BIOEQUIVALENCE OF TWO FORMULATIONS OF GLICLAZIDE IN A RANDOMIZED CROSSOVER STUDY IN HEALTHY CAUCASIAN SUBJECTS UNDER FED CONDITION

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Abstract

This study was aimed to assess the bioequivalence of a test product, Gliclazide 60 mg modified release tablets (Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India) and a reference product Diamicron\textsuperscript{®} 60 mg modified release tablets (Les Laboratoires Servier Industrie, France) in 26 healthy Caucasian volunteers under fed condition. The design of the study was single-dose, two-treatment, two-period, and two-sequence crossover study in fed condition with a washout period of 21 days. Blood samples were collected for a period of 96 h after drug administration in each period. Gliclazide plasma concentrations were determined by a LC-MS/MS method. Pharmacokinetic analysis used a non-compartmental model. The logarithmically transformed data of $C_{\text{max}}$ and AUCs were analysed using ANOVA. The 90\% confidence intervals were within the acceptance range of 80.00 - 125.00\% and there is no significant difference in pharmacokinetic characteristics between the products. The investigated products are bioequivalent under fed condition.

Resumat

Acest studiu a urmărit să evaleze bioechivalența unui produs test, Gliclazide 60 mg comprimate cu eliberare modificată (Ranbaxy Laboratories Limited, în prezent Sun Pharmaceutical Industries, India) și un produs de referință, Diamicron\textsuperscript{®} 60 mg comprimate cu eliberare modificată (Les Laboratoires Servier Industrie, Franța) pe 26 de voluntari caucazieni în condiții post-prandiale, cu o perioadă de eliminare de 21 de zile. Probelor de sânge au fost recoltate într-un interval de 96 ore după administrarea medicamentului în fiecare perioadă. Concentrațiile plasmatice ale gliclazidei au fost determinate prin metoda LC-MS/MS. Analiza farmacocinetice a utilizat un model non-compartmental. Datele transformate logaritmice ale $C_{\text{max}}$ și ASC au fost analizate utilizând ANOVA. Intervalele de incredere 90\% se încadrează în intervalul acceptat de 80.00 - 125.00\% și nu există diferențe semnificative în ceea ce privește caracteristicile farmacocinetice între produse. Produsele investigate sunt bioechivalente în condițiile post-prandiale.

Keywords: pharmacokinetics, bioequivalence, gliclazide, modified release tablets

Introduction

The incidence of type 2 diabetes increased dramatically due to urbanization, aging of population, changes in diet, obesity, sedentary lifestyle. Diabetes is associated with a large number of chronic complications [6, 10]. Treatment of type 2 diabetes mellitus according to the therapeutic guidelines developed by various international organizations may include non-pharmacological treatment (diet and exercise) and medication [10]. Oral hypoglycaemic agents and insulin are generally available in only few low-income countries. For improvement of equitable access, there is need for policy and programme interventions [10, 11]. The most frequently used oral hypoglycaemic agents are sulfonylureas. Trials of gliclazide modified release tablets support its use as first-line treatment in type 2 diabetes, including to elderly, obese and mild-to-moderate renal insufficient patients. The compliance to treatment is significantly improved due to the simplicity of the dose regimen and its tolerance and efficacy, especially when T2DM is associated with other comorbidities [1, 2]. As a mechanism of action, gliclazide decreases the blood glucose levels by stimulating the secretion of insulin from the β-cells of the Langerhans islets. Gliclazide has high affinity with strong selectivity and it is reversible bound to the β-cell KATP channels. Gliclazide has low affinity for vascular and cardiac...
and Pharmacokinetics Department of Terapia SA, Romania.

Materials and Methods

The clinical study protocol was approved by National Agency for Medicines and Medical Devices, Romania and the Ethics Committee of the “Iuliu Hațieganu” University of Medicine and Pharmacy Cluj-Napoca, Romania.

The study was performed at the Clinical Pharmacology and Pharmacokinetics Department of Terapia SA, Romania.

A written consent has been obtained from each volunteer prior to any screening procedures. The Investigator included in the study only healthy volunteers after examining their health on the basis of medical history, physical examination, ECG and routine blood and urine tests. Volunteers were also tested for drugs of abuse and alcohol and in case of female volunteers, for pregnancy. The volunteers were asked about consumption of any pharmacological agent and/or dietary product and it was evaluated the risk of drug interaction.

Test product was a new generic modified release formulation containing Gliclazide 60 mg developed by Ranbaxy Laboratories Limited, now Sun Pharmaceutical Industries, India and the reference product was Diamicron® modified release tablets (containing gliclazide 60 mg) manufactured by Les Laboratoires Servier Industrie, France.

In each period of the study the subjects received alternatively, according to the generated randomization list, the test formulation (T) or the reference formulation (R).

Following a fasting period of at least 10 hours, the subjects started the recommended high-fat high-calorie standard meal, 30 minutes prior to administration of the drug product. The drug product was administered with 240 mL of 20 % glucose solution. During the first 4 hours post dose at every 15 minutes it was administered approximately 60 mL of 20% glucose solution. The fasting period continued for 4 hours post-dose. Water was also restricted 1 hour before drug administration until 2 hours post-dose. Subjects received standard meals, identical for both the periods during the housing period.

Throughout the study, the subjects were monitored for adverse events and vital signs (sitting blood pressure, radial pulse and axillary body temperature) and blood glucose levels were measured periodically according to the study protocol. During each period of the study, principal investigator/subinvestigator was available for at least 24 hour post-dose at the investigation site. The clinical examination, urine analysis, biochemistry and haematology tests and pregnancy test (in case
of females) were repeated for the safety assessment at the end of the study.

Blood samples were collected in K$_3$EDTA vacutainers: pre-dose and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 20, 24, 36, 48, 72 and 96 hours post-dose in each period. The samples were collected either through indwelling cannula or through a new vein puncture.

Blood samples were centrifuged for 15 min, under refrigeration, at 4000 rpm for plasma separation which was kept at -50°C until assay. For the determination of Gliclazide in plasma it was used a validated LC-MS/MS method using Gliclazide D4 as internal standard.

The relationship between concentration and peak area was found to be linear in the range of 5.00 ng/mL to 5016.48 ng/mL. The limit of quantification was 5.00 ng/mL [7].

HPLC Agilent 1200 from Agilent Technologies, MS API 3200 from Applied Biosystem MDS SCIEX and Analyst software version 1.4.2 were used for sample analysis and data processing. All the sample processing was done under low light conditions. Retention times were for Gliclazide and Gliclazide D4 between 0.3 - 2.0 minutes [7].

During validation it has been reported a between-run precision of 1.83% to 4.69%, between-run accuracy of 90.53% to 110.14%, within-run precision of 0.96% to 4.38% and a within-run accuracy of 89.10% to 114.98% [7].

The noncompartmental pharmacokinetic analysis was performed for Gliclazide using WinNonlin® PK software version 5.2 and the following parameters were calculated: C$_{\text{max}}$, T$_{\text{max}}$, AUC$_{0-t}$, AUC$_{0-\infty}$, AUC% Extrapolated and T$_{1/2}$.

The statistical analysis was performed for log-transformed pharmacokinetic parameters (C$_{\text{max}}$, AUC$_{0-t}$ and AUC$_{0-\infty}$) using SAS software version 9.1.3. The analysis used Type III sum of squares from ANOVA.

Ratios of means were calculated using the antilog of the differences of LSM for log-transformed C$_{\text{max}}$, AUC$_{0-t}$ and AUC$_{0-\infty}$. For pharmacokinetic parameters C$_{\text{max}}$ and AUC$_{0-t}$ of log-transformed data the confidence interval 90% for the test (T) and reference (R) product ratio averages should be in 80% - 125% interval.

**Results and Discussion**

26 Caucasian subjects were enrolled in the study and 23 subjects finalized the study. The mean age, mean weight, mean height and mean BMI of the subjects completing the study were 25 years (range 19 to 35 years), 74 kg (range 56 to 92 kg), 180 cm (range 166 to 195 cm), 22.9 kg/m$^2$ (18.6 to 28.7 kg/m$^2$), respectively.

In this study there were reported the following not-serious adverse events: excoriations, hypoglycaemia, diarrheic syndrome, increased BUN, leukocyturia, increased ALT, increased direct bilirubin, increased total bilirubin, decreased serum glucose, haematuria and proteinuria.

The plot of Gliclazide mean plasma concentration versus time under the fed condition is showed in Figure 1 and pharmacokinetic characteristics are summarized in Table I. The products have similar values for C$_{\text{max}}$ and AUC$_{0-t}$.

![Figure 1](image_url)

**Figure 1.**
Mean plasma concentration of gliclazide after administration of test product (Gliclazide 60 mg modified release tablets), and the reference product (Diamicron® modified release tablets), in 23 volunteers under fed condition.
Pharmacokinetic characteristics of Gliclazide in 23 volunteers following administration of Gliclazide modified release tablets 60 mg under fed condition

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>C_{max} (ng/mL)</th>
<th>AUC_{0-4} (hr*ng/mL)</th>
<th>AUC_{0-∞} (hr*ng/mL)</th>
<th>AUC % Extrapolation (hr*ng/mL)</th>
<th>T_{1/2} (hr)</th>
<th>T_{max} (hr)</th>
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<tr>
<td>Mean</td>
<td>1862.9±1844.3</td>
<td>35205.1</td>
<td>34156.0</td>
<td>36772.0±35702.5</td>
<td>3.9±4.2</td>
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<td>SD</td>
<td>401.0±405.2</td>
<td>12096.2</td>
<td>11322.4</td>
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<td>1.8±2.3</td>
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<td>Min</td>
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<td>15151.0</td>
<td>15765.0</td>
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<td>CV%</td>
<td>21.5±22.0</td>
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T represents Test Product which is Gliclazide 60 mg modified release tablets. R represents Reference Product which is Diamicron® 60 mg modified release tablets.

The ratios of LSM (with 90% confidence intervals) for C_{max}, AUC_{0-4}, and AUC_{0-∞} for Gliclazide were 99.04% (93.17% - 105.28%), 97.43% (93.13% - 101.93%), respectively 97.78% (93.48% - 102.27%). The reported adverse events in this study are unlikely to have any impact on the subjects’ safety.

Based on the results, it was concluded that Gliclazide 60 mg modified release tablets manufactured by Ranbaxy Laboratories Ltd, now Sun Pharmaceutical Industries, India is bioequivalent to Diamicron® modified release tablets (containing gliclazide 60 mg) manufactured by Les Laboratoires Servier Industrie, France are bioequivalent in healthy, adult, human subjects under fed condition.

**Conclusions**

Gliclazide 60 mg modified release tablets manufactured by Ranbaxy Laboratories Ltd, now Sun Pharmaceutical Industries, India and Diamicron® modified release tablets (containing gliclazide 60 mg) manufactured by Les Laboratoires Servier Industrie, France are bioequivalent in healthy, adult, human subjects under fed condition.

**Conflict of interest**

The authors declare no conflict of interest.

**References**


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**Table I**

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