Pharmacokinetics and relative bioavailability of metformin hydrochloride extended-release tablets in healthy volunteers

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Abstract

Aim To evaluate the pharmacokinetic and relative bioavailability of metformin hydrochloride extended-release tablets made in two formulations (test drugs, T1, T2) to metformin hydrochloride extended-release tablets already for sale (reference product, R).

Methods An open-label, randomized and crossover clinical trials recruited 18 healthy volunteers were carried out for single-dose (1000mg) and multiple-doses (1000mg·d−1 × 7d) administration study. The plasma metformin concentrations were determined using HPLC method.

Results Various pharmacokinetic parameters were determined from plasma metformin concentrations. After single-dose administration (T1, T2, R), there was no statistical significant difference in AUC, Cmax and tmax either among the three treatments or among formulations by ANOVA after log-transformation of the data and two one-sided t-test, and the relative bioavailabilities for test drugs to the reference drug, were 98.8%±13.0% and 96.7%±14.6% respectively. After multiple-doses administration (T2, R), no statistically significant difference was found in AUCss, Cmax and tmax between test drug and reference product evaluated by ANOVA after log-transformation of the data and two one-sided t-test. Besides, there was no statistically significant difference in Cmin, Cav and DF between test drug and reference product evaluated by paired-sample t-test. The relative bioavailability for the test drug to the reference product was 94.6%±14.0%.

Conclusion The reference metformin product is bioequivalent to the test two formulations of metformin hydrochloride tablets, and it is suggested that the test drugs and reference product are of equal clinical efficacy.

Key words metformin hydrochloride; tablets; pharmacokinetics; bioavailability; high performance liquid chromatography

Introduction

Metformin (dimethylbiguanide), N,N-dimethylimidodicarbonimidic biamide, was introduced into clinical practice in 1957 as an oral antihyperglycaemic agent for the treatment of non-insulin-dependent diabetes mellitus (NIDDM or type 2 diabetes) by lowering both basal and postprandial-elevated blood glucose[1]. Pharmacodynamics studies show that metformin may act by improving peripheral and hepatic sensitivity to insulin and delaying gastrointestinal absorption of glucose[2,3].

In common with the other biguanides, metformin does not increase plasma insulin levels[4] and it may actually reduce hyperinsulinaemia[5]. Metformin may also have a beneficial effect on the serum lipid profile and has putative vasoprotective properties[5].

Lactic acidosis is the biguanide-related adverse effects of most concern[6,7]. However, because of differences in chemical structure and pharmacokinetic profile between the various biguanides, this serious adverse reaction is much rarer with metformin than with phenformin and buformin[1,4,8]. Other common side effects existing in metformin therapy include gastrointestinal symptoms, such as abdominal discomfort, nausea, and diarrhea that especially occur during the initial weeks of treatment[9]. While the employing of extended-release technique in preparing metformin formulations could reduce the direct
drug stimulation to the gastrointestinal tract, and may reduce the above gastrointestinal symptoms due to the lower peak exposure of intestinal tissue to the drug. Thus preparation of metformin extended-release form is a good way to reach the same therapeutic effects as the immediate-release forms with favorite patient compliance.

Gastrointestinal absorption of metformin is incomplete with an absolute bioavailability of 50-60% (under fasting conditions) and 20-30% of an oral dose being recovered in faeces\[4,10]\.

Absorption is estimated to be complete with 6 hours of administration and is presumably confined to the upper part of the intestine\[10]\.

And recent work indicated that metformin was well absorbed throughout the small intestine but rapidly decreased in the colon\[11]\.

It has also been reported that metformin is poorly absorbed from the stomach and that the delivery process was highly correlated with the rate-limiting factor for metformin absorption from the duodenum, while that the whole intestine was necessary for sufficient absorption of drug\[12]\.

These research findings are consistent with the requirement for candidates for extended-release or controlled-release forms, which describes the appropriate candidates as molecules that have poor colonic absorption but are characterized by better absorption properties at the upper parts of the gastrointestinal tract\[3]\.

Studies using single oral doses of metformin tablets of 500mg and 1500mg, and 850mg to 2550mg indicate that there is a lack of dose proportionality with increasing doses, which is due to decreased absorption rather than an alternation in elimination. Co-administration of food has been reported to slightly decrease the rate and extent of absorption of metformin\[14]\.

Bioequivalence of two formulations of the same drug comprises equivalence with respect to the rate and extent of their absorption. The area under concentration time curve (AUC) generally serves as the characteristic of the extent of absorption while the peak concentration ($C_{\text{max}}$) and the time of its occurrence ($T_{\text{max}}$), reflect the rate of absorption\[15,16]\.

In the current study, the pharmacokinetics of two formulations of generic metformin extended-release tablets (250mg metformin hydrochloride and 500mg metformin hydrochloride, respectively) and a commercial sustained-release metformin tablet product (500mg metformin hydrochloride) were compared in fasting, healthy human volunteers after single-dose and multi-dose administration.

**Materials and methods**

**Drugs and reagents**

Test drug (T): two formulations of metformin hydrochloride extended-release tablets: 250mg metformin hydrochloride (T$_1$, Lot: 03040501, purity: 100.8%) and 500mg metformin hydrochloride (T$_2$, Lot: 03040601, purity: 99.9%), were manufactured and produced by Jiangsu HengRui Pharmaceutical scientific Co., Ltd. (Jiangsu, China). Reference drug (R): BeiShun\textsuperscript{®} metformin hydrochloride sustained-release tablets (500mg metformin hydrochloride) were manufacture by Chengdu Hengrui Pharmaceutical Co., Ltd. (Chengdu, China). Lot: 031202. Internal standard: metronidazole was provided by Shanghai Second Military Medical University (Shanghai, China). Acetonitrile and methanol (both HPLC grade) were purchased from Concoct Chemical Reagent Company (Tianjin, China). Ammonium acetate (analytical grade) was purchased from Tianjin Chemical Reagent No.1 Plant (Tianjin, China). Ion pair reagent, 1-Heptane sulfonic acid sodium salt (IPR-B$_{7}$, 0.25mol·L$^{-1}$) was purchased from Tianjin Chemical Reagent Co, Ltd. (Tianjin, China).

**Instruments**

Shimadzu LC-10A auto high performance liquid chromatography (HPLC) system consists of LC-10A auto solvent delivery module pump, SIL-10AXL autosampler, SPD-10Avp UV spectrophotometric detector, TC Column oven as well as AnaStar chromatograph data system. Vortex mixer (KH-851, Shanghai), ultrapure water system (Milipore, Japan), high-speed centrifuge (TGL-16, Shanghai), and low-speed autobalance centrifuge (B600, Hebei).
Test subjects
Eighteen healthy Chinese male subjects with mean age of (21.9±1.2) years, mean body weight of (68.1±4.8) kg, and mean height of (173.4±5.2) cm were enrolled in the study. All subjects were determined healthy at the screening visit by medical history, physical examination, and results of clinical laboratory tests and electrocardiographs (ECGs). No medications were used for at least two weeks before the study. During the study, subjects were fed with same foods. Tobacco, alcohol as well as beverage containing ethanol were forbidden during the study. Following the guidance on the Helsinki declaration, all subjects signed the informed consent before the study.

Study design
Single-dose study An open-label, single-dose, randomized, three-way crossover study was carried out in 18 subjects. Subjects were divided into three groups according to the three formulations (T1, T2, R). All subjects were fasted overnight before treatment, and then were given corresponding tablets (T1, 4 tablets, T2 and R, 2 tablets) with 150 mL water in the next morning. Drinking water was allowed 2 hour after drug administration, then after another 2 hour, subjects were fed with same foods. Venous blood samples (4 mL) were collected in heparinized tubes prior to drug administration (0 hour), and at 0.5, 1, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 9.0, 12.0, 24.0 hour after drug administration. Blood samples collected were centrifugated at 2500 r·min\(^{-1}\) to separate the plasma, which was then frozen and stored at -20°C for future determination. There was a 7-day washout period between treatments. Adverse events of each subject during the study were carefully observed and recorded.

Multiple-doses study An open-label, multiple-doses, randomized, two-way crossover study was carried out in 18 subjects. Subjects were divided into two groups according to two formulations (R and T2). All subjects were fasted overnight before treatment, then were given corresponding tablets (both 2 tablets) with 150 mL water at 8:00 in the next morning and continued for 7 days. Venous blood samples were collected before drug administration (at 7:50) in the 4th, 5th and 6th day during the treatment. In the last day of treatment, drinking water was allowed 2 hour after drug administration, then after another 2 hour, subjects were fed (content and quantity of food were specified for each subject). Coffee, tea and beverages were forbidden during the treatment. Venous blood samples (4 mL) were collected in heparinized tubes prior to drug administration (0 hour), and at 0.5, 1, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 9.0, 12.0, 24.0 hour after drug administration. Blood samples collected were centrifugated at 2500 r·min\(^{-1}\) to separate the plasma, which was then frozen and stored at -20°C for future determination. There was a 7-day washout period between treatments. Adverse events of each subject during the study were carefully observed and recorded.

The above study protocol was approved by ethic committee, clinical pharmacological base.

Analysis of blood samples
Blood sample preparation 200μL spiked blood sample with 600μL acetonitrile containing metronidazole (2.0μg·mL\(^{-1}\), internal standard) were vortex-mixed for 1min, the mixer were then centrifugated at 12000 r·min\(^{-1}\) for 10min to separate the supernatant, 600μL supernatant was removed to another tube and was evaporated to dryness under a nitrogen stream at 60°C in a water bath. The residues were dissolved with 150μL mobile phase (without B-), and 30μL sample solution was injected into the HPLC column.

HPLC conditions Blood samples were analyzed by the HPLC system at UV 234nm, oven temperature 40°C. Analytes were separated on a Diamonsil™ ODS C\(_18\) column (5μm, 200mm×4.6 mm I.D.). The mobile phase was made up of methanol: 20mmol·L\(^{-1}\) NH\(_4\)AC (containing 0.45% 0.25mol·L\(^{-1}\) B-)=10:90 (V:V). The flow rate was 1.0mL·min\(^{-1}\). Metronidazole (2.0μg·mL\(^{-1}\) dissolved in acetonitrile) was used as internal standard. Peaks of metformin and metronidazole were well separated (retention time were 10.5min and 14.6min, respectively), there is no interference of endogenous substances in plasma, see Fig 1.
Method Validation

Standard curve According to the above method, the linearity was evaluated by constructing a calibration curve with seven different concentrations of metformin. Metformin standard were added to blank human plasma to obtain plasma metformin standard solution of 20, 50, 100, 200, 500, 1000 and 2000ng·mL$^{-1}$. Then the plasma samples (containing metformin) were prepared as the above method, and the calibration curve was obtained:

$$Y = 0.01581 + 0.000758 X \quad (n=7, R=0.995)$$

which indicated good linear correlations between concentration and analytical response. The method validation was suitable for the PK study$^{[17,18]}$.

Recovery, accuracy and precision Absolute recovery, accuracy and precision were calculated at three concentrations (50, 200, 1000ng·mL$^{-1}$). The absolute recovery of metformin extracted from plasma were (89.3% ± 6.4%), (89.3% ± 6.4%) and (89.3% ± 6.4%) for the three concentrations. Accuracy and precision assays were performed intra- and inter- batch, five replicates for each concentration in a batch, totally three batch. Precision was expressed as the relative standard deviation (RSD%). Accuracy was expressed as the mean relative error (RE%). Results showed that RSD% in three batch were all less than 5.5%, and RE% ranged from −12.0% to 16.0% (around LLOQ), indicating high precision and accuracy of the method.

Stability tests The stability tests contained tests of plasma metformin concentration after the samples were placed at room temperature for 8h and 24h, as well as after the samples were frozen and defrosted for one and three times. Tests were performed at 50, 200 and 1000ng·mL$^{-1}$, results showed that RSD% of all the items tested were less than or equal to 6.1%, indicating metformin was quite stable in plasma during the whole study.
Sensitivity
Limit of quantification was defined as the lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision (≤ 20%) of the true concentration. Lower limit of quantification (LLOQ) was 20 ng·mL⁻¹ for determination in plasma.

Data analysis
Plasma metformin concentration profiles of 18 subjects were presented as mean±SD. Main pharmacokinetics parameters were calculated for each subject. The maximum plasma concentration, $C_{\text{max}}$, is the highest observed plasma concentration, and $t_{\text{max}}$ is the time at which $C_{\text{max}}$ occurred, they were obtained directly from the observed data. Plasma metformin concentrations below LLOQ (20 ng·mL⁻¹) were assigned a value of zero for calculation of pharmacokinetic parameters and plasma concentrations. The area under the plasma metformin concentration-time curve ($AUC$) was calculated by the linear trapezoidal method during the 24 hours postdosing ($AUC_{(0-24)}$). The $AUC$ extrapolated to infinity ($AUC_{(0-\infty)}$) was calculated as the sum of $AUC_{(0-24)}$ plus the last detectable concentration, $C$, divided by $k$ ($C/k$). Pharmacokinetic parameters other than $C_{\text{max}}$, $t_{\text{max}}$, and $AUC$ were treated by the Practical Pharmacokinetic Program (3P97). The relative bioavailability of metformin hydrochloride sustained-release tablets was determined by:

$$F = \frac{AUC_T}{AUC_R} \times 100\%$$

Statistical analysis
Based on the guidance on bioequivalence studies from FDA and WHO, $AUC$, $C_{\text{max}}$ and $t_{\text{max}}$ were evaluated by ANOVA after log-transformation of the data and Two one-sided t-test, and DF, $C_{\text{min}}$ and Cav were evaluated by Paired-samples t-test for statistical analysis.

Results
Metformin was well tolerated by the volunteers, no serious adverse events occurred during the study. There was no drop-out, all volunteers continued to the end and were all in good health.

Single-dose study
Mean plasma metformin concentration-time profiles for the 18 subjects in the three study treatments are shown in Fig.1. The mean pharmacokinetic parameters are summarized in Table 1. Mean $C_{\text{max}}$ values for $T_1$ and $T_2$ were 1258 and 1285 ng·mL⁻¹, respectively, compared with 1300 ng·mL⁻¹ for the reference treatment; corresponding mean $t_{\text{max}}$ values were 3.8, 3.9 and 3.6 hours, respectively. There was no statistical significant difference in $AUC$, $C_{\text{max}}$ and $t_{\text{max}}$ either among the three treatments or among formulations by ANOVA after log-transformation of the data and two one-sided $t$-test, which proved that the two formulations of test drug and the reference drug were bioequivalent with single-dose administration.

The relative bioavailabilities for metformin based on a comparison of $AUC_{(0-24)}$ values for test drugs to the reference drug, were 98.8% ± 13.0% and 96.7% ± 14.6% for $T_1$ and $T_2$, respectively.

Table 1. Mean (±S.D.) pharmacokinetic parameters for metformin from $T_1$, $T_2$ and R treatment (n=18)

<table>
<thead>
<tr>
<th>Parameters (units)</th>
<th>$T_1$ (250mg/tablet)</th>
<th>$T_2$ (500mg/tablet)</th>
<th>R (500mg/tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng·mL⁻¹)</td>
<td>1258±334</td>
<td>1285±377</td>
<td>1300±347</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>3.8±0.5</td>
<td>3.9±0.7</td>
<td>3.6±0.8</td>
</tr>
<tr>
<td>$K_e$ (h⁻¹)</td>
<td>0.174±0.031</td>
<td>0.164±0.037</td>
<td>0.196±0.033</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>4.1±0.9</td>
<td>4.5±1.2</td>
<td>3.7±0.8</td>
</tr>
<tr>
<td>$AUC_{(0-24)}$ (ng·h·mg⁻¹)</td>
<td>8461±1853</td>
<td>8309±2075</td>
<td>8616±1830</td>
</tr>
<tr>
<td>$AUC_{(0-\infty)}$ (ng·h·mg⁻¹)</td>
<td>8815±1865</td>
<td>8703±1983</td>
<td>9096±1881</td>
</tr>
<tr>
<td>Relative bioavailabilities (%)</td>
<td>98.8±13.0</td>
<td>96.7±14.6</td>
<td></td>
</tr>
</tbody>
</table>
Multiple-doses study

Mean plasma metformin concentration-time profiles for the 18 subjects in the two study treatments are shown in Fig 2. The mean pharmacokinetic parameters are summarized in Table 2. Mean $C_{\text{max}}$ values for $T_2$ and $R$ were 1093 and 1218 ng·mL$^{-1}$, respectively; corresponding mean $t_{\text{max}}$ values were 4.2 and 3.6 hours, respectively. There was no statistical significant difference in $AUC_{\text{ss}}, C_{\text{max}}$ and $t_{\text{max}}$ between test drug and reference product evaluated by ANOVA after log-transformation of the data and two one-sided $t$-test. Besides, there was no statistical significant difference in $C_{\text{min}}, C_{\text{av}}$ and $DF$ between test drug and reference product evaluated by paired-sample $t$-test. The above results demonstrated that the test drug and the reference product were bioequivalent with multiple-dose administration.

The relative bioavailability ($Fr$) for metformin based on a comparison of $AUC_{\text{ss}}$ values for the test drug to the reference product was $94.6\% \pm 14.0\%$.

<table>
<thead>
<tr>
<th>Parameters (units)</th>
<th>$T_2$</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{min}}$ (ng·mL$^{-1}$)</td>
<td>88±41</td>
<td>94±40</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng·mL$^{-1}$)</td>
<td>1093±247</td>
<td>1218±227</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>4.2±0.8</td>
<td>3.4±1.0</td>
</tr>
<tr>
<td>$K_e$ (h$^{-1}$)</td>
<td>0.150±0.047</td>
<td>0.144±0.027</td>
</tr>
<tr>
<td>$t_{1/2e}$ (h)</td>
<td>5.0±1.2</td>
<td>4.9±0.8</td>
</tr>
<tr>
<td>$AUC_{[0\rightarrow\infty]}$ (ng·h·mg$^{-1}$)</td>
<td>9749±2102</td>
<td>10410±2026</td>
</tr>
<tr>
<td>$AUC_{\text{ss}}$ (ng·h·mg$^{-1}$)</td>
<td>9270±1988</td>
<td>9886±1826</td>
</tr>
<tr>
<td>$C_{\text{av}}$ (ng·mL$^{-1}$)</td>
<td>386±83</td>
<td>412±76</td>
</tr>
<tr>
<td>$DF$ (%)</td>
<td>264±62</td>
<td>279±59</td>
</tr>
<tr>
<td>$Fr$ (%)</td>
<td>94.6±14.0</td>
<td></td>
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</tbody>
</table>
Fig 1. The plasma concentration-time curve of 18 volunteers after multiple-doses administration of 1000mg test drug (500mg metformin hydrochloride) and reference product

Discussion

Metformin hydrochloride sustained-release tablet is a new formulation for biguanides drugs treating NIDDM. The reference product used in the present study is a new commercial product which has just come into market. Determination of metformin plasma concentration using HPLC method has been reported previously\(^{19-21}\). The described analytical method was proven sensitive and accurate for determination of metformin plasma concentration. In the present study, acetonitrile was used to deposit protein when extracting metformin from human plasma. Compared with the published methods, this would be easier, and have higher sensitivity and accuracy.

Bioequivalence study is to test the rate and extent of absorption for different formulations of the same drug. The most important objective of bioequivalence testing is to assure the safety and efficacy of generic formulations. When two formulations of the same drug are equivalent in the rate and extent to which the active drug becomes available to the site of drug action, they are considered bioequivalent, and thus considered therapeutically equivalent. In bioequivalence study, the result may be affected by many factors, such as the nature of the drug studied, patient population, and clinical end points, etc, thus certain limits should be set depending on these factors. Generally, the standard equivalence range is 80%-125% for basic pharmacokinetic characteristics, such as \(AUC\), \(C_{max}\), \(t_{max}\)\(^{[22]}\).

In the present study, results of single-dose administration showed that the relative bioavailability of metformin hydrochloride for the test drugs with the reference product were 98.8%±13.0% and 96.7%±14.6% for \(T_1\) and \(T_2\), respectively. No statistically significant difference was found in \(C_{max}\), \(AUC_{(0,24)}\), \(AUC_{(0→∞)}\), \(T_{max}\) of the three formulations by ANOVA and two one-sided \(t\)-test. 90% confidence interval (90% CI) of each parameter of test drugs were all within the range of 80%-125% of that of reference product, thus it could be concluded that the two formulations of test drug and the reference drug were bioequivalent with single-dose administration. For multiple-dose administration study, the relative bioavailability for metformin hydrochloride for the test drug with the reference product was 94.6%±14.0%.
There was no statistically significant difference in $C_{\text{max}}$, $C_{\text{min}}$, $AUC_{\text{ss}}$, $T_{\text{max}}$, and $DF$ between test drug ($T$) and reference product, with 90% CI of each parameter of test drugs were all within the range of 80%-125% of that of reference product. The results demonstrated that when the steady-state was achieved after multiple-dose administration of the two formulations, the rate and extent of absorption, the plasma metformin concentration of steady-state ($C_{\text{ss}}$), as well as DF did not differ significantly, thus it could be concluded that these two formulations ($T$ and $R$) were bioequivalent with multiple-doses administration. The results of this study suggest equal clinical efficacy of test drugs and reference product either with single-dose administration or multiple-doses administration.

References