Bioequivalence Study of Generic Metformin Hydrochloride in Healthy Nigerian Volunteers

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Abstract

Background and objectives: Metformin is key in the management of type 2 diabetes mellitus but also represents additional financial burden, particularly with the use of branded products. The availability of generic products permits generic substitution with a much-reduced cost of treatment. However, only generic products that offer similar bioavailability with the innovator should be considered. This study was designed to assess the bioequivalence of generic metformin tablets within Nigeria.

Methods: Metformin tablets selected from the Nigerian market were appraised for quality following British and United States Pharmacopoeia guidelines. In vivo bioequivalence study in healthy volunteers was applied for a generic and the innovator brand in an open-label, 2-arm, 2-treatment crossover fashion with a 1-week washout period. Blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 24 h post-dose. Plasma concentrations of metformin were analysed using a validated high-performance liquid chromatography method, and pharmacokinetic parameters were obtained using the non-compartmental approach. The formulations were considered bioequivalent based on the guidelines by United States Food and Drug Administration, Centre for Drug Evaluation and Research.

Results: Nine generic products met the quality assessment standards, and the in vivo bioequivalence study was carried out in 17 healthy volunteers. The mean values for $C_{\text{max}}$, $T_{\text{max}}$, $AUC_{0-24}$, and $AUC_{0-\infty}$ for the innovator brand of metformin were $0.43 \pm 0.14 \mu g/mL$, $1.35 \pm 0.46 h$, $2.03 \pm 0.68 \mu g/mL*h$ and $2.63 \pm 1.11 \mu g/mL*h$ respectively; for the generic product, the values were $0.44 \pm 0.13 \mu g/mL$, $1.41 \pm 0.59 h$, $2.04 \pm 0.68 \mu g/mL*h$ and $2.85 \pm 1.37 \mu g/mL*h$. The 90% confidence intervals for the test formulation/reference formulation ratio for Log $C_{\text{max}}$, Log $AUC_{0-10\ h}$ and $AUC_{0-\infty}$ were within the bioequivalence limits of 80% to 125% (95.8–106.8, 94.8–105.5 and 96.3–108.4 respectively).

Conclusions: The bioavailability of the test product was not inferior to innovator metformin.

Keywords: Metformin; Bioequivalence; Antidiabetic; Pharmacokinetics.

Introduction

Metformin is an oral antidiabetic drug (OAD) belonging to the biguanide class. Other biguanides are phenformin and buformin, but the former was withdrawn from market due to reported links with serious cases of lactic acidosis.1,2 Metformin, however, remains the drug of choice in the management of type 2 diabetes mellitus, particularly in patients whose renal functions have not been compromised. According to the United Kingdom Prospective Diabetes Study (UKPDS), metformin is superior to other OADs in lowering both the macrovascular- and microvascular-related complications that characterize the disease progression in diabetic patients.3,4 Recently, some researchers have spoken out against the claims by UKPDS, citing methodological shortcomings.5 Despite
this dichotomy, metformin remains a first-line drug in obese and non-obese diabetic patients, alongside lifestyle adjustment. It is considered superior to sulphonylurea because it causes no weight gain and it is rarely associated with hypoglycaemia. Moreover, it is safer than the thiazolidinediones because it offers a cardio-protective effect instead of cardiotoxicity.

Metformin, 1,1-dimethylbiguanide (Fig. 1), has a low molecular weight (129.1 g/mol), good solubility (about 300 mg/mL) in polar solvents, resulting in solution with a pH range of 1.2–6.8 at 25 °C; however, its lipophilicity and permeability are unacceptably low. Metformin is provided as 500, 850 and 1,000 mg tablets, either as immediate release (IR) and extended release (ER) formulations. Glucophage® (a descriptive name to describe its role as a ‘glucose-eater’) is an innovator product that stands out in terms of quality and efficacy over time, but is relatively unaffordable for some patients. In general, the high-price associated with some branded products may predispose patients to opt for generic products, often registered by the drug regulatory body. In Nigeria, many generic products are in circulation, and they are often preferred by the populace because of the prevailing poor socioeconomic status. This trend has helped to curtail rising in pharmaceutical expenditure, especially in low- to middle-income countries. However, generic substitution should not be based solely on the initial cost of treatment but on the overall cost effectiveness of pharmacological treatment. As a result, a standard has been set for generic substitution. Interchangeability is permitted when the generic product demonstrates bioequivalence (BE) and therapeutic equivalence with the innovator.

BE of a generic product could be determined by either in vivo or in vitro studies. In vivo BE studies are frequently used to establish therapeutic equivalence, but this approach is usually expensive and more rigorous and may require clinical trial or study expertise. In vitro dissolution profiles are proxies for establishing BE when the drug meets the criteria prescribed for a Biopharmaceutics Classification System (BCS) biowaiver. The BCS considers three major factors—dissolution, solubility and intestinal permeability—which influence the rate and extent of drug absorption from IR solid oral dosage forms. Metformin is highly soluble in water with poor permeability, and as such it is classified as a BCS class 3. It may enjoy a biowaiver if dissolution of 85% or more of the labelled amount of the active pharmaceutical ingredient (API) in both the generic and the innovator products is attainable within 15 min in standard dissolution media at pH 1.2, 4.5 and 6.8.

An in vitro dissolution study on four generic products of metformin showed that none of the four brands of metformin tested met this requirement because the innovator product and two others did not achieve 85% dissolution in 15 min. In a similar study conducted by Olusola et al. in 2012 on eight generic products of metformin, only three met the criteria for BCS biowaiver after a physiochemical equivalence testing. Thus, using an in vitro dissolution profile as a surrogate for in vivo BE is still debatable as in vivo-in vitro correlation has not been established for metformin in most cases. Developing countries will benefit from generic products, unfortunately the resources for testing drug quality is limited. Thus, this study aimed to assess the bioavailability of generic formulations of metformin versus that of the innovator product.

Materials and methods

Metformin HCl was obtained from AK Scientific chemicals (United States), high-performance liquid chromatography (HPLC) grade acetonitrile and methanol were obtained from Scharlau® Chemicals (Spain). Cimetidine and potassium hydrogen phosphate were purchased from Sigma-Aldrich® Chemical Company (Germany). The innovator product of metformin (coded as A) and 13 other generic products of metformin tablets (coded as B, C, D, E, F, G, H, I, J, K, L, M and N) were purchased from retail pharmacies in Ile Ife, Ilesa and Ibadan South-West, Nigeria. All were IR tablets and the products’ manufacturers and their batch numbers are as follows: Merck Sanite, France(5009, manufactured in July 2012); Hovid BDH, Malaysia (03-536 BD, manufactured in March 2013); Jiangsu Ruiniun Pharm, China (111208, manufactured in December 2011); Fredun Pharmaceutical, India (FT 362, manufactured in July 2012); NGC Plc, Nigeria (F0802, manufactured in June 2013); Medopharm, India (2G31, manufactured in July 2012); Drugfield Pharm., Nigeria (580302, manufactured in March 2011); Vatphos Lab Ltd, Nigeria (V054, manufactured in August 2012); Rajat Pharmchem, India (RA 2001, manufactured in June 2012); Juhel Nig. Ltd, Nigeria (0015, manufactured in October 2012); Henan Topfond Ltd, China (120810740, manufactured in August 2012); Vapicare Pharm. India (FUV1201, manufactured in April 2012); Vapicare Pharm., India (EF21002, manufactured in October 2012); and Watson Global Pharm., Nigeria (20120801, manufactured in 2012).

Chemical assay and dissolution testing

Assay and in vitro BE comparison of the innovator and generic products of metformin were carried out as prescribed by the British Pharmacopoeia (BP) 2013. The potency of metformin in each product was established using ultraviolet (UV) spectrophotometric techniques by measuring the absorbance of the stated solution of metformin at 232 nm, with A1 cm taken as 798. The dissolution system complied with the requirements in the Monographs of the United State Pharmacopoeia for the dissolution test for tablets. Potassium dihydrogen phosphate buffer (0.68% w/v) was adjusted 1% taken as 798. The dissolution

In vivo BE study design

Ethical approval for the study was given by the Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Nigeria. Written informed consent was obtained from each voluntary subject before commencement of drug administration and sample collection. Healthy volunteers (n = 22) between the ages of 18 and 28 years-old, with body weight ranging from 45 to 75 kg, were recruited.

Fig. 1. Chemical structure of metformin.
for the study. Each subject underwent a physical examination and medical history-taking, both conducted by a physician. After an overnight fast, the subjects were given a single oral dose of 500 mg metformin HCl tablet. Two products, consisting of a test product and a reference product, were administered to the subjects in a crossover fashion.

Study inclusion criteria included strict adherence to the following parameters: healthy adults; 18–45 years of age; non-smokers; not pregnant; and body mass index (BMI) between 18–32 kg/m². Medically healthy was determined according to medical history and findings of physical examination. All study participants were required to provide voluntary written informed consent and show willingness and ability to fast overnight. Exclusion criteria included: history of renal impairment; pregnancy or lactation; recent significant blood donation; recent participation in similar studies (within 28 days); evidence of alcoholism or drug abuse, especially of drugs that could cause hypoglycaemic effect; and history of hypersensitivity to biguanides.

Study treatment
The voluntary subjects were invited to the study centre at about 7:30 am on the study day. The subjects were told to observe overnight fasting prior to that day. The study was implemented as a single dose, two-period and two-treatment with a test and a reference product in crossover design. The test product was chosen based on its equivalence with the reference product as determined by the quality assessment using assay and dissolution profiling of the two products. During the first period, half of the subjects received 500 mg metformin as the reference product, which was given as a single oral dose, while the remaining half received orally 500 mg metformin as the test product. Venous blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 24 h following drug administration. A 1-week washout period could ensure that the level of metformin in plasma had fallen far below the limit of quantitation. Then, subjects who received the test product in period 1 were treated with the reference product and vice-versa. Venous blood samples of the subjects were collected into ethylenediaminetetraacetic acid bottles at predetermined time intervals over 24 h. The samples were centrifuged to obtain plasma and the plasma was stored at −20 °C until analysis in our Therapeutic Drug Monitoring (TDM) laboratory.

Quantification of metformin in human plasma
The analytical procedure involved modification of an extraction and HPLC method previously reported in the literature. A 1 mg/mL stock solution of metformin and cimetidine were prepared by dissolving 25 mg of each in 25 mL volumetric flask using methanol, and the solutions were stored at 4 °C. The chromatographic system consisted of an Agilent 1,100 series liquid chromatography system (Agilent Technologies, United States) fitted with a quaternary pump and a diode array UV detector (DAD; at 190–900 nm). Chromatographic separation was achieved at 25 °C on a reverse-phase Agilent Zorbax (C18) column (5 µm × 4.6 mm) while the mobile phase was acetonitrile-potassium hydrogen phosphate buffer (0.01 M, adjusted to pH 6.67), 55:45, applied at a flow rate of 1.2 mL/min. Sample was injected through a Rheodyne model 7725 valve (United States) fitted with a 20 µL loop.

The eluents were monitored with UV detection at 234 nm λmax, while chromatograms were recorded with HP Chemstation software. To 100 µL of plasma in a 2-mL Eppendorf tube was added 50 µL of 20 µg/mL cimetidine solution (internal standard), 100 µL of 8M NaOH and 1.25 mL of l-butanol/n-hexane (50:50, v/v), followed by shaking for 2 min. After centrifugation at 10,800 g for 5 min, the whole organic layer was separated and transferred into another tube. Metformin was back-extracted with 100 µL of 1% acetic acid. The mixture was vortex-mixed and centrifuged for 1 min. The organic phase was removed and a 20 µL volume of aqueous phase was injected into the chromatograph. The peak area ratio for each sample was generated from the peak response of metformin and cimetidine using UV detection at 234 nm.

A 100 µL aliquot of drug-free plasma samples were spiked with 50 µL of internal standard solution (20 µg/mL cimetidine) and standard solutions (between the range of 0.05–5.0 µg/mL) of metformin.

For each sample, the above-stated extraction procedure was carried out and the supernatant (20 µL) was injected into an HPLC column. A plot of peak area ratios versus concentrations of the standard solutions was made. The limit of detection (LOD) and limit of quantification (LOQ) were generated based on regression analysis of the calibration curve. Intra-day precision and accuracy were determined by analysis of five replicates of each QC level at 0.05 µg/mL, 0.5 µg/mL and 5.0 µg/mL concentrations. Inter-day precision was measured by analysis of duplicates of each QC concentration on three different days.

Pharmacokinetic and statistical analysis
The plot of plasma concentration (C) against time (t) data of metformin was carried out using Microsoft Excel 2010. The data were analysed to obtain pharmacokinetic parameters using the non-compartmental model by means of the KINETICA Pharmacokinetic Software (United States). The results were recorded as mean ± standard deviation (SD). AUC (0–t) was computed using the linear method. The trapezoidal rule was applied when C > 0. The AUC (0–t) was estimated as the sum of AUC (0–t) h. The AUC (0–t) h was defined as C × t. The T_half was estimated as the sum of AUC (0–t) h. The AUC (0–t) h was obtained as the slope of linear regression of the ln concentration on three different days.

Comparisons of the pharmacokinetic parameters for the two products for determining BE were made using t-test and ANOVA by means of the SPSS 16 Software. After transforming BA parameters (C, AUC (0–t), AUC (0–t) h, AUC (0–t) h) to the logarithm scale, the data from both arms were compared by the 90% confidence intervals (CIs) using the ratio of geometric means. The test product was consid- ered to be BE compared with the reference sample if the 90% CIs for AUC and C_max were within the predetermined BE range of 80% to 125% (CDER, 2014).

Results
The innovator product and 13 generic brands of metformin were selected for preliminary quality appraisal screening by weight uniformity test, quantitative analysis and dissolution profiling. Twelve
Overall, nine generic products met all the standards stipulated in the official guidelines. The results for the assay and dissolution test are presented in Table 1 and Figure 2. Also, the quantity of metformin that went into solution within 15 min is presented in Table 1, in order to show if the products rapidly dissolved to attain 85%
total release within 15 min with the aim of determining whether the products meet criteria for bio waivers. Though similar dissolution profiles were observed for all the products, except products C and K, only three generic products rapidly released ≥85% metformin API. This led to the extension of this project to in vivo study.

For lack of resources, only product B was considered for in vivo comparative study. Seventeen healthy volunteers, including six females and eleven males, completed the in vivo study (Table 2). Treatment with both generic and innovator products was well tolerated. Metformin was quantifiable in all the subjects from 30 min to 10 h post-dose sampling points. The bioanalytical procedure for metformin analysis was validated based on FDA/CDER guidelines. The LOD and LOQ were 10.2 and 30.9 ng/mL respectively. Accuracy, recovery and intra-day and inter-day precision are presented in Table 3. The average plasma concentrations-time profile of metformin for the innovator (reference) and the generic (test) products is depicted in Figure 3. Derived pharmacokinetic parameters, 90% CI and geometric mean ratio (GMR) of the test/reference products for logarithm-transformed BE parameters (Cmax, AUC0–10 hr and AUC0–∞) are presented in Tables 4 and 5.

Discussion

Out of 14 brands of metformin tablets in the Nigerian market, 10 products were found to be fit according to weight uniformity test, UV-spectrophotometric quantitative analysis and dissolution test. Eight of those products were generic and they were found to demonstrate pharmaceutical equivalence with the innovator brand by releasing 75% or more within 45 min using a basket rotating at 100 rpm. The products could have enjoyed in vivo bio waivers but some, including the innovator brand, were not sufficiently released in pH 6.8 phosphate buffer during the dissolution test to meet the specification of 85% or more release within 15 min.

Metformin is highly hydrophilic, with poor permeability and it belongs to class III of BCS. To establish in vitro BE—in vivo BE correlation, metformin products should release ≥85% of API within 15 min.15 The reason for this is that if the products rapidly dissolved under all physiological conditions, one will expect such products to behave like oral solutions in vivo. Only three generic products released ≥85% of its API within 15 min. This pattern was similar to that of previously reported dissolution studies on metformin tablets in Nigeria, where the products were not noted not to be rapidly dissolving in any of the three media having pH 2.0, 4.5 and 6.8.16,17 The feasibility of generic substitutions of OAD with the same amount and quality of API in the management of diabetes relies on the fact that the products are therapeutically equivalent and are able to offer glycaemic control of <7.0% HbA1C and prandial capillary plasma glucose of 80–130 mg/dL.21

As in vitro BE—in vivo BE correlation is still debatable for metformin, further in vivo BE study was launched in accordance with theICH guidelines on Good Clinical Practice and Guidance for Industry Bioavailability and Bioequivalence Studies.22,23 The test product was selected based on the prequalification dissolution test and assay of metformin tablets, while the innovator brand of 500 mg metformin IR tablet served as the reference product. The healthy subjects treated with the test and reference products in a crossover fashion seemed to tolerate the treatment very well, as no adverse events were recorded. We started with 22 healthy volunteers, but we had to exclude 5 subjects because they contradicted the rule for this study either by taking other drugs during the study period, taking a meal unduly, or not being available at the time required for pharmacokinetic sampling.

In this BE study, metformin quantification from human plasma was achieved by adapting a simple, sensitive and selective HPLC method by Amini et al.24 This method was slightly modified and validated in our TDM laboratory. Samples were pre-treated by basification with sodium hydroxide, extraction with 50% v/v butanol-hexane, and back-extracted with 1% acetic acid. Cimetidine was used in lieu of ranitidine. Though the LOD and LOQ in our study were slightly above the corresponding data generated by Amini’s group, 10.2 and 30.9 ng/mL respectively compared with 5 and 15.6 ng/mL, quantification of plasma levels of metformin at 0–10 h after a single oral dose of 500 mg metformin tablet was adequately realizable.

All the findings for pharmacokinetic parameters in this study are in concordance with the other data reported previously. This study found 4.31 ± 2.2 h as the half-life, 221.86 ± 86.5 L/h as the clearance, and 1,190.33 ± 421.75 L as the volume of distribution respectively after a single oral dose of 500 mg of reference product. The corresponding data for a single oral dose of test product are 5.67 ± 1.74 h, 214.29 ± 96.91 L/h and 1,273.35 ± 468.87 L. The within-subject variability during the two treatments among the Nigerian healthy volunteers was low, as the average clearance and the average volume of distribution of metformin determined for both products were not statistically significantly different from each other.

However, wide variations in both clearance and volume of distribution do exist between subjects in this study. Similar findings

*means ± the standard deviation of Demographic features.
have been reported for metformin pharmacokinetics in another population. The reason put forward to explain these variations is inter-subject variability in the oral bioavailability (F) of metformin and inter-subject variation in the ratio of the renal clearance of metformin to creatinine clearance which is independent of F. Another strong rationale is the single nucleotide polymorphisms in metformin transporters-organic cation transporters in either healthy subjects and diabetic or obese individuals.

Other pharmacokinetic parameters, such as maximum plasma concentration (C_{max}), time to reach the maximum concentration (T_{max}) and plasma exposure (AUC), were considered for the determination of BE of the two products. There was no significant difference for C_{max}, T_{max} and AUC, as shown in Table 3. After logarithm transformation of BE parameters, the GMR and 90% CI was within the BE acceptable range, from 80% to 125%.

Future research directions/recommendations

As a result of the great importance of generic drugs in healthcare, it is imperative that their pharmaceutical quality and in vivo performance be reliably assessed before they could be used interchangeably with the innovator product in the marketplace. It must be demonstrated that the safety and efficacy of the generic drugs are comparable to those of the innovator drugs.

Conclusions

This BE study found that the 500 mg of the test product is equivalent to 500 mg of the reference product of metformin. The outcome of the in vitro study correlates well with the in vivo study and both formulations met the regulatory standards for assuming BE in healthy volunteers.

Acknowledgments

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Designing the research (JOS, AJA, BAA), recruiting volunteers (JOS, AJA, BAA, OJA, BSO, ARO), certifying medical fitness of volunteers (ARIO), analysing samples (JOS, AJA, BAA, OJA, BSO), preparing the manuscript (JOS, AJA, BAA, OJA, BSO),

Table 4. Derived pharmacokinetic parameters of metformin after oral administration of 500 mg tablet of innovator and a generic product

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference A (Mean ± SD)</th>
<th>Test B (Mean ± SD)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max}, µg/mL</td>
<td>0.43 ± 0.14</td>
<td>0.44 ± 0.13</td>
<td>0.8133</td>
</tr>
<tr>
<td>T_{max}, µg/mL</td>
<td>1.35 ± 0.46</td>
<td>1.41 ± 0.59</td>
<td>0.5795</td>
</tr>
<tr>
<td>AUC_{t0-10}, µg/mL * h</td>
<td>2.03 ± 0.68</td>
<td>2.04 ± 0.68</td>
<td>0.9718</td>
</tr>
<tr>
<td>AUC_{t0-}, µg/mL * h</td>
<td>2.63 ± 1.11</td>
<td>2.85 ± 1.37</td>
<td>0.4434</td>
</tr>
<tr>
<td>t_{1/2}, h</td>
<td>4.31 ± 2.2</td>
<td>5.67 ± 1.74</td>
<td>0.0003</td>
</tr>
<tr>
<td>CL, L/h</td>
<td>221.86 ± 86.5</td>
<td>214.29 ± 96.91</td>
<td>0.6126</td>
</tr>
<tr>
<td>V_{ss}, L</td>
<td>1,190.33 ± 421.75</td>
<td>1,273.35 ± 468.87</td>
<td>0.4426</td>
</tr>
<tr>
<td>V_{SS}, L</td>
<td>1,335.17 ± 441.00</td>
<td>1,371.33 ± 382.01</td>
<td>0.7110</td>
</tr>
</tbody>
</table>

*Threshold of significance was set at <0.05.

Table 5. Derived pharmacokinetic parameters of the test/reference products for logarithm-transformed BE parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>90% CI</th>
<th>GMR Test/Reference</th>
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<tbody>
<tr>
<td>Log C_{max}</td>
<td>95.8–106.8</td>
<td>101.3</td>
</tr>
<tr>
<td>Log AUC_{0-10 hr}</td>
<td>94.8–105.5</td>
<td>100.2</td>
</tr>
<tr>
<td>Log AUC_{0-IN}</td>
<td>96.3–108.4</td>
<td>102.3</td>
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</tbody>
</table>

Abbreviations: CI, confidence interval; GMR, geometric mean ratio.
References


