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DETERMINATION OF BIOEQUIVALENCE OF METFORMIN TABLETS USING URINARY EXCRETION DATA

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Abstract: The aim of the present study was to assess the bioequivalence of two metformin tablet formulations available in the Albanian market (product R as reference formulation and product T as test formulation). The bioequivalence study was performed in eighteen healthy volunteers in a two - treatment, open, crossover design. Single oral dose (tablet containing 850 mg of metformin) of each product was administered with one week of washout period. Urinary concentrations of metformin were measured by high-performance liquid chromatography (HPLC) method and pharmacokinetics parameters were estimated by urinary excretion data. The bioequivalence was determined by the following parameters: the cumulative amount of metformin excreted in the urine, the total amount of metformin excreted in the urine and the maximum urinary excretion rate of metformin. Various pharmacokinetic parameters like peak excretion rate $[(dDU/dt)_{max}]$, time for peak excretion rate (t_{max}), cumulative amount ($D_{cum0-24}$), total amount of drug recovered from urine ($D_{cum0-\infty}$), elimination half-life ($t_{1/2}$), and terminal elimination rate constant (kel), were calculated for both the formulations. The average cumulative amounts of metformin excreted in urine after administration of Formulation R and Formulation T were found to be 346.3 mg (40.74% of dose) and 358.7 mg (42.2% of dose), respectively. The urinary excretion profiles of metformin up to 24 h for both the formulations were found to be similar. Statistical comparison (90% confidence intervals of ratio) of pharmacokinetic parameters were in compliance with the international standards, indicating that products R and T can be considered bioequivalents and therefore interchangeable.

Keywords: Metformin, urinary excretion, bioequivalence study

Introduction

Metformin is an oral antihyperglycemic agent that has been widely used in the management of type 2 diabetes mellitus for decades (Davidson & Peters 1997; Kirpichnikov et al. 2002). It mainly works on islet tissues to inhibit the absorption of glucose by intestine and increases the utilization of glucose by peripheral tissues to reduce hepatic glycogenesis, so as to achieve the purpose of lowering blood sugar (Musi et al. 2002).
Metformin is slowly absorbed after oral administration, about 60% of an oral dose is excreted in the urine as unchanged drug within 24 h, and about 30% of the dose is nonabsorbed and eliminated unchanged in feces (Scheen 1996).

The pharmacokinetic parameters can be calculated from the accumulated amount of excreted drug in the urine sample in a particular time interval. However, it is necessary that a signifcant amount of the unchanged drug be excreted in the urine, that the analytical method be specifc for the unchanged drug, that the samples be collected with larger frequency to determine the excretion profle and that the sampling be made until the almost complete elimination of the drug (practically seven half-lives). The decline of the plasmatic concentration curves and drug urinary excretion rate can be described mathematically by the same equation. Thus, it is possible to assume that

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the parameters obtained from urinary excretion data reflect the drug absorption (Cawello *et al.,* 2013; Shargel & Yu,1993).

Urine provides a non-invasive sample collection method, and determination of drug levels in urine is comparatively less complex than plasma and other body fluids (Shah et al. 2002). Several reports indicate that urinary excretion data can be used to arrive at bioequivalence decision of different drug formulations (Shah et al. 2002; Maher et al. 2012).

The aim of this study was to evaluate bioequivalence of two tablet formulations of metformin (850 mg) designated as product R (reference formulation) and product T (test formulation) available in the Albanian market using urinary data from healthy human volunteers. Previously, HPLC method was developed and validated in order to quantify metformin in urine samples (Troja et al 2016).

Methods

Metformin quantifcation in human urine

Urinary concentrations of metformin were determined using a validated ion-paired HPLC method described in details elsewhere (Troja et al. 2016).The separation was performed on a Superspher 100 RP 18 (250/× 4.0 mm i.d. C18 (5 µm, particle size) column (ISS, Surrey, UK). The mobile phase was prepared by mixing 0.01 M of sodium phosphate buffer (pH=6.0), 0.3% SDS, and acetonitrile in a ratio of 70:30, adjusting with H_3PO_4 to 6.0 as necessary. The mobile phase was prepared daily, filtered through a 0.45 µm porosity Nylon filter membrane, and ultrasonicated for 30 minutes before use. The flow rate and the column temperature were 1.0 mL/minute and 50°C, respectively. The detection of metformin was carried out at 236 nm.

Study protocol

The protocol of the *in vivo* assay was approved by the National Medical Ethics Committee. Tablets of metformin 850 mg available in the Albanian market were designated as product R (Glucophage, reference formulation) and product T (Metformine, test formulation) and they were used in this study. Metformin was administered in a single dose of 850 mg to 18 healthy volunteers (male and female) after over night fasting. The study was conducted in an open, randomized, two period, cross-over design. Two brands of metformin 850 mg in conventional tablets were employed: the reference formulation (Glucophage) and the test formulation (Metformine). The subjects were divided into two groups. In the first period of the study, volunteers from one group received product R and volunteers from the other group received product T. A week later, this procedure was repeated by inverting the groups. Each subject fasted overnight prior to the experiment, and the drug was administered with 250 mL of water.
Urine samples were collected at various time periods after dosing and were analysed for metformin. Blank urine samples were obtained from each volunteer prior to dosing. Quantitative urine collections were obtained during each of the following time intervals: 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 12, 12 to 24 h. A standard lunch was ingested by all subjects 4 hours after dosing. Urine volume was measured and an aliquot of each sample was kept at 2-8°C until analysis by HPLC.

Pharmacokinetic Analysis

Pharmacokinetics parameters used in this evaluation were obtained from urinary excretion data of metformin. According to specification of FDA/USA, the evaluation of the bioavailability implies in determination of the amount of drug absorbed and in the rate of this process (FDA, 2003; USP, 2010). As bioequivalence refers to the comparative study of bioavailability of two dosage forms or products that contain the same drug in the same amount, the pharmacokinetic parameters selected should reflect the absorption process (Shargel & Yu, 1993). Cumulative amount of excreted metformin (*Duc*) in the urine up to each sample collection time was determined by adding the amount of drug excreted in each time interval to the amount of drug excreted recovered in the previous time intervals. The total amount of drug recovered from urine after all excretion period was designed by $Du\infty$. In this study the $Du\infty$ was obtained from the cumulative excretion at 24 hours (Arancíbia, 1991; Shargel & Yu, 1993). The observed total amount of the drug recovered in the urine from time 0 up to 24 h ($D_{cum0-24}$) was determined by multiplying the concentration with the urine volume of the respective sample in each collection interval and summing up all intervals after dosing subsequently. The fraction of orally administered drug in urine within 24 h (% dose) was calculated by dividing $D_{cum0-24}$ by the dose of the drug administered (Swarbrick 2007; Gieschke 1999). The peak excretion rate [(dDu/dt)max] and peak excretion time (tmax) values were obtained from the urinary excretion rate (dDu/dt) versus time curves obtained for each volunteer after administration of Formulation R and Formulation T. Other pharmacokinetic parameters such as terminal elimination rate constant (kel) and elimination half life ($t_{1/2}$) were obtained from linear regression of the

ln-transformed terminal segment of urinary excretion rate [ln(dDu/dt)] versus midpoint of time (h) curves. Both $D_{cum0-24}$ and $D_{cum0-\infty}$ were calculated using untransformed (dDu/dt) data. $D_{cum0-24}$ was calculated using linear trapezoidal rule and was extrapolated to infinite time, $D_{cum0-\infty}$. kel was calculated from the slope of terminal linear portion of log(dDu/dt) versus midpoint of time curve. The elimination half life ($t_{1/2}$) was calculated using the formula, $t_{1/2} = 0.693/kel$ (Swarbrick 2007; Gieschke 1999).

Statistical Analysis

Student's t-test (paired) at 5% level of significance was used for testing the differences between the mean values obtained from two treatments using statistical software STATA 13 (StataCorp LLC, College Station, Texas, USA). Pharmacokinetic parameters generated for the two treatments were also compared for significant differences using ANOVA. Analysis of variance (ANOVA) was performed for the values of the following pharmacokinetic parameters: cumulative amount of excreted metformin (*Duc*); total cumulative amount of metformin excreted (*Du*∞); maximum excretion rate of metformin [(*dDu/dt*)max]. Factors accounting for the following variation sources were considered: sequence, subjects, period and treatment. The 90% confidence interval (90% CI) for the ratio between the test and the reference [*Duc*, *Du*∞ and (*dDu/dt*)max] were calculated. Bioequivalence is confirmed if the 90% CI are within 80-125%.

Results and Findings

All volunteers successfully completed the trial according the protocol. Both metformin formulations were well-tolerated at the administered dose and no clinical or biological side effects were reported during the study. Urinary excretion levels of metformin after administration of Formulation R and Formulation T (both containing 850 mg of metformin) were estimated using ion pairing HPLC method. The pharmacokinetic parameters for both products are presented as mean (±SD) in Table 1.

Table 1. Mean of pharmacokinetics parameters after oral administration of metformin (R and T products, n=18). values represent mean ± standard deviation

Pharmacokinetic parameter	Mean ± SD (CV%) Formulation R	Mean ± SD (CV%) Formulation T
(dDu/dt)max (µg/min)	977.50 ± 264.14 (27.02%)	1019 ± 321.90 (31.58%)
Dcum0-24 (mg)	346.28 ± 79.66 (23.01%)	358.67 ± 98.91 (27.58%)
Dcum0-∞ (mg)	446.27 ± 104.88 (23.50%)	458.60 ± 153.86 (33.55%)
T1/2 (hrs)	3.258 ± 0.639 (19.599)	3.11 ± 0.668 (21.481)
Kel (hrs-1)	0.221 ± 0.047 (21.39)	0.234 ± 0.058 (24.644)

Mean values of $D_{cum0-24}$ were 358.7 mg and 346.3 mg after oral administration of Formulation T and Formulation R, respectively. Maximum excretion rates of metformin $(dDu/dt)_{max}$ were 977.50 and 1019 µg/min after oral administration of Formulation R and Formulation T, respectively. It was observed that Tmax occurred after 2.4 ± 0.93 hrs (39.16) for formulation R and 2.3 ± 0.65 (28.15) hrs for Formulation T. Elimination rate constants were 0.221 ± 0.047 hrs^{-1} and 0.234 ± 0.058 hrs^{-1} for Formulation R and Formulation T, respectively. The values found in our study are very close to those found by other authors (Holguin et al., 2011; Gopi et al., 2012; Najib et al., 2002). In this study, the elimination half-life ($T_{1/2}$) was 3.258 ± 0.639 hrs and 3.11 ± 0.668 hrs after oral administration of Formulation R and Formulation T, respectively. İn previous studies, the elimination half-life was 2.3 hrs (He et al., 2008) and 2.77 hrs (Holguin et al., 2011). In our study, this value is comparable with the value described by Holguin (Holguin et al., 2011). Values of other pharmacokinetic parameters of test formulation were also comparable with that of reference formulation. The plot of cumulative amount of excreted metformin over a period of 24 hours versus mid-point of time intervals for each product is shown in Figure 1. Urinary excretion rates of metformin (dDu/dt) for each product are shown in Figure 2. From these figures, it is evident that both formulations show similar excretion pattern, which in turn, indicates similarity in their bioavailability.

The average amount of unchanged metformin excreted was found to be 40.74 % and 42.2 % after oral administration of Formulation R and Formulation T, respectively. This is lower than the value described by El-Gindy et al., 2010 for a 850 mg tablet of the administered drug within 24 hrs after oral administration. The

fraction dose absorbed was 52.5% and 53.95% after administration of Glucophage and Metformine products, respectively. This indicates a similarity with the values given by the literature (Dunn & Peters 1995; Hundal & Inzucchi 2003).

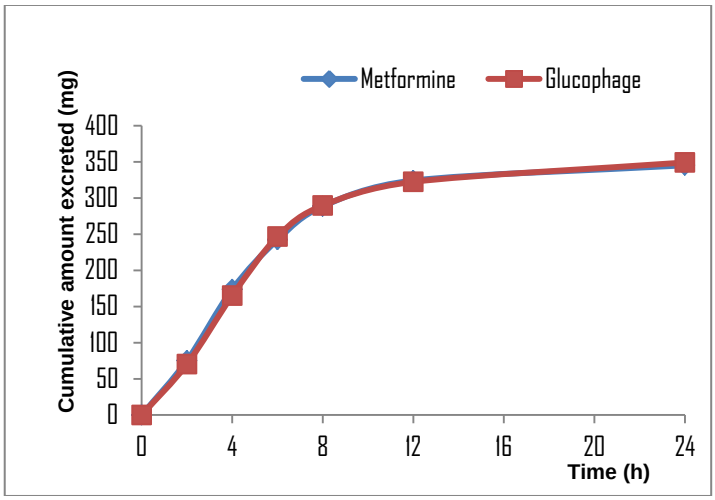

Figure 1. Linear plot of cumulative amount ($D_{cum0-24}$) versus mid-time of metformin in 18 healthy volunteers in 24 hours after oral administration of tablets [Glucophage (R) dhe Metformine (T), N=18].

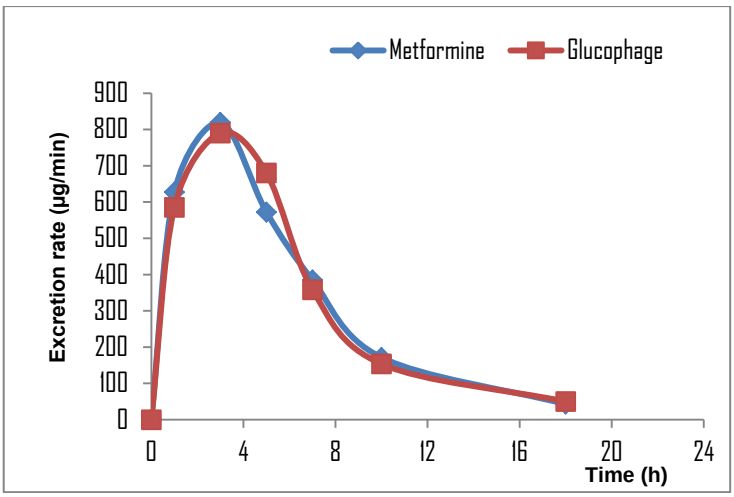

Figure 2. Linear plot of rate of excretion (dDu/dt) versus mid-time of metformin in 18 healthy volunteers in 24 hours after oral administration of tablets [glucophage (R) dhe metformine (T), N=18].

Analysis of variance (ANOVA) for $D_{cum0-24}$, $D_{cum0-\infty}$ and $(dDu/dt)_{max}$, after ln-transformation of the data, showed no statistically significant difference between Formulation T (Metformine) and formulation R (Glucophage) either in periods, formulations or sequence. 90% confidence intervals also demonstrate that the ratios of $D_{cum0-24}$, $D_{cum0-\infty}$ and $(dDu/dt)_{max}$ of both formulations lie within the regulatory acceptable range of 80–125% (Table 2). So the test product showed good bioavailability as compared to the reference one.

Table 2. Statistical analysis of ln-transformed data for pharmacokinetic parameters after oral administration of metformin ımmediate-released tablets (R and T products, N=18)

Pharmacokinetic parameter	**gMean ratio (T/R) %**	**90% confidence interval** **Lower limit** **Upper limit**
Dcum0-24	103.5	89.4 119.5
Dcum0-∞	100.6	86.8 117
(dDu/dt)max	103.2	88.3 120.9

Conclusion

Statistical analysis of various pharmacokinetic parameters calculated using urinary excretion data of metformin revealed that Formulation T is bioequivalent with Formulation R.

Recommendations

Based on the results obtained from the study, it results that Metformine 850 mg tablets produced by Profarma Sh.A are bioequivalent to Glucophage 850 mg tablet, manufactured by Merck Santé S.A.S. Their costs are respectively 2.5 ALL/tab and 10.4 ALL/tab. Based on the pharmacoeconomic criteria we recommend Metformine tablets (Formulation T) as an antihyperglycemic agent because it has lower cost and no significant difference in the rate and extent of absorption of the therapeutic ingredient. The bioequivalence studies should also be made for other important drugs on the reimbursement list.

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