed for Cinacalcet concentration level and utilised the data for pharmacokinetic and statistical evaluations.
The pharmacokinetic parameters observed in this study are consistent with that reported in the literature published. Bioequivalence was assessed using standard equations. The 90% confidence intervals for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for Cinacalcet were within the usual acceptable limit for 80-125 %.

CONCLUSION

The test product, single dose of Cinacalcet Hydrochloride Tablets 90mg (each tablet contains Cinacalcet 90 mg) manufactured by Macleods Pharmaceuticals Ltd, India is bioequivalent to the reference product of Sensipar™ (Cinacalcet Hydrochloride) Tablets 90mg (each tablet contains Cinacalcet 90 mg) Manufactured by Amgen USA Inc. in healthy, adult, human subjects under fed conditions.

Both the formulations are well tolerated following a single dose administration of the investigational product. No serious clinical adverse events causing death, disability, hospitalization, or dropouts of the subjects were encountered.

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