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A Single Dose, Bioavailability Study Mounjaro (Tirzepatide) Solution for Injection in a Pre-Filled Pen 2.5 Mg/0.5 Ml in Normal Healthy Adult Human Subjects Under Fasting Condition

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ABSTRACT

Background: Tirzepatide is a novel long-acting agonist of the gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptors, exhibiting high selectivity for both human receptors. Its pharmacological profile includes a strong affinity for both the GIP and GLP-1 receptors, which are pivotal in the regulation of glucose homeostasis and appetite control. Notably, the activity of Tirzepatide at the GIP receptor closely mimics that of the endogenous GIP hormone, facilitating insulin secretion in a glucose-dependent manner. In contrast, its action at the GLP-1 receptor is somewhat reduced when compared to the native GLP-1 hormone. Both receptors are widely distributed throughout the body, being present on pancreatic α and β cells, as well as in the heart, vasculature, immune cells (such as leukocytes), gastrointestinal tract, and kidneys. Additionally, GIP receptors are localized to adipocytes, emphasizing their role in lipid metabolism and energy balance. Importantly, both GIP and GLP-1 receptors are expressed in brain regions integral to appetite regulation, suggesting a complex interplay between these pathways and body weight management. The primary objective of this study was to evaluate the pharmacokinetics and bioavailability of tirzepatide following subcutaneous administration in healthy subjects. Moreover, the impact of tirzepatide on weight reduction was assessed by comparing the subjects' weight measurements taken at study check-in and again at the end of the intervention period. Materials and methods: Materials: Dose and Mode of Administration: Mounjaro (Tirzepatide) solution for injection in a prefilled pen 2.5 mg/0.5 mL, subcutaneous injection in sitting posture under fasting condition. Methods and Findings: The study was conducted as a single-dose bioavailability study under fasting conditions to evaluate the pharmacokinetics of Tirzepatide. A total of four male subjects, aged between 31 and 40 years, who met the protocol defined eligibility criteria were enrolled in the study after a written informed consent was obtained. Each participant was administered with a single dose of subcutaneous injection of Tirzepatide, administered in the right side of abdomen to ensure standardisation

for consistent bioavailability and pharmacokinetic profiling. Blood samples were collected at various time points, up to 96 hours post-administration, enabling a comprehensive analysis of the drug's pharmacokinetic parameters. To evaluate safety, thorough assessments were conducted prior to enrolment in study, during the study and post study, including clinical examinations, vital sign measurements, laboratory tests, and close monitoring of each subject's overall well-being during the conduct. Any symptoms or signs of adverse events were carefully evaluated throughout the study duration and documented. The plasma concentrations of Tirzepatide were quantified using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, ensuring reliable and accurate measurement of the drug concentration levels. The concentration data obtained were then used for pharmacokinetic analysis using the Phoenix WinNonlin version 8.4 software tool. Conclusion: The study was conducted to evaluate the bioavailability and pharmacokinetic profile of Tirzapatide, a novel long-acting agonist of GIP and GLP-1 receptors, when a single dose is administered via subcutaneous route to healthy male subjects. The findings from this study will contribute to the understanding and provide insights into Tirzepatide's therapeutic efficacy in weight reduction and its safety profile.

Keywords: bioavailability, bioequivalence, tirzepatide, single dose, healthy subject, AZIDUS

INTRODUCTION

Tirzepatide is a novel therapeutic agent designed for the management of type 2 diabetes mellitus, administered via subcutaneous injection on a once-weekly basis. This medication functions as a dual agonist of the glucose-dependent insulinotropic peptide (GIP) receptor and the glucagon-like peptide-1 (GLP-1) receptor, leveraging the intricate pathways of glucose homeostasis to enhance glycemic control [9].

Pharmacokinetic evaluations of tirzepatide have demonstrated comparable profiles between healthy individuals and patients diagnosed with type 2 diabetes, suggesting its consistent absorption and distribution characteristics across different populations. Notably, steady-state plasma concentrations of tirzepatide are achieved after four weeks of weekly administration, indicating a predictable pharmacokinetic behavior that supports its dosing regimen.

Upon subcutaneous administration, tirzepatide exhibits a time to maximum plasma concentration (Tmax) ranging from 8 to 72 hours, with a notable mean absolute bioavailability of 80%. This efficient absorption has been observed irrespective of the injection site—whether administered in the abdomen, thigh, or upper arm. Furthermore, the drug displays a mean apparent steady-state volume of distribution of approximately 10.3 L and is highly bound to plasma albumin (99%), underscoring its extensive distribution within the body compartments. The pharmacokinetics of tirzepatide also reflect a favourable elimination profile, with a mean clearance of 0.061 L/h and an elimination half-life of approximately five days, facilitating the convenience of once-weekly dosing. Metabolically, tirzepatide undergoes proteolytic cleavage, beta-oxidation [8], and amide hydrolysis, with its metabolites primarily excreted through urine and faeces, while intact tirzepatide remains undetectable in these excretory pathways.

METHODS

Volunteers

A total of four subjects were selected and willing to participate in the study.

Key criteria for inclusion/exclusion considered for this study were:

a) Healthy male volunteers as evaluated by medical history, vitals, and general clinical examination and ECG were enrolled in the study.
b) Subjects with significant clinical findings and had recent history of surgery, alcoholism, smoking and with known hypersensitivity to Tirzepatide were excluded from the study during screening

Informed Consent

The study protocol and informed consent forms (ICFs) were reviewed and approved by an independent ethics committee under the New Drugs and Clinical Trials Rules, 2019, prior to study initiation. The study informed consent documents were distributed to all the eligible subjects, and the consent documents were carefully read and explained by Trained staff members to ensure comprehension. Participants were awarded adequate time to review and understand the consent documents. Written informed consent was systematically obtained from each participant, affirming their understanding and willingness to engage in the study [6].

The entire study was conducted in strict compliance with the Declaration of Helsinki, Good Clinical Practice (GCP) guidelines, and relevant national regulatory requirements, maintaining the highest ethical standards in clinical research [4, 5].

Study Design

A single-dose, bioavailability study of Mounjaro (Tirzepatide) solution for injection in a pre-filled pen 2.5 mg/0.5 mL in normal healthy adult human subjects under fasting conditions.

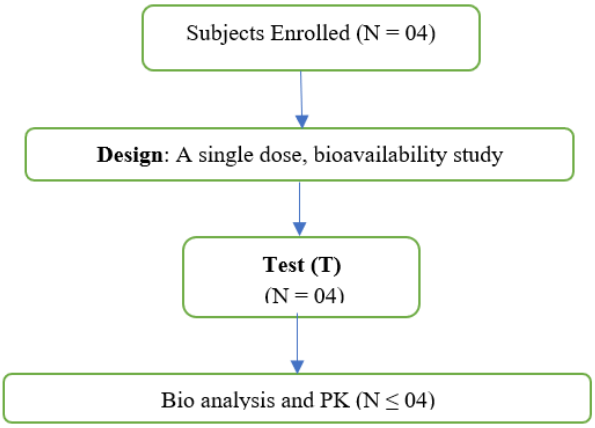

Figure 1: Illustration of Study Design

Drug Administration

After an overnight fast of 10 hours, all four subjects were administered with a single dose of Tirzepatide in sitting posture via subcutaneous route in the abdomen, as specified in the product information. Each subject's drug dosing details were documented in their individual

case report forms to ensure accurate record-keeping. The study was conducted under the vigilant supervision of the Principal Investigator, ensuring protocol adherence and participants' safety with the help of a sub-investigators, physicians and a study team. Subjects were not restricted for posture during the study.

Blood Sampling, Handling and Processing

A total of 19 blood samples of 03 mL each were collected at 00.00 (pre-dose), 02.00, 04.00, 06.00, 08.00, 10.00, 12.00, 14.00, 16.00, 18.00, 20.00, 24.00, 28.00, 32.00, 36.00, 42.00, 48.00, 72.00, and 96.00 hours post dose for measurement of pharmacokinetic parameters. The samples were collected from an intravenous cannula or by direct vein puncture using pre-labeled K3EDTA vacutainers at the scheduled times. The blood samples were centrifuged at 3500 rpm for 10 minutes at 4°C. The plasma harvested was separated into two aliquots, 01 mL of plasma was transferred to the first aliquot and the remaining volume to the second aliquot. The samples were stored at -70oC in an ultra-low temperature freezer. The plasma samples were quantified using a validated bio-analytical method.

Assessment of Weight

The weight of the participants was carefully recorded both at check-in and again at the end of the study, coinciding with the final pharmacokinetic sample collection.

Analytical Method for Quantification

A validated LC-MS/MS bio-analytical method was used for the estimation of Tirzepatide in plasma. Bioanalytical method validation was done in compliance with the ICH M10 guidance. This validation process includes a thorough evaluation of key parameters such as specificity, sensitivity, precision, and accuracy, stability assessments, recovery, and dilution integrity.

Pharmacokinetic Analysis

The pharmacokinetic parameters - C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, $t½$, K_{el}, and $AUC_{\_\%Extrap\_Obs}$ were calculated using the Non-compartmental Model in Phoenix® WinNonlin® v 8.4. The AUC was calculated employing the linear trapezoidal rule, a widely recognized method for determining drug exposure over time. The C_{max} and T_{max} were derived directly from the individual concentration versus time data, ensuring an accurate representation of the pharmacokinetic profile. The elimination rate constant (Kel) was estimated by log-linear least squares regression of the terminal part of the plasma concentration versus time curve using the best fit method.

Safety Analysis

Safety was assessed through continuous health monitoring and the systematic recording of safety measurements throughout the study period. Trained staff members monitored and recorded the pulse rate, blood pressure, and subjects' well-being during check-in, at 00.00 (pre dose), 02.00, 08.00, 25.00, 31.00-, and 47.00 hours post-dose, ambulatory, post-study and at discretion of clinical staff. Temperature was recorded during check-in, prior to check-out and post-study and at the discretion of clinical staff.

General examination and systemic examination of subjects were performed during check-in, prior to check-out and post study with the primary goal of ensuring safety. Subjects were actively encouraged to inform clinic personnel of any untoward medical symptoms and/or

events that occurred throughout the study duration. The Principal Investigator evaluated the adverse events and graded them as mild and for their relationship to the study drug. Post-clinical laboratory tests (serum chemistry and haematology), post-study general and systemic examination including vital sign measurements (blood pressure, pulse rate, and temperature) were performed, ensuring a thorough understanding of the safety profile associated with the study interventions.

RESULTS

Pharmacokinetics

In the present study, four subjects participated and all completed the study. The concentrations obtained for the subjects were subjected to pharmacokinetic analysis to evaluate the absorption, distribution, metabolism, and excretion of the administered compound in healthy individuals. The calculated pharmacokinetic parameters C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max} and t½ for Tirzepatide are presented in **Table 1**.

Table 1: Pharmacokinetic Results - Tirzepatide

Parameters	Test (T)
C_{max}	226.007 ± 20.416 ng/mL
AUC_{0-t}	17779.421 ± 2044.805 hr.ng/mL
$AUC_{0-\infty}$	50869.845 ± 22799.897 hr.ng/mL
T_{max}	39.00 (32.00 – 48.00) hr
t½	135.515 ± 82.681 hr

The pharmacokinetic parameters obtained in the study are consistent with the reported values for tirzepatide [10]. The concentration vs time plot of Tirzepatide obtained in the study is presented below as figure 1.

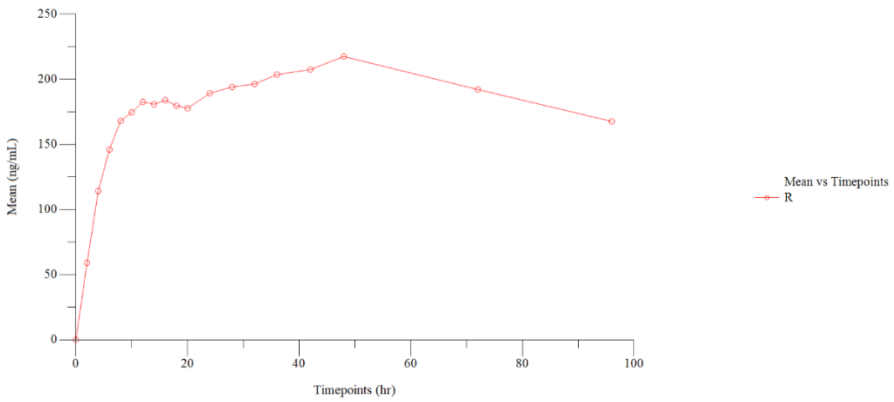

Figure 1: Bioavailability curve (Mean plasma concentration vs. time) of Tirzepatide injection

Safety - Brief Summary of Adverse Events:

Safety was assessed throughout the duration of the study. One adverse event was reported, mild in severity and evaluated to be unrelated to the study drug. There were no serious adverse events in the study, underscoring the overall safety of the intervention.

DISCUSSION

This study was designed to evaluate the bioavailability of tirzepatide when administered subcutaneously as a single dose to healthy subjects. Blood samples were meticulously collected at predetermined time points following dosing to construct a comprehensive pharmacokinetic profile using concentrations obtained using a validated LC-MS/MS method. Additionally, weight assessments were done for all participants to observe the potential impact of the drug on body weight. These assessments involved comparing the subjects' weights at check-in and check-out.

The results of the study were promising, providing valuable insights into the pharmacokinetic and pharmacodynamic properties of Tirzepatide. The study significantly contribute to the understanding of quantification of Tirzepatide in the human plasma samples using mass spectrometric methods and safety profile in healthy adults.

CONCLUSION

This study successfully evaluated the pharmacokinetic profile of Tirzepatide. The analysis revealed valuable insights into the absorption, distribution, metabolism, and excretion patterns, with established safety profile contributing to our understanding of the drug's behavior in a clinical context in healthy adults of Indian origin. These findings pave the way for future research and potential clinical applications of the compound, highlighting the need for further studies to expand on these preliminary results.

Conflict of Interest Disclosure

All technical, clinical, and analytical executions of the bioequivalence studies were performed independently by Azidus Laboratories Ltd with no conflict of interest.

ACKNOWLEDGMENT

We extend our deepest gratitude to the healthy volunteers who generously participated in this study. Their willingness to contribute to scientific advancement made this research possible. We sincerely appreciate their time, commitment, and trust. Additionally, we acknowledge the invaluable support of the research team, clinical staff, and institutions that facilitated this work.

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