Chapter-5

Summary and Conclusions
5.1 Summary

Secondary hyperparathyroidism (SHPT) is a major complication of Chronic Kidney Disease (CKD), resulting from disturbances in the regulation of PTH, calcium, phosphorus, and vitamin D. SHPT arises in most patients during the progression of CKD and is associated with several comorbidities, including renal osteodystrophy (ROD), extraskeletal calcification, and cardiovascular disease (CVD), resulting in increased mortality.

The most difficult challenge in the treatment of SHPT is that of patient acceptance and adherence. Complicated medication regimens (Phosphate binding agents, Vitamin D and Its Derivatives, Ergocalciferol, Selective Vitamin D Analogues) that involve taking medicines multiple times each day, a high pill burden, comorbid conditions, financial constraints, psychosocial issues, and dietary restrictions are all factors that increase the rate of nonadherence and thwart treatment success. Maintaining bone and mineral metabolism is a challenge for all health care providers and requires a multidisciplinary team approach.

Cinacalcet was proven safe and effective in clinical trials, cinacalcet has been shown to substantially suppress PTH levels without concomitant elevations in calcium, phosphorus, or Ca x P.

Currently Cinacalcet is not available in the Indian market. There is no published data for the efficacy and safety of Cinacalcet in Indian Population. So, the Calcimimetic drug Cinacalcet was selected for the research work.

Ischemic heart disease (IHD), also called coronary heart disease or coronary artery disease, is an imbalance between myocardial oxygen demand and supply.

The majority of patients with IHD (nearly 60%) suffer from angina pectoris, or simply angina. Patient compliance and physician use of conventional treatments, as well as that of nitrates, may however be limited by contra-indications, development of tolerance or common side effects.

In view of these side effects, a new pharmacological target was sought as a mechanism of lowering HR.

In view of the current data on the efficacy and safety of Ivabradine, there appears to be an important clinical role for the drug in patients with chronic stable angina.
Currently Ivabradine is not available in the Indian market. Also there is no published data for the efficacy and safety of Ivabradine in Indian Population. So, Ivabradine was selected for the research work.

To approve or to launch a drug first time in India, a bioequivalence study and phase-III clinical study is need to be carried out in Indian population to bring the product in Indian market hence the objective of the work was to perform A Phase-III clinical and Bio-equivalence studies to evaluate efficacy and safety of Antihyperparathyroidism and Antianginal drug in Indian Population.

1) Bio-Equivalence Studies:

The following steps are involved in conduct of bioequivalence studies,

a) To develop simple, accurate, precise, specific and sensitive methods for the estimation of Antihyperparathyroidism and Antianginal Drugs of stated drug.

b) To validate the developed method as per the international guidelines for bio-analytical methods.

c) To evaluate the comparative bioavailability of the stated drug / drugs with the reference formulation in healthy, adult, human subjects.

d) Incurred Sample Reanalysis

e) Comparison of pharmacokinetic data with the published data.

f) Statistical analysis of all the collected data.

2) A Phase-III clinical studies:

The following steps are involved in conduct of Phase-III studies,

a) A Phase-III clinical studies in Indian Population of stated drug.

b) Population Pharmacokinetic and Pharmacodynamic study to show the variability at different centers.

c) Statistical analysis of all the collected data

Innovation in the Study

The method development started with the selection of analytical instrument i.e. HPLC, LC-MS/MS. Further steps involved in method development were optimization of mobile phase,
column selection, and finally sample purification techniques. All the developed methods were evaluated for, specificity / selectivity, sensitivity, linearity, accuracy and precision, recovery, hemolysis effect, ruggedness, dilution integrity, stability studies like stock solution stability, bench top stability, freeze thaw stability, auto-sampler stability, post preparative stability and long term stability. All the bioequivalence studies were conducted in accordance with their respective study protocols and to comply with all requirements of international, national and ICH guidelines (Guideline for Good Clinical Practice’ and Declaration of Helsinki, 2008). The independent ethics committee approved each of these study protocol and all the activities in conduct of studies were complied with all relevant standard operating procedures. Analysis of the study samples was performed in compliance to GLP and was in accordance with the international guidelines (USFDA, ANVISA) available for method validation and study sample analysis. Statistical evaluation of ANOVA test, 90 % CI was performed for $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ for test and innovator formulations for all the study molecules. It was performed with the help of SAS software version 9.1.3. For a product to be bioequivalent, the 90% CI for $C_{\text{max}}$ and AUC should fall within the range of 80-125%.

Complicated current medication regimens for the treatment of Secondary hyperparathyroidism (Phosphate binding agents, Vitamin D and Its Derivatives, Ergocalciferol, Selective Vitamin D Analogues) that involve taking medicines multiple times each day, a high pill burden, comorbid conditions, financial constraints, psychosocial issues, and dietary restrictions are all factors that increase the rate of nonadherence and thwart treatment success. Cinacalcet was proven safe and effective and has been shown to substantially suppress PTH levels without concomitant elevations in calcium, phosphorus, or Ca x P. So, it will be very helpful full in the Indian Population for the treatment of Secondary hyperparathyroidism in CKD patients. There were no published data available on the efficacy and safety of Cinacalcet for the treatment of Secondary hyperparathyroidism in Indian patients.

So, an Open, Comparative, Randomized, Phase-III, Multicentric Clinical Study to evaluate the efficacy and safety of Cinacalcet Vs Calcitriol for the treatment of Secondary hyperparathyroidism in Indian patients was conducted.

The majority of patients with IHD (nearly 60%) suffer from angina pectoris, or simply angina.
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The main goals in the treatment of patients with chronic stable angina are improving prognosis by preventing myocardial infarction and/or death and improving the functional status and quality of life by relieving the symptoms of angina.

Conventional pharmacologic options to reduce symptoms are beta-blockers, calcium-channel antagonists and organic nitrates. Patient compliance and physician use of these treatments, as well as that of nitrates, may however be limited by contra-indications, development of tolerance or common side effects.

In view of the current data on the efficacy and safety of Ivabradine, there appears to be an important clinical role for the drug in patients with chronic stable angina. So, it will be very helpful in the Indian Population for the treatment of patients with Chronic stable angina. There were no published data available on the efficacy and safety of Ivabradine for the treatment of chronic stable angina in Indian patients.

So, an Open, Comparative, Randomized, Phase-III, Multicentric Clinical Study to evaluate the efficacy and safety of Ivabradine Vs Atenolol for the treatment of chronic stable angina in Indian patients was conducted.

5.1.1 Cinacalcet Hydrochloride

Simple specific and sensitive method was developed. The system used was API 3200 LC-MS/MS attached to Shimadzu Prominence HPLC System consisting of LC-20AD Prominence pump, SIL-HTc autosampler, CTO 10ASvp column oven and DGU-A3 degasser was used to setting the reverse-phase LC conditions. The separation of analyte and internal standard was performed on Hypurity Advance, 50 x 4.6 mm, 5µm column at 35°C. The mobile phase consisted of Acetonitrile: 0.1% formic acid solution (90:10v/v). Flow rate of mobile phase was kept at 1.2mL/min with split. The autosampler temperature was maintained at 10°C. Injection volume was 10µL and rinsing solution was acetonitrile and mili-Q water in the volume ratio of 90:10 v/v.

The developed method was further validated. It was found that the method is precise and accurate. No carry over was observed in the optimized chromatographic conditions. The recovery of analyte and internal standard was consistent. The mean overall recovery of Cinacalcet was 49.480% with a precision of 0.90% and the mean recovery of internal standard was 49.480% by the optimized method. Correlation coefficients (r²) were greater than 0.98 in the concentration range of 0.52 ng/mL to 59.95 ng/mL. Stability studies were performed to
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check the stability of the drug in the required experimental conditions. Cinacalcet was found to be stable in plasma for 30 days in deep freezers maintained below –50°C.

Cinacalcet was also stable in plasma undergoing 3 freeze thaw cycles. The processed samples of Cinacalcet were stable for 23 hours at 4°C in auto-sampler and for 5 hours when stored at bench top. Dry extract stability of the samples was found to be 13 hours in refrigerator.

The stock solution of Cinacalcet and Carbamazepine were stable in refrigerator for 70 hrs and for 6 hours when stored at room temperature. Precise and accurate results were obtained even after sample dilution. The application of the validated method was evaluated on study samples.

The bioequivalence study was conducted as per the protocol approved by the independent ethics committee. The study design 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.

It was an open labeled study because it was not possible to blind the appearance of the products. The analysts, 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. 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).

The study samples were analysed and the concentrations obtained were subjected to statistical analysis. The study sample analysis was completed within long-term stability period.

The study need to conduct on 24 subjects for that 4 subjects were kept as standby to replace dropouts if any. Means total 24+4 as stand by volunteers need to enroll in the study.

As per the protocol 28 (24+4 stand by) subjects would be enrolled but only 24 subjects reported to the facility at the time of check in.

So, 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 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 based on the literatures.
The ratio of geometric mean and 90% confidence interval for the ln-transformed pharmacokinetic parameters were for Cmax and AUC0-t was within 80% to 125%. The mean Cmax for test and innovator formulation was 41.373 and 38.795 ng/mL respectively. The mean AUC0-t for test and innovator formulation was 397.063 and 391.590 ng hr/mL respectively. The test to reference ratio was found to be 106.64 for Cmax and 101.40 for AUC0-t. 90% C.I. was found to be 94.55% to 120.29% for Cmax and 90.89% to 113.12% for AUC0-t. The power for ln-transformed pharmacokinetic parameters Cmax and AUC0-t was found to be 85.95% and 91.54% respectively. The test and reference products were well tolerated by all the subjects. The intra subject variability was found to be 20.19% for Cmax and 18.31% for AUC0-t. None of the subject showed any clinically significant adverse event after post study safety evaluation. The efficacy and safety of the test product is equivalent to that of the innovator product.

The adverse event vomiting which was observed in 7 subjects during study is common as per the literature.

A Phase-III Clinical Study of Cinacalcet was performed on the Indian patients. The study design was An Open, Comparative, Randomized, Phase-III, Multicentric Clinical Study. Diagnosis and main Criteria for Inclusion in the study was Patients with the confirmed diagnosis of secondary hyperparathyroidism were eligible to be enrolled for the study. Inclusion Criteria was Patient of either sex with an age older than 18 year, Predialysis patients suffering from Chronic Kidney Disease (CKD), Patients with plasma parathyroid hormone level (PTH) of at least 200 pg/ml i.e. PTH > 200 pg/ml or histological evidence of secondary hyperparathyroidism, Serum calcium > 7.1 mg/ml and < 11.0 mg/dl, Serum phosphorus > 2.5 mg/dl and Patient willing to provide informed consent to be included in the study. An oral dose of study drug from Test & Reference were administered to the patients as per the randomization schedule. Dose of Cinacalcet tablet was 30 mg (once a day) increased sequentially every four weeks To 60, 90, 120 & 180 mg once daily and the dose of Calcitriol tablet was 0.25 mcg daily. May be increased by 0.25 mcg daily at intervals of 4-8 weeks. Primary End Points were Proportion of randomized patients who had a mean parathyroid hormone level of 150 pg/milliliter or less at the end of treatment and Secondary End Points were Proportion of patients with a reduction from base line of at least 30% in mean parathyroid hormone level. Medical examination and clinical laboratory tests were performed to cater to the post study safety assessments at the end of trial.
Mean age of the patients was 45.67 years for Cinacalcet treated group and 44.88 for Calcitriol treated group. Average weights of the patients were comparable. The mean weight of patients was 55.25 kg & 57.2 respectively. Out of 50 patients, 33 patients were males and the remaining 17 were females. A total of 50 patients were enrolled in the study. 100% (30 out of 30) patients in cinacalcet treatment group and 90% (18 out of 20) patients in calcitriol treatment group completed the 24 week treatment. Reason for 2 drop out patients from Calcitriol group was lost to follow-up. All 48 patients completed the study were included for statistical analysis.

The proportions of patients who had treatment related adverse events were higher in the group receiving Cinacalcet than in the Calcitriol group (treatment related adverse events: 33.33 percent and 30.00 percent, respectively).

Mean PTH level for patients treated with Cinacalcet tablet was significantly decreased from 404.43pg/ml to 304.74pg/ml (i.e. 24.64%) at the end of treatment. In the same line there was significant decreased PTH level from 495.09pg/ml to 387.99pg/ml (i.e. 21.63%) for patients treated with Calcitriol tablet at the end of treatment. 36.66% of patients receiving Cinacalcet (11 out of 30) reached the primary end point i.e. a mean parathyroid hormone level of 150 pg/ml or less at the end of treatment as compared with 11.11% (2 out of 18) of those receiving Calcitriol. Mean parathyroid hormone levels decreased by 30% or more in 46.67% of patients given Cinacalcet (14 out of 30), as compared with 38.88% (7 out of 18) of those given calcitriol at the end of treatment.

Logistic regression analysis confirmed that the center of enrollment had no effect on the likelihood of achieving the primary end point i.e. Achieving PTH level of 150pg/ml or less at the end of treatment.

Population pharmacodynamic shows, there was no variability in the data in all the three centers for effect of Cinacalcet on PTH, Serum-Calcium and Serum-Phosphorus calculated by Proc Mix method using SAS program.

Cochran-Mantel-Haenszel test shows that there is no significant evidence of association between age and achieving primary end point, Baseline PTH and achieving primary end point and Baseline calcium level and achieving primary end point.

Mean Serum calcium level for patients treated with Cinacalcet tablet was significantly decreased from 8.83mg/dL to 8.07 mg/dL (i.e. 8.61%) at the end of treatment.
Conclusions:

Bio-equivalence Study of Cinacalcet

A simple, fast but accurate and precise method for the estimation of Cinacalcet from human plasma was developed. The developed method was validated as per the international guidelines of method validation. The application of this validated method was evaluated on samples of bioequivalence studies.

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 Innovator product in healthy, adult, human subjects under fed conditions.

The formulation was well tolerated following a single dose administration of the investigational product. No serious clinical adverse events causing disability, hospitalization, or dropouts of the subjects were encountered.

A Phase-III Clinical Study of Cinacalcet

Study results indicate that cinacalcet effectively reduces parathyroid hormone levels in patients with secondary hyperparathyroidism who were receiving hemodialysis and ameliorates disturbances in serum calcium and phosphorus that have been associated with adverse clinical outcomes. Our patients had been treated with dialysis for an average of 1-3 months and had persistently elevated parathyroid hormone levels. Nevertheless, PTH levels declined rapidly during treatment with cinacalcet, and this response was sustained for the duration of study. The reduction in parathyroid hormone levels in those given cinacalcet were accompanied by decrease in serum calcium.

There were no serious adverse effects found throughout the study. Treatment with cinacalcet was generally well tolerated.

By directly targeting the molecular mechanism that regulates the secretion of parathyroid hormone, the calcimimetic agent cinacalcet provides a novel therapeutic approach for controlling secondary hyperparathyroidism in patients with chronic kidney disease in Indian population.
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5.1.2 Antianginal Drug-Ivabradine

Optimized Method: Simple specific and sensitive method was developed. The system used was API 3200 LC-MS/MS attached to Shimadzu Prominence HPLC System consisting of LC-20AD Prominence pump, SIL-HTc autosampler, CTO 10ASvp column oven and DGU-A3 degasser was used to setting the reverse-phase LC conditions. The separation of analyte and internal standard was performed on Hypurity Advance, 50 x 4.6 mm, 5µm column at 35°C. The mobile phase consisted of ACN: 5 mM Ammonium Acetate Buffer Solution (80:20 v/v). Flow rate of mobile phase was kept at 0.8 mL/min with split. The autosampler temperature was maintained at 5°C. Injection volume was 10µL and rinsing solution was ACN and mili-Q water in the volume ratio of 50:50 v/v.

The developed method was further validated. It was found that the method is precise and accurate. No carry over was observed in the optimized chromatographic conditions. The recovery of analyte and internal standard was consistent. The mean overall recovery of Ivabradine was 82.797% with a precision of 1.56% and the mean recovery of internal standard was 31.583% by the optimized method. Correlation coefficients (r2) were greater than 0.99 in the concentration range of 1.01 ng/mL to 60.44 ng/mL. Stability studies were performed to check the stability of the drug in the required experimental conditions. Ivabradine was found to be stable in plasma for 50 days in deep freezers maintained below –50°C. Ivabradine was also stable in plasma undergoing 3 freeze thaw cycles. The processed samples of Ivabradine were stable for 30 hours at 4°C in auto-sampler and for 6 hours when stored at bench top. Dry extract stability of the samples was found to be 13 hours in refrigerator.

The stock solution of Ivabradine and Carbamazepine were stable in refrigerator for 5 days and for 24 hours when stored at room temperature. Precise and accurate results were obtained even after sample dilution. The application of the validated method was evaluated on study samples.

The bioequivalence study was conducted as per the protocol approved by the independent ethics committee. The study design was An open label, balanced, analyst blind, randomized, two-treatment, two-period, two sequence, single dose, crossover bioequivalence study on 12 + 2 (standby) healthy, adult, human subjects under fasting condition.

It was an open labeled study because it was not possible to blind the appearance of the products. The analysts, 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. The blood samples were collected during the study at sampling hours at pre-dose and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 6.00, 8.00, 12.00, 16.00, 24.00 and 48.00 hours post dose (Time points being relative to the formulation dosing).

The study samples were analyzed and the concentrations obtained were subjected to statistical analysis. The study sample analysis was completed within long-term stability period.

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 based on literatures.

The ratio of geometric mean and 90% confidence interval for the ln-transformed pharmacokinetic parameters were for Cmax and AUC0-t was within 80 % to 125 %. The mean Cmax for test and innovator formulation was 41.899 and 39.392 ng/mL respectively. The mean AUC0-t for test and innovator formulation was 142.611 and 146.245 ng hr/mL respectively. The test to reference ratio was found to be 101.84% for Cmax and 96.66% for AUC0-t. 90 % C.I. was found to be 88.40% to 117.32% for Cmax and 85.13% to 109.74% for AUC0-t. The power for ln-transformed pharmacokinetic parameters Cmax and AUC0-t was found to be 72.86% and 81.95% respectively. The test and reference products were well tolerated by all the subjects. The intra subject variability was found to be 19.30% for Cmax and 17.29% for AUC0-t. None of the subject showed any clinically significant adverse event after post study safety evaluation. The efficacy and safety of the test product is equivalent to that of the innovator product.

The pharmacokinetic parameters observed in this study are consistent with that reported in the published literature.

The test formulation, is bioequivalent to the reference in terms of both the rate and extent of absorption.

A Phase-III Clinical Study of Ivabradine was performed on the Indian patients. The study design was An Open, Comparative, Randomized, Phase-III, Multicentric Clinical Study. Diagnosis and main Criteria for Inclusion in the study was Patients with the confirmed diagnosis of chronic stable angina were eligible to be enrolled for the study. Inclusion Criteria was Patients of both sexes with an age of 18-65 years, Ambulatory patients with clinical diagnosis of above mentioned disease condition, Patients willing to provide informed consent and come for follow up to be included in the study, Patient who had at least a three-month history of effort angina responding to beta blockers, calcium channel blockers and/or long
acting nitrates and Patient willing to provide informed consent to be included in the study. An oral dose of study drug from Test & Reference were administered to the patients as per the randomization schedule. Dose of Ivabradine was 5 mg twice daily for 4 weeks and then 7.5mg bd for next 8 week and the dose of Atenolol was 50 mg (one tablet once daily) for first 4 weeks and then 100mg od for next 8 weeks. Efficacy was evaluated on the basis of Exercise duration, Time to angina, angina attacks per week and Short Acting Nitrates Consumption. Medical examination and clinical laboratory tests were performed to cater to the post study safety assessments at the end of trial.

The mean age of the patients were 57 years for patients treated with Ivabradine and 46.01 years for patients in Atenolol group. Average weights of the patients were Comparable. The mean weight of patients was 63.42Kg and 64.75 Kg respectively. 23 out of 30 patients were males and the remaining 7 were females. A total of 30 patients were enrolled at these centers. All the patients completed the study. There were no dropout in the study. All 30 patients completed the study were included for statistical analysis.

Exercise duration for those taking Ivabradine was increased approximately from 406.4 to 515.75 sec (Increased by 109.35 seconds) in the Ivabradine taking group after 12 weeks, as compared with a slight increase in exercise duration in Atenolol group i.e. from 407.65 to 478 sec (Increased by 70.35 seconds).

Time to angina in patients treated with Ivabradine for 12 weeks increased from 319.54 to 462.56 sec (Increased by 143.02 seconds) while from 315.93 to 416.86 sec (Increased by 100.93 sec) increase in time to angina was observed in the group on Atenolol. Angina attacks per week for those taking Ivabradine was decreased from 2.32 to 1 in the Ivabradine taking group after 12 weeks, as compared with a slight decrease in Angina attacks per week in Atenolol group i.e. from 2.2 to 1.1. Short Acting Nitrates Consumption in patients treated with Ivabradine for 12 weeks decreased from 2.12 to 0.92 while from 1.93 to 1.08 was observed in the group on Atenolol.

The proportions of patients who had treatment related adverse events were higher in the group receiving Atenolol than in the Ivabradine group (treatment related adverse events: 73.33 percent and 33.33 percent, respectively).
Conclusions:

Bio-equivalence Study of Ivabradine

A simple, fast but accurate and precise methods for the estimation of Ivabradine was developed. The developed method was validated as per the international guidelines of method validation. The application of this validated method was evaluated on samples of bioequivalence studies.

The test product, single dose of Cinacalcet Hydrochloride Tablets 90mg Ivabradine tablet 7.5 mg (each film-coated tablet contains Ivabradine hydrochloride equivalent to Ivabradine 7.5 mg) manufactured by Macleods Pharmaceuticals Ltd. comparing with is bioequivalent to the Innovator product in healthy, adult, human subjects under fed conditions.

The formulation was well tolerated following a single dose administration of the investigational product. No serious clinical adverse events causing disability, hospitalization, or dropouts of the subjects were encountered.

A Phase-III Clinical Study of Ivabradine

In this study, the novel selective and specific I$_I$ inhibitor Ivabradine demonstrated antianginal and anti-ischaemic efficacy in the treatment of patients with chronic stable angina pectoris. Ivabradine improved exercise tolerance, reduced the frequency of angina attacks and reduced the consumption of short-acting nitrates. In ivabradine treated patients, the improvements in measured ETT criteria were maintained for the duration of the study.

Results from this study suggest that ivabradine may be an effective and safe alternative to the range of options available for the treatment of stable angina and potentially, other myocardial ischaemic conditions for Indian population.