Bioequivalence Study of Vildagliptin and Metformin Fixed Dose Combinations in Healthy Volunteers

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Abstract

Aim: **To evaluate the comparative oral bioavailability of single dose of Vildamet® (fixed dose combination of Vildagliptin and Metformin hydrochloride 50 mg/1000 mg, Macleods Pharmaceuticals Limited, India) to Eucreas® (Vildagliptin and Metformin hydrochloride 50 mg/1000 mg film-coated tablets Novartis Pharmaceuticals UK Ltd., UK) in healthy, adult, human volunteers under fed condition. Additionally, Safety and tolerability of test and reference product was also evaluated.**

Methods: **This was an open label, balanced, analyst blind, randomized, two- treatment, two-period, two-sequence and crossover bioequivalence study with 07 days wash out period in 12 healthy, adult, human volunteers. The study compared oral bioavailability of two formulations Vildamet® versus Eucreas® as two tablets in single dose administered in fed condition.**

Results: **The 90% confidence intervals for the ratio (Test/Reference) of Cmax and AUC_{0-48} for Vildamet® were within the acceptable limits of bioequivalence 80.00% - 125.00%. Ratio (T/R) for C_{max} found to be 101.99%. The highest intra subject C.V. for Vildamet® combination was observed to be 8.95%. There was no adverse event occurred during the study and both test and reference products were safe and well tolerated in fed condition.**

Conclusion: **Vildamet® the test formulation was found to bioequivalent with the reference product Eucreas®.**

Registration: **35-5/WZ-2016/BE EXP/Macleods-70.**

Keywords: **Vildagliptin, Metformin, Bioequivalence, C_{max} and AUC_{0-48}**

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Introduction

Vildagliptin is a selective dipeptidyl peptidase IV (DPP-IV) inhibitor and currently being used for the management of diabetes. It inhibits the enzyme responsible for the degradation and inactivation of the incretin hormones, glucagon-like peptide-1 (GLP-1), and gastric inhibitory peptide (GIP). [1,2] GLP-1 and GIP are responsible for release of insulin, reduction in glucagon concentration and delay in gastric emptying and thus inhibition of DPP-IV helps in maintaining normal glucose levels.[3] Previously published reports have shown that daily dose of vildagliptin 100 mg results in significant decrease in pre and post prandial glucose levels in type 2 diabetes patients.[4,5] Another study reported that monotherpay of vildagliptin 100 mg for 12 week significantly reduced fasting and postprandial glucose in drug-naive patients with type 2 diabetes.[6]

According to type 2 diabetes management guidelines from European Association for the Study of Diabetes (EASD) and the American Diabetes Association (ADA), use of two different anti-hyperglycemic agents with different mechanism of actions result in effective management of type 2 diabetes. It is recommended in these guidelines that a combination of metformin with other anti-hyperglycemic agents results in better control of blood sugar levels in type 2 diabetes patients compared to monotherpy.[7] In such cases, a combination of vildagliptin and metformin may prove an attractive option because of respective complementary mechanism of actions.[8] In a study, it was reported that combination of vildagliptin 100 mg with metfromin 1500 mg was significantly better in managing type 2 diabetes compared to metformin 1500 mg monotherapy after 24 weeks of treatment. Therefore, it is convenient to use a fixed dose combination of vildagliptin and metformin compared to individual drug formulations.[9]

The current bioequivalence standards recommend use of two-treatment, two-period, two-sequence, crossover design and compare blood levels of drug for test and reference products over time after a single dose in volunteers to establish bioequivalence of generic drug products. By using this design, the maximum observed blood concentrations (C_{max}) and the area under the blood level vs time curve (AUC) are calculated for individual product using logarithmic transformation. If the blood level curves of test product are considered superimposable to reference product based on inspection of the curves and the calculated average C_{max} and AUC parameters, then the test product is deemed bioequivalent to the reference product.[10]

This study was conducted with following objectives

1. **Pharmacokinetic:** To evaluate the comparative oral bioavailability of single dose of Vildamet® (fixed dose combination of Vildagliptin and Metformin hydrochloride 50 mg/1000 mg, Macleods Pharmaceuticals Limited, India) to Eucreas® (Vildagliptin and Metformin hydrochloride 50 mg/1000 mg film-coated tablets, Novartis Pharmaceuticals UK Ltd., UK) in healthy, adult, human volunteers under fed condition
2. **Safety:** To monitor the safety and tolerability of single oral dose of fixed dose combination of Vildagliptin and Metformin hydrochloride 50 mg/1000 mg when administered in healthy, adult, human volunteers under fed condition.

Materials and Methods:

Formulations in the study

Test Drug: Vildamet® (A fixed dose combination of Vildagliptin and Metformin hydrochloride 50 mg/1000 mg) tablet: manufactured by Macleods Pharmaceutical Limited, India.

Reference Drug: Eucreas® (Vildagliptin and Metformin hydrochloride 50 mg/1000 mg) tablet: manufactured by Novartis Pharma, India.

Volunteers

A total of 12 healthy volunteers were enrolled in the study and all of them completed both periods of the study. All the healthy volunteers were aged between 18 to 45 years both inclusive. The body mass index of all the healthy volunteers was between 18.50 kg/m^2 – 29.99 kg/m^2 (both inclusive) and body weight was equal or above 50 kg. No volunteer had significant smoking, alcoholism and drug abuse history. Volunteers were excluded from the study in case of any allergy or hypersensitivity to study treatments. Volunteers were also excluded from the study if they had consumed alcohol or tobacco within 48 hours prior to dosing. A thorough physical and medical examination was done before enrolment of volunteer in the study. Physical examination included assessment of blood pressure, pulse rate, respiratory rate, 12-lead ECG and systemic examination. Blood samples of volunteers were collected to perform haematology (leukocyte count, erythrocyte count, PCV [packed cell volume], ESR [erythrocyte sedimentation rate], platelet count, haemoglobin, and DLC [differential leucocyte count]) and Biochemistry (Blood sugar, triglycerides

and cholesterol, Hepatic profile: SGOT [serum glutamic oxaloacetic transaminase], SGPT [serum glutamic pyruvic transaminase], Alkaline Phosphatase, GGT [gamma-glutamyl transpeptidase] and serum bilirubin [direct, indirect and total], Serum creatinine, BUN [blood urea nitrogen], serum calcium, serum electrolytes [sodium, potassium, chlorides].

Obtaining informed consent

Volunteers were informed about the consent form in detail by the designated personnel before the initiation of the study. Volunteers were explained in detail about the purpose of the study, possible benefits, risks and discomforts, alternative treatment and confidentiality. All the queries / clarifications asked by the volunteers were solved so they could freely decide to participate in the study. Volunteers signed the informed consent form with date. Copies of the signed and dated informed consent form were given to the respective volunteers for their reference. Audio visual recording of the informed consent process of all the volunteers was done.

Ethical conduct of the study

This study was conducted in compliance with Guideline on the investigation of Bioequivalence, 2010; Directive 2001/20/EC, and ICH, "Guideline for Good Clinical Practice', Schedule Y of Drug and Cosmetic Act of India and in accordance with Indian GCP, European guidelines (EMA) and Declaration of Helsinki (2013).

Study Design

This was an open label, balanced, analyst blind, randomized, two-treatment, two-period, two- sequence, single dose, crossover bioequivalence study on 12 healthy adult males. Healthy volunteers were randomized to receive a single dose test formulation (1 tablet of fixed dose combination of Vildagliptin and Metformin hydrochloride 50 mg/1000 mg) and single dose of reference formulation (1 tablet of Eucreas®) separately in each treatment period. There were two treatment sequences and a 7 days washout between the two treatment periods. Medical examination was carried out at the time of check-in, check-out and at 48.00 hours post dose ambulatory visit of each period of the study.

The study was conducted at BA/BE facility after approval from an independent ethics committee. Volunteers had a screening visit within 21 days prior to the first dose of study drug, two treatment periods with each containing a single dose of study drug, followed by 48 hours of serial pharmacokinetic sample collection. The subject returned for the next treatment period or for the final follow-up visit, as appropriate. The final follow-up visit occurred 7 days after the last dose of study drug. Volunteers were assigned to each of the two treatments randomly as per the randomization schedule.

Drug Administration

A single dose of test formulation (Vildamet® tablet) and reference formulation (Eucreas® tablet) was administered orally at 0.00 hours during each period with 240 ml 20% w/v glucose solution under fed condition at room temperature. Volunteers were dosed while in sitting posture and instructed to avoid any strenuous activity following the investigational product administration. Sitting position restriction up to 2 hours post dose was maintained for all volunteers. Blood samples (1 x 5 ml) were collected in 5 mL blood collection tube containing K_2EDTA as anticoagulant during each period. The venous blood samples were withdrawn pre-dose and at 0.33, 0.67, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.33, 3.67, 4.00, 4.50, 5.00, 5.50, 6.00, 8.00, 10.00, 14.00, 18.00, 24.00, 30.00, 36.00 and 48.00 hours post dose (time points being relative to the investigational product dosing).

The blood samples collected at each time point were centrifuged between 4 to 8^0C and at 4000 rpm for 10 minutes to separate plasma. These samples were centrifuged within 30 minutes after collection of last blood sample; for any delay in centrifugation, samples were kept in cold condition. The separated plasma was aliquoted in pre-labelled polypropylene tubes during each period and then being transferred to a deep freezer for storage.

LC-MS/MS Method for Estimation of Vildagliptin and Metformin Fixed Dose Combination in Human Serum Samples

A LC-MS/MS method was developed by using solid phase extraction technique and vildagliptin D7 and metformin D6 as internal standards. Chromatographic separation was achieved by kinetex PFP (50×4.6) mm, 5µ column with mobile phase consisting methanol and buffer solution (5mM ammonium trifluroacetate) in volume ratio of 70:30 (V/V). The total run time was 3.00 minutes. Detection was carried out in positive turbo-spray ionization mode using MRM transitions of 130.1/71.1 for metformin, 136.1/77.1 for metformin D6, 304.1/154.2 for vildagliptin and 311.1/161.1 for vildagliptin D7.

The analytical method was validated over the range of 5-600 ng/mL for vildagliptin and 10-3500 ng/mL for metformin. The results of validation parameters including selectivity, analytical standard purity, exchange reaction, carryover effect, linearity, precision and accuracy, bench top stability, freeze thaw stability and dilution integrity test were within the acceptance range. The above said analytical method is valid for the estimation of Vildagliptin and Metformin in human serum for above mentioned concentration range.

Pharmacokinetic Assessments

Pharmacokinetics parameters including C_{max}, AUC_{0-48}, and T_{max} were calculated for both test and reference formulations.

Statistical Analysis

Statistical analysis was performed using SAS® version 9.4. Standard ANOVA model was used to analyse the log-transformed pharmacokinetic parameters (C_{max} and AUC_{0-48}) for Vildagliptin and Metformin fixed dose combination with the main effects of "sequence, period, formulation and volunteer nested within sequence."

The following bioequivalence criteria were established on the protocol: a) the products claimed to be bioequivalent if the 90% confidence intervals are included in the range of 80.00% -125.00% for Cmax and AUC_{0-48} log-transformed.[11]

Adverse events

Any untoward reactions during study were planned to be reported.

Result and Discussion

Vildagliptin is a potent anti-diabetic drug being used to reduce elevated blood glucose levels on type 2 diabetes patients. Previous studies have reported that when vildagliptin is combined with metformin, the combination provides synergistic effect and thus effective management of diabetes.

The ADA and EASD guidelines recommend using other hypoglycaemic

Table-1A: Comparative Mean Pharmacokinetic Data of Test Formulation

Pharmacokinetic Parameters	Vildamet®						
	N	Mean	Median	S.D.	C.V.	Minimum	Maximum
C_{max} (ng/mL)	12	1630.97	1681.81	307.87	18.88	947.35	2006.20
AUC_{0-48} (ng*hrs/mL)	12	18248.30	17789.59	2477.59	13.58	15230.38	21629.18
T_{max} (hrs)	12	5.62	5.75	1.33	23.71	2.00	8.00

Table-1B: Comparative Mean Pharmacokinetic Data of Reference Formulation

Pharmacokinetic Parameters	Eucreas®						
	N	Mean	Median	S.D.	C.V.	Minimum	Maximum
C_{max} (ng/mL)	12	1589.45	1593.96	260.12	16.37	1098.40	2048.73
AUC_{0-48} (ng*hrs/mL)	12	17316.10	18174.73	2592.88	14.97	13676.95	20853.08
T_{max} (hrs)	12	5.77	6.00	1.47	25.50	2.33	8.00

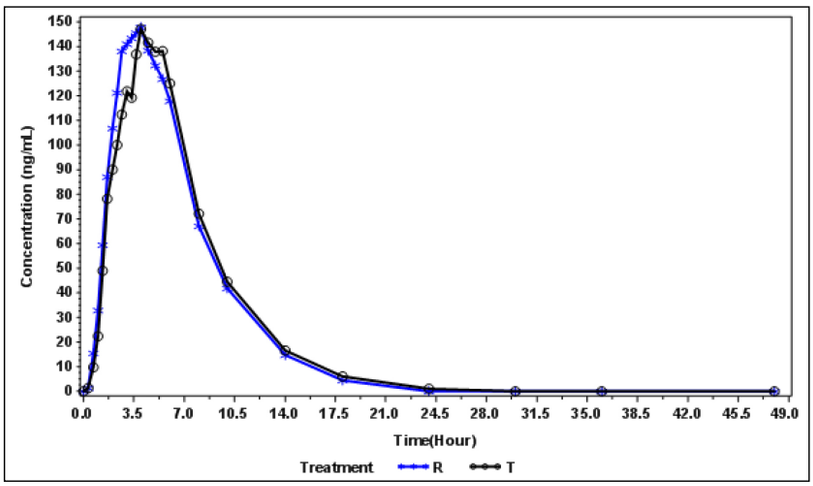

Fig 1A: Comparative Linear Plot of Vildagliptin Mean Plasma Concentration (ng/ml) Vs Time (hrs)

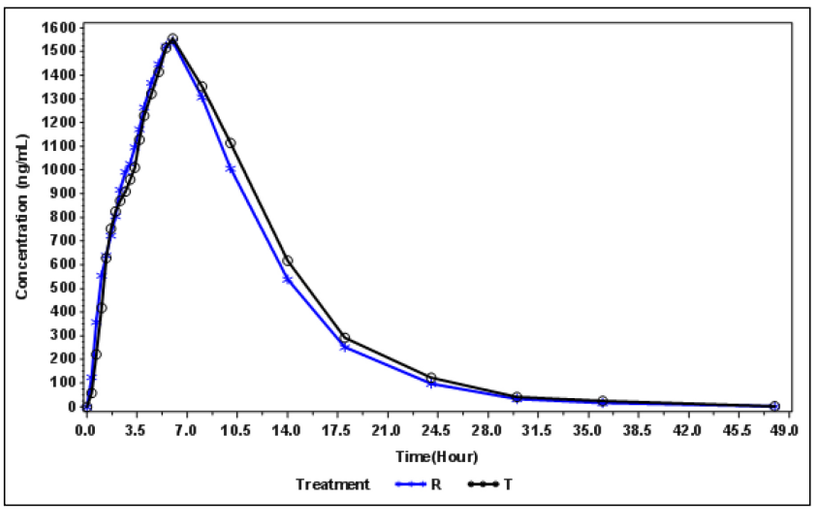

Fig 1B: Comparative Linear Plot of Metformin Mean Plasma Concentration (ng/ml) Vs Time (hrs)

Table-2: Confidence Intervals of Ln-Transformed Parameters for Test Formulation and Reference Formulation

Geometric Means, Ratio, Intra-subject C.V., Power & 90% Confidence Interval							
Pharmacokinetic Parameters	Geometric Mean		Ratio (T/R) (%)	Intra Subject C.V. (%)	Power (%)	90% Confidence Interval (%)	
	Reference(R)	Test (T)				Lower	Upper
C_{max}	1569.24	1600.47	101.99	8.95	99.85	95.46	108.96
AUC_{0-48}	17133.79	18094.72	105.61	4.53	100.00	102.13	109.21

T: Vildamet®; R:Eucreas®

drug along with metformin in type 2 diabetes patients. Patient compliance is an issue when multiple treatments are needed for the management of ongoing disease. In such conditions, a fixed dose combination of these therapies can be used to improve patient compliance. However, fixed dose combination of drug should possess the same pharmacokinetic profile as that of individual treatments to achieve desired efficacy goals. This study was performed to evaluate the bioequivalence of fixed dose combination of Vildamet® of Macleods Pharmaceutical Limited with Eucreas® of Novartis Pharma India.

Results of the study showed that a single dose of test formulation had similar pharmacokinetic profile to that of reference standard. The comparative pharmacokinetic profiles of both test and reference products are shown in Table-1A, Table-1B and Figure-1. The key pharmacokinetic profile were similar in both test and reference formulations. The C_{max} was found to be 1630.97 ng/mL in test formulation compared to 1589.45 ng/mL in reference formulation. The AUC_{0-48} was 18248.30 ng*hrs/mL in test formulation compared to 17316.10 ng*hrs/mL. The Tmax was found to be 5.62 hrs in test formulation compared to 5.77 hrs in reference formulation.

The 90% confidence interval (CI) of geometric mean of C_{max} and AUC_{0-48} for test and reference products were 87.87 (90% CI 86.57-109.29) and 99.92 (90% CI 95-107.49) respectively. There was no statistical significant difference observed in pharmacokinetic parameters between test and reference products. Ratio (T/R) for C_{max} found to be 101.99%. The highest intra subject C.V. for Vildagliptin and Metformin combination was observed to be 8.95% (Table-2).

No adverse events were experienced by any of the patient during entire study period

Conclusion

Vildamet® Macleods Pharmaceuticals Ltd, India (Test product) and Eucreas® of Novartis Pharma, India (Reference Product) demonstrated the 90% confidence interval for the ratio test/reference of pharmacokinetic parameters of C_{max} & AUC_{0-48} was within the acceptable limits (80.00%-125.00%), thereby establishing bioequivalence between both the formulations.

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