Bioequivalence assessment of the two brands of glimepiride tablets

Ispitivanje bioekvivalentnosti dva preparata glimepirida u obliku tableta

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Abstract

Background/Aim. Glimepiride, as an antidiabetic from the group of sulfonylurea, is administered perorally in the treatment of diabetes mellitus. The aim of this study was to compare pharmacokinetic profiles and relative bioavailabilities of the two oral formulations of glimepiride, generic and innovator tablets, after a single dose of the active drug.

Methods. An oral dose of 6 mg glimepiride was given under fasting conditions to 24 healthy volunteers. A one-week washout period was applied between the two consecutive periods. The serum samples obtained before dosing, and at various time points up to 48 hours, were analyzed for glimepiride concentration using the validated high-performance liquid chromatographic method with ultraviolet detection. Pharmacokinetic parameters representing early (maximal concentration, time to reach maximal concentration) and total exposure (area under the curve from the time 0 to the infinite time) to glimepiride were obtained and further analyzed using the multifactorial analysis of variance and the non-parametric Wilcoxon signed ranks test. Comparison of the secondary kinetic variables was only descriptive.

Results. The point estimates of the ratios of geometric means (test/reference) of maximal concentrations and areas under the curve were 1.046 (90% confidence interval: 0.906–1.208) and 1.022 (90% confidence interval: 0.856–1.220), respectively, while the median values of times to reach maximal concentration, at 5% level of significance, did not differ significantly. Both formulations were well tolerated. Transient mild hypoglycaemia, which had been noted in 6 participants, resolved spontaneously within 30–60 minutes.

Conclusion. Since all the parametric 90% confidence intervals for the log-transformed main variables of glimepiride were within the 0.80 and 1.25 interval, accepted as the definition of bioequivalence, and the differences in times to reach maximal concentration also did not reach statistical significance, studied tablets were considered bioequivalent.

Key words: diabetes mellitus, type 2; drugs, generic; sulfonylurea compounds; therapeutic equivalency; biological availability.

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Apstrakt

Uvod/Cilj. Glimepirid je antidiabetik iz grupe sulfonilurea koji se primjenjuje peroralno u lečenju dijabetes mellitus. Cilj ispitivanja bio je da se nakon peroralnog davanja jednokratne doze uporedi farmakokinetičko ponašanje i relativna bioraspoloživost generičkog proizvoda i referentnih tableta koje kao aktivnu materiju sadrže glimepirid. Metode. Doza od 6 mg glimepiridaordinirana je zdravim dobrovoljcima (n = 24) na tačne. Pauza između dva uzastopna perioda ispitivanja iznosila je sedam dana. Uzorci krvi krvnih sazivki su prikupljeni u neposredno pre davanja lekova, a posle ingestije, krv je uzimana u prethodno definisanim vremenskim intervalima, tokom 48 sati. Koncentracije glimepirida određivane su metodom tečne hromatografije visoke rezolucije sa UV detekcijom. Primarni farmakokinetički parametri, koji u potpunosti opisuju faze rane (maksimalna koncentracija, vreme postizanja maksimalne koncentracije i ukupne (površina ispod krive od nultog vremena do beskonačnosti) izloženosti glimepiridu, statistički su upoređeni korišćenjem analize varijanse u više pravaca i neparametrijskog Wilcoxonovog testa sume rangova. Rezultati. Kritične vrednosti za relativne razlike (ispitivani lek/referentni lek) pojedinačnih vrednosti maksimalnih koncentracija i površina ispod krive koncentracije su 1,046 (90% interval poviranja = 0,906–1,208) i 1,022 (90% interval poviranja = 0,856–1,220). Pri nivou značajnosti od 5%, medijani vremena postizanja maksimalnih koncentracija nisu se, takođe, statistički razlikovali (p = 0,8026). Podnošljivost oba farmaceutska oblika glimepirida bila je dobra. Prolazna, umerenih hipoglikemija, koja je zapravo kod šest ispitanih, normalizovala se spontano tokom 30–60 minuta. Zaključak. Budući da su se parametrijski 90% intervali poviranja za relativne razlike logaritamski transformisanih vrednosti primarnih farmakokinetičkih parametara glimepirida nalazili u rašponu 0,80–1,25, što je stručno prihvaćeno kao kriterijum za definisanje bioekvivalentnosti lekova, kao i da nije postojala statistički značajna razlika među vremenima postizanja maksimalnih koncentracija glimepirida u krvi, zaključeno je da su ispitivani farmaceutski oblici dva proizvođača međusobno bioekvivalentni.
**Introduction**

Glimepiride is a sulfonylurea antidiabetic. It is given orally for the treatment of type 2 diabetes mellitus (NIDDM). Initial oral doses of 1 to 2 mg daily may be increased, if necessary, to 4 mg daily for maintenance. The maximal recommended dose is 6 mg in the UK, 1 and 8 mg in the USA.

After oral administration, glimepiride was completely absorbed from the gastrointestinal tract. Studies with single oral doses in normal subjects and with multiple oral doses in NIDDM patients had shown significant absorption of glimepiride within 1 hour after administration and peak drug levels at 2 to 3 hours. When glimepiride was given with meals, the mean time to reach maximal concentration (T(max)) was slightly increased and the mean C(max) and area under the curve (AUC) were decreased less than 10%, respectively. After intravenous dosing in normal subjects, the volume of distribution was 113 ml/kg, and the total body clearance was 47.8 ml/min. Protein binding was greater than 99.5%. Glimepiride was metabolized to M1 and M2 metabolites accounting for about 40% of the dose. About 10 to 20% of the dose was eliminated in feces as metabolites.

The pharmacokinetic parameters of glimepiride obtained from a single-dose, crossover, dose-proportionality study in normal subjects and from a single- and multiple-dose, parallel, dose-proportionality studies in NIDDM patients indicated that glimepiride did not accumulate in serum, and that its pharmacokinetics was not different. The oral clearance of glimepiride did not change over the 1-8 mg dose range, indicating linear pharmacokinetics.

Differences in gender, age, and race or obesity did not change significantly the pharmacokinetics of glimepiride. However, pathologic conditions (e.g., renal failure with creatinine clearance < 20 ml/min), interactions with concurrently used drugs or genetic CYP polymorphism might affect the pharmacokinetic profile of glimepiride and thereby contribute to the changes in therapeutic efficacy or in the adverse events profile. Therapeutic failure might also occur when a patient is switched between an innovator drug and a non-bioequivalent generic formulation. Having in mind that pharmacokinetic studies investigating the bioequivalence of generic and innovator drugs could minimize such risks, the aim of this study was to evaluate the bioequivalence of generic glimepiride, prepared as 6 mg tablets by the manufacturer Re-mevita d.o.o., Niš, Serbia (Glimepirid; Test), with the brand drug (Amarily 6 mg tablets, Aventis Pharma Deutschland GmbH, Frankfurt ab Main, Germany; Reference). Eligible volunteers were randomly assigned to one of the two sequence groups so that, upon conclusion of the study, each subject received both regimens. Dosing in each of the two consecutive periods was separated by one-week washout period.

A written informed consent was obtained from all subjects prior to any study-related procedures. The study was approved by the Drug Commission and the Ethics Committee of the Military Medical Academy, Belgrade, Serbia, on November 22, 2005.

Prior to the clinical part of the study, the in vitro dissolution comparison of the test and reference drug confirmed their pharmaceutical similarity and prompted the initiation of the clinical pharmacokinetic trial.

**Methods**

**Study design**

This was a single-dose, open-label, randomized, two-sequence, two-period crossover pharmacokinetic study aimed to evaluate the bioequivalence of generic glimepiride, prepared as 6 mg tablets by the manufacturer Remevita d.o.o., Niš, Serbia (Glimepirid; Test), with the brand drug (Amarily 6 mg tablets, Aventis Pharma Deutschland GmbH, Frankfurt ab Main, Germany; Reference). Eligible volunteers were randomly assigned to one of the two sequence groups so that, upon conclusion of the study, each subject received both regimens. Dosing in each of the two consecutive periods was separated by one-week washout period.

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**Subject population**

Twenty-four Caucasian subjects of both sexes (15 males, 9 females) in good physical condition, as confirmed by the complete medical and laboratory examinations before the study, were enrolled. The volunteers were between 20 and 50 years of age, their mean body mass was 78.1 kg and the body mass index ranged from 20 to 26 kg/m². Prescription and over-the-counter drugs, except paracetamol, were prohibited within 14 days and 48 hours of study entry, respectively.

**Glimepiride dosing**

Since the study was conducted as a crossover comparison, each subject had to receive the 2 respective oral doses of 6 mg glimepiride. Thus, after the two consecutive periods a total exposure of each volunteer to glimepiride was 12 mg.

Under the supervision of medical staff, a single dose of 6 mg glimepiride was given with 200 ml of non-carbonated mineral water, following an overnight fast of at least 10 hours. The dose chosen for the study was selected because it is still below the maximal recommended dose for glimepiride (in the USA) in NIDDM patients, and was expected to produce measurable serum levels for a sufficient portion of the terminal elimination phase.

**Blood sampling schedule**

Venous blood samples (8 ml each) were collected prior to dosing (hour 0) by direct venous puncture and at 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 9, 12, 24 and 48 hours. The samples were allowed to clot at room temperature for 20 minutes. Within 1 hour of collection all samples were centrifuged at 3 000 rpm for 15 minutes; the serum was then separated and frozen at −20 °C until assayed. Total volume of blood drawn during

**Reference**

the study (including blood taken for the 2 clinical tolerability analyses) was approximately 300 ml.

Blood glucose content was determined regularly at time points 0, 3, 6 and 12 hours after glimepiride ingestion or, additionally, whenever it seemed to be appropriate due to a suspected hypoglycaemic effect of the ingested formulation.

Assay Method

The serum samples were analyzed for glimepiride concentrations by high-performance liquid chromatography combined with ultraviolet spectrometry (HPLC/UV). The HPLC/UV set was equipped with a pump (model 2150, LKB, Bromma, Sweden), an automatic sample system (model AS-100, BioRad Laboratories, Inc., Hercules, CA, USA), and a Dual λ Absorbance Detector (model 2487, Waters Corporation, Milford, MA, USA). A Clarity Lite - 2.4.0.195 software (DataApex Ltd., Prague, Czech Republic), running on a PC, was used for data acquisition and integration.

After being refrigerated, the samples were allowed to melt spontaneously at room temperature for 20 minutes. Aliquots of 1 ml of serum were dispensed into glass tubes, and 5 ml of a solution containing ethyl acetate and ether (1:1) was added. The mixture was stirring for 15 minutes using a mechanical mixing extractor (model KS-50, IKA Werke GmbH & Co.KG, Staufen, Germany), and then centrifuged at 3 000 rpm for the next 15 minutes to express the organic layer. The aqueous phase extracts were evaporated to dryness in the stream of air, reconstituted in a mobile phase and filtrated through 13 mm Nylon 0.45 µm filters (Waters Corporation). Separations were performed on a reversed-phase analytical column (SpheriSorb S5 ODS2, 4.6 × 100 mm, particle size 5 µm; Waters Corporation), with a guard column (SpheriSorb S5 ODS2, particle size 5 µm; Waters Corporation), at room temperature and at the flow rate of 1.0 ml/min. The injector loop volume was 200 µl, and the UV detector wavelength was set at 228 nm. Mobile phase was a 50:50 mixture of acetonitrile and phosphate buffer (pH 2.55), while the assay was performed using an external standard. Under these conditions the retention time for glimepiride was approximately 8.86 minutes. All chemicals were of HPLC and p.a. purity and were purchased from Merck KGaA, Darmstadt, Germany.

Pharmacokinetic analysis

The pharmacokinetic parameters of glimepiride were estimated by noncompartmental calculations with Pharm package, version 1.4 (Simed SA, Paris-Crétteil, France). The parameters were not further corrected for weight or administered dose.

The peak serum concentration [C(max)] and the time elapsed to peak concentration [T(max)] were obtained directly from the data. The elimination rate constant [K(el)] was obtained from the slope of the terminal log-linear phase of the semilog plot of concentration versus time. The half-life [T (1/2)] was calculated as ln2/K(el). The area under the glimepiride serum concentration-time curve [AUC (0-48)] was computed using the linear trapezoidal rule while the area under the serum concentration-time curve from the time 0 to the infinite time [AUC (0-inf)] was calculated as the sum of AUC (0-48) and Ct/K(el), where t was the time of the last measurable concentration (Ct) and K(el) was the elimination rate constant.

The MRT (mean residence time) was defined as the ratio of the area under the first moment curve [AUMC], and AUC [MRT = AUMC/AUC]. The first moment curve was the AUC of the product of concentration [Cp], and the time versus time on a linear scale [Cp (t) * t].

Statistical analysis

In general, a multiplicative model was assumed for concentration-dependent parameters, implying a logarithmic normal distribution, whereas an additive model with the normal distribution of non-transformed data was assumed for the time-related parameters. The pharmacokinetic parameters that describe the early and total exposure to glimepiride, C(max), T(max), and AUC (0-inf), were derived from the individual serum concentration-time profiles and were subjected to statistical analysis. The comparison of the secondary kinetic parameters [AUC (0-48), K(el), T (1/2), and MRT] was only descriptive.

After the logarithmic transformation, AUC (0-inf) and C(max) values were subjected to the analysis of variance (ANOVA), including the terms for subjects, treatment (sequence) and the time period, the residuals of which were then tested for normality, as described by Chow and Liu. For the evaluation of bioequivalence, the point estimates and 90% confidence intervals (CIs) for the relative difference between the test and reference formulations (test/reference) in each subject were constructed, using the residual mean square error obtained from the multi-factorial ANOVA. The point estimates and the 90% CIs were then transformed back to give the estimates of the ratio of the geometric means and the corresponding 90% CIs for the individual ratios of the 2 formulations. A non-parametric test (Wilcoxon signed ranks test) was performed for T(max).

Bioequivalence between the two formulations was accepted if 90% CIs transformed back for the geometric mean ratios of AUC (0-inf) and C(max) were within the 0.80–1.25 range, and if the differences in T(max) were not statistically significant.

Results

Dissolution test

In a phosphate buffer (pH 7.8) at 37°C (± 0.5°C), the 2 formulations under investigation dissolved at 93.3% (test) and 94.2% (reference) within 15 minutes (USP Apparatus 2 with paddles; rotations, 75 rpm; replication, 6).

HPLC

Calibration curves (standard solution, spiked serum) were derived from peak area ratios using a least squares regression of the ratio versus the nominal concentration of glimepiride. After the analysis of 6 triplicate samples at concentrations ranging from 0.005 to 0.750 µg/ml, linearity was

observed in the calibration curves of glimepiride with the correlation coefficients being both greater than 0.999.

A validated liquid chromatographic method had lower limit of quantification (LLOQ) of 0.01 µg/ml and the limit of detection was 0.005 µg/ml. Concentrations below LOQ were reported as 0.0 µg/ml. The mean recovery of glimepiride extraction from serum samples was 87.64% (range, 84%-90%). For within-day and between-day analysis, the mean percent accuracy values of quality control (QC) samples were less than 15% of theoretical values, with precision (expressed as relative standard deviation [RSD]) of 8.97% and 7.24% at 0.05 µg/ml of glimepiride, respectively.

**Study population and tolerability**

All 24 subjects, having no statistically significant differences in baseline characteristics such as age, weight, or heights, had completed the study, and were included in the pharmacokinetic analysis. Six volunteers (test drug, n = 4; reference drug, n = 2) reported headache, sweating and weakness over the first 2 hours of the treatment. The corresponding glycaemia was, on average, about 15% lower (range, 7.9%−34.2%) when compared to the pre-treatment value. Blood glucose-lowering effect gradually and spontaneously decreased at 30 to 60 minutes afterwards, probably because of the homeostasis mechanism in the healthy volunteers.

**Comparative pharmacokinetics and bioequivalence**

Figure 1 shows the mean concentration-time profiles of the 2 oral formulations of glimepiride 6 mg in serum in 24 healthy volunteers. At any of the time-points evaluated, neither the averages nor the concentrations of glimepiride differed significantly in the individual study subjects after the administration of each of the 2 formulations. The mean C(max) values amounted to 398.3 ng/ml (test), and 399.3 ng/ml (reference), and were attained after 3 hours. At the last blood sampling time (48 h), serum concentrations of glimepiride were below the LLOQ in all of the tested subjects.

The relevant pharmacokinetic parameters for the 2 formulations are listed in Table 1. The residual area [difference between AUC (0-48) and AUC (0-inf)] of glimepiride (test: 18.9%; reference: 17.8%) accounted for < 20% of the AUC (0-48). Therefore, the stated criterion [AUC (0-48)/AUC (0-inf) * 100% > 80%] was fulfilled, and the residual area had no sizeable impact on the calculation of AUC (0-inf) and, consequently, on bioavailability.

![Fig. 1 – Concentration (mean ± SD) vs. time profiles of glimepiride 6 mg in serum in 24 healthy volunteers](image)

Neither the early exposure to glimepiride, measured as C(max) and T(max), nor the parameters that describe its elimination [K(el), T (1/2)] were statistically different. The differences in MRT after the administration of the test and reference formulations were negligible.

The results of statistical evaluation of the main pharmacokinetic variables of glimepiride are shown in Table 2.

Further evaluation of pharmacokinetic variables that described the early and total exposure [AUC (0-inf)] to glimepiride showed point estimates of the ratios of geometric means of C(max) and AUC (0-inf) (glimepiride test vs. glimepiride reference) to be 1.046 (90% CIs: 0.906−1.208) and 1.022 (90% CIs: 0.856−1.220), respectively. For median T(max) values, at 5% level of significance, no significant differences were found between the two formulations in the study, as determined by the nonparametric Wilcoxon signed ranks test (p = 0.8026).

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test tablets</th>
<th>Reference tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(max) [ng/ml]</td>
<td>532.5</td>
<td>518.6</td>
</tr>
<tr>
<td>T(max) [h]</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>K(el) [L/h]</td>
<td>0.0894</td>
<td>0.0853</td>
</tr>
<tr>
<td>T(1/2) [h]</td>
<td>8.8</td>
<td>8.9</td>
</tr>
<tr>
<td>AUC (0-48) [ng/ml · h]</td>
<td>3535.2</td>
<td>3346.6</td>
</tr>
<tr>
<td>AUC (0-inf) [ng/ml · h]</td>
<td>4399.6</td>
<td>4260.7</td>
</tr>
<tr>
<td>MRT [h]</td>
<td>12.7</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Mean – arithmetic mean
SD – standard deviation
Statistical evaluation of bioequivalence of the 2 oral formulations of glimepiride

<table>
<thead>
<tr>
<th>Statistical test</th>
<th>Pharmacokinetic parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C(max)</strong></td>
<td><strong>AUC(0-inf)</strong></td>
</tr>
<tr>
<td>Point estimate, ratio of geometric means</td>
<td>1.046</td>
</tr>
<tr>
<td>90% CI, ratio of geometric means*</td>
<td>0.906–1.208</td>
</tr>
<tr>
<td>Wilcoxon signed ranks test**</td>
<td>–</td>
</tr>
</tbody>
</table>

* Acceptance range (bioequivalence): 0.80 – 1.25  
** Bioequivalence: \( p > 0.05 \)

Discussion

The analysis of the assay data gathered in this study indicated that the chosen HPLC/UV method was sufficiently simple, precise (RSD range 7%–9%), and accurate (CV of QC samples, \(-13.8\% \text{ to } +10.3\%\)) for performing a valid bioequivalence study. The lack of interference with other peaks at the retention time of glimepiride, when blank serum samples were tested, supported the specificity of the method. According to the literature, the sensitivity of the HPLC method applied in this study (LLOQ, 0.01 µg/ml) was comparable to that of HPLC methodologies used by other investigators to determine concentrations of glimepiride. As expected, however, the HPLC method we used was less sensitive compared with HPLC coupled to tandem mass spectrometry.

Twenty-four subjects were included in this study because this number arbitrarily represented a reliable sample to assess the bioequivalence of the two products. A post study calculations based on the log-ANOVA error revealed that sample size of > 23 subjects was sufficient to show the difference of 20% between the [C(max)] and AUC (0-inf) values of the test and reference products. Type I and type II errors would not exceed 5 and 20%, respectively.

Intra-individual variabilities [CV-intra] of [C(max)] and AUC (0-inf) for glimepiride were 30.32 and 36.95%, respectively. The CV-intra value for the [C(max)] parameter and the individual [C(max)] ranges (reference, 226–1009 ng/ml; test, 218–1034 ng/ml) obtained in the study indicated that the absorption rate of glimepiride was highly variable. The overall pharmacokinetic profile of the test formulation of glimepiride in the present study, however, was very similar to the data previously published. Based on the results of this study, [C(max)] and AUC (0-inf) values of glimepiride were not significantly different, with the power (derived from ANOVA) of 0.864 and 0.897, respectively.

Conclusion

In this small, selected population of healthy volunteers, 90% CIs for geometric means of AUC (0-inf) and [C(max)] ratios were within the 0.80 to 1.25 interval proposed by the U.S. Food and Drug Administration as the definition of bioequivalence, and the difference between [T(max)] values was not statistically significant. On that basis, considering both early and total exposure to glimepiride 6 mg, these two oral formulations were concluded to be bioequivalent. Both treatments were well tolerated. Transient mild hypoglycaemia that had been noticed in 6 out of 24 participants was not recognized as the unexpected or serious adverse effect of glimepiride.

REFERENCES


The paper was received on August 29, 2006.