In Vitro Dissolution and In Vivo Bioequivalence Evaluation of Two Brands of Trimetazidine Tablets

Sally A. Helmy¹ and Noha O. Mansour²

Abstract

Trimetazidine is an effective anti-anginal and anti-ischaemic effect. The objective of this study was to assess the in vitro dissolution and to evaluate the bioavailability of two brands of trimetazidine dihydrochloride tablets. Prior to the in vivo PKs study, an in vitro comparative dissolution test was performed for 2 oral brands of trimetazidine dihydrochloride tablets (20 mg). In vivo PKs study was evaluated in 24 healthy male volunteers after a single dose oral administration in an open, randomized, two-way crossover study with a washout period of 1 week. After an overnight fast, human volunteers were randomly allocated to receive a single dose of either test or reference product. Blood samples were collected over a 24-hour period following drug administration and plasma was analyzed for trimetazidine concentrations using a validated high-performance liquid chromatography assay method. The PK parameters Cmax, AUC0–t, AUC0–∞, tmax, and t1/2 were determined from plasma concentration-time profiles. The 90% confidence intervals for the ratio of log transformed values of Cmax, AUC0–t, and AUC0–∞ of the test product over those of reference were within the acceptable range (0.8–1.25) for bioequivalence. As a result, the 2 trimetazidine formulations are considered bioequivalent and thus could be prescribed interchangeably in the medical practice based on its PK effect and biopharmaceutical performance.

Keywords
trimetazidine, pharmacokinetics, dissolution, bioequivalence, HPLC

The method was successfully applied to a bioequivalence study on Egyptian populations. According to the World Health Organization guidelines, comparative dissolution tests and PK analyses are preferred in comparative pharmacodynamic studies and clinical trials in humans.¹ The objective of this study was to assess the in vitro comparative dissolution and determine the in vivo comparative PK parameters of two brands of trimetazidine dihydrochloride tablets (20 mg) and to compare these parameters statistically to evaluate the bioequivalence between the two brands.

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Materials and Methods

Study Products, Reagents, and Instruments

Study products. The test product was Vastor tablets (20 mg trimetazidine dihydrochloride; PHARCO Pharmaceuticals, Alexandria, Egypt). The reference product was Vastarel tablets (20 mg trimetazidine dihydrochloride; Servier, Neuilly-sur-Seine, France).

Reagents. Trimetazidine and pseudoephedrine (IS) were purchased from Sigma-Aldrich Co. (St. Louis, MO); All chemicals and reagents used were of LC grade and were purchased from Merck Chemicals (Darmstadt, Germany); Milli-Q grade (Millipore, Bedford, MA) water was used in all cases. Serum was prepared from normal human blood, procured freshly from healthy volunteers through a local blood bank of Tanta University Hospital, Egypt and pooled for spiking and using as control blank samples.

Instruments. The analysis was performed in HPLC system equipped with a variable-wavelength ultraviolet (UV) detector and an automatic injector (LC-10 VP, Shimadzu Scientific Instrument, Kyoto, Japan). Data analysis program Class-VP (Shimadzu Scientific Instruments) was used for data acquisition and processing. Other instruments used in this study include Bath sonicator (UltraSonik 57X, Elmsford, USA); Balance (Sartorius, Goettingen, Germany); Magnetic stirrer (Stuart, Stone, Staffordshire, UK); Centrifuge (Hettich, Tuttingen, Germany); Vortex tube mixer (Heidolph, Schwabach, Germany); Brand auto micro-pipettes (Labnet, Edison, USA); and micro liter syringes from Hamilton (Bonaduz, Switzerland).

Study Population

Twenty-four healthy male volunteers were selected for this study after clinically assessing their health status by physical examination, electrocardiography, hematology, biochemistry, electrolyte analysis, and urinalysis. Subjects with a history of drug allergies or drug idiosyncrasies, renal or hepatic impairment, or drug or alcohol abuse were excluded. Subjects who had used medications of any kind within 2 weeks of the start of or during the study were also excluded. The volunteers had the following clinical characteristics, expressed as means ± SD; age of 22.2 ± 3.63 years (range, 18–30 years) and weight of 70.1 ± 7.4 kg (range, 55–58 kg) and were chosen to participate in the present study. All enrolled volunteers were healthy, and none of the participants showed any signs of adverse drug reactions during or after completion of the study.

Study Design

In Vitro Dissolution Study. Dissolution of the both tablets was tested in vitro in acidic pH (1.2) using the dissolution apparatus II (paddle method) specified by the United States Pharmacopeial Convention 12 at 50 rpm at 1, 2, 3, 4, 5, 10, 15, 30, 45, 60, and 90 minutes using 0.1 N HCl (pH 1.2) as dissolution medium. Five-milliliter samples of the dissolution medium were withdrawn at specified time intervals. The samples were filtered through a 0.45 μm Millipore filter and fresh release medium preheated at 37°C was added to compensate for the withdrawn volume. Trimetazidine content was determined spectrophotometrically (Jasco V-530 UV/VIS spectrophotometer, Tokyo, Japan) at 232 nm. The release profiles were plotted as the cumulative percent of drug dissolved versus time. Dissolution profile similarity was calculated using a statistical similarity factor f2, as defined in the international guidelines. 13 The similarity factor (f2) is a logarithmic reciprocal square root transformation of the sum of the squared error. It is a measure of similarity in the percentage dissolution between two curves and is calculated as:

\[ f_2 = 50 \log \left( \frac{1}{n} \sum_{i=1}^{n} \left( \frac{R_i - T_i}{C_0} \right)^2 \right)^{-0.5} \times 100 \]

where n is the number of time points, R, the dissolution value of the reference product at time t, and T, is the dissolution value of the test product at time t. When >85% of the drug is dissolved within 15 minutes, dissolution profiles are considered similar without further mathematical evaluation.

In Vivo Bioequivalence Study. This study was carried out at Bioavailability Unit of the Faculty of Pharmacy, Tanta University and monitored in accordance with the International Conference of Harmonisation (ICH) guidance on general considerations for clinical trials. The protocol was approved by the ethical committee of Tanta University Hospital. All participants gave their written informed consent to study participation after they were well informed about the study objectives, methods, and possible risks. The study was conducted according to a single-dose, standard two-way, split-group crossover design with 12 participants in each of the two treatment groups and a washout period of 1 week between the two phases. The volunteers were randomly selected to receive a single dose of two Vastor tablets (each contains 20 mg trimetazidine dihydrochloride) as the test drug, or two Vastarel tablets (each contains 20 mg trimetazidine dihydrochloride) as the reference drug followed by 200 mL of water after a 12-hour overnight fast. Food and drinks were withheld for at least 2 hours after dosing. Blood samples were collected in heparinized tubes at 15 minutes predose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, and 24 hours postdose. Plasma was directly separated by centrifugation at 3,000 rpm for 10 minutes, transferred into polypropylene plastic tubes, and stored at −20°C until assay.

Analytical Method. The plasma level of trimetazidine was measured using a validated high-performance liquid chromatography (HPLC) method developed in our
laboratory. The mobile phase consisted of 20 mM ammonium dihydrogen orthophosphate, and the pH was adjusted to 3.0 by phosphoric acid and acetonitrile at a ratio of (88:12%, v/v). The analysis was run at a flow rate 1.5 mL/min on a Hypersil® BDS C18 column (250 mm × 4.6 mm, 5 µm; Thermo Fisher Scientific, Waltham, MA). The effluent was monitored using a model SPD-10AVP UV–VIS detector at 207 nm for trimetazidine and IS, and peak areas were integrated electronically using the Class-VP data analysis program (all Shimadzu Scientific Instruments) and used to calculate the peak area ratio of the drug and IS.

The calibration standards of trimetazidine dihydrochloride were prepared by transferring 50 µL from each working solution and 50 µL IS (pseudoephedrine) to a set of test tubes. The solvent was evaporated, and 0.5 mL of blank plasma was added to each tube to form a set of calibration standards with concentrations of 10, 20, 30, 50, 75, 100, 150, 200, and 300 ng/mL. The calibration standards were extracted by adding 5 mL of dichloromethane after addition of 100 µL of 1.0 N NaOH and shaken for 1 minute, then centrifuged at 3,000 rpm for 7 minutes. The dichloromethane layer was transferred to clean test tubes and was evaporated in a water bath at 60°C, then reconstituted with 300 µL of the mobile phase. The resulting solution was transferred to clean HPLC vials and 50 µL was injected into the HPLC.

The assay validation was determined according to the spirit of the Food and Drug Administration (FDA) guidelines for the validation of a bioanalytical method in terms of linearity, selectivity, precision, accuracy, limit of detection, limit of quantification, and stability. Pharmacokinetic Analysis. A non-compartmental model was used to determine the PK parameters of trimetazidine. The elimination rate constant (Kₑ) was estimated by least squares regression of plasma concentration-time data points in the terminal log-linear region of the curves. Half-life (t₁/₂) was calculated as 0.693 divided by Kₑ. The area under the concentration-time curve from zero to the last measurable plasma concentration (AUC₀₋ₜ) was calculated using the linear trapezoidal rule. The AUC from zero to infinity (AUC₀₋∞) was calculated as AUC₀₋∞ (AUC₀₋ₜ + C/Kₑ), where C is the last measured concentration. Peak plasma concentration (Cₘₐₓ) and time to reach peak plasma concentration (tₘₐₓ) were obtained directly from the individual plasma concentration-time curve.

Statistical Analysis. For the purpose of bioequivalence analysis, Cₘₐₓ, AUC₀₋ₜ, and AUC₀₋∞ were considered primary variables. After logarithmic transformation of Cₘₐₓ, AUC₀₋ₜ, and AUC₀₋∞ values were analyzed according to the FDA guidelines. Bioequivalence was assessed by means of an analysis of variance using Minitab Statistical Package version 13 (Minitab, Inc., State College, PA) for crossover design and calculating the standard 90% CI of the ratio test/reference. The products were considered bioequivalent if the difference between 2 compared parameters was found statistically insignificant (P > 0.05) and the 90% CI for these parameters fell within 80–125%. In accordance with standard criteria, bioequivalence was assumed when the log transformed mean test-to-reference ratio was between log (0.8) and log (1.25).

Results

This assay method is valid within a wide range of plasma concentrations and may be proposed as a suitable method for the PK, bioavailability, and bioequivalence studies. The calibration curves include all drug concentrations measured in clinical practice with within- and between-day accuracies and precision were in accordance with FDA guidelines, making the assay method reliable and rugged.

Under the chromatographic condition described before, the retention times for trimetazidine and IS were 4.0 ± 0.3 and 6.0 ± 0.2 minutes, respectively. There were no peaks for any endogenous compound appeared at the same retention time for trimetazidine and IS. The calibration curves were linear with a correlation coefficient R² < 0.999 throughout the course of the assay (300–10 ng/mL). Study samples were treated as the calibration standards. The within- and between-day coefficients of variance (CV) were always within ±15% in the entire range of the calibration curve. The within-day %CV ranged between 1.048% and 14.90% whereas the between-day %CV ranged between 1.339% and 12.21%. The within-day accuracy ranged between 99.63% and 109.1%, whereas the between-day accuracy ranged between 93.41% and 111.3%. The concentrations...
of the quality control samples were 10, 50, and 150 ng/mL, respectively. The limit of quantification was 10 ng/mL.

In Vitro Comparative Dissolution Tests. Release of trimetazidine from Vastor and Vastarel tablets is shown in Figure 1. Comparison of test with the reference products suggested that the test dissolution matches the reference. The results indicated that both test and reference tablets are >85% dissolved in the dissolution medium within 15 minutes and similarity factor \( f_{2} \) is 51.1% (>50%), signifying acceptable similarity of the compared formulations.

In Vivo Bioequivalence Study. The mean timed plasma concentrations of trimetazidine following administration of a single oral dose of either test or reference products are shown in Figure 2. A summary of the PK parameters of trimetazidine is shown in Table 1.

**Discussion**

The objective of this study was to determine the PK parameters of two brands of trimetazidine tablets and then to compare these parameters statistically to evaluate the bioequivalence between them. Two pharmaceutical products are considered bioequivalent if their bioavailabilities, in terms of \( C_{\text{max}} \), \( t_{\text{max}} \), and AUC after administration of the same molar dose under the same conditions, are similar to such a degree that their effects could be expected to be the same. The AUC generally serves as the characteristic of the extent of drug absorption, whereas the \( C_{\text{max}} \) and \( t_{\text{max}} \) reflect the rate of drug absorption. The PK results of this study were in good agreement with the results of the other studies in normal volunteers,\(^3\),\(^5\) which emphasized the validity of the present results. The study revealed that the 90% confidence intervals of \( C_{\text{max}} \), \( \text{AUC}_{0,\infty} \), and \( \text{AUC}_{0,t} \) were always between 80% and 125%. Therefore, the two formulations can be considered bioequivalent with regard to the extent and rate of absorption. No statistically significant difference was observed regarding subjects within sequence, treatments and time periods for \( C_{\text{max}} \), \( \text{AUC}_{0,\infty} \), and \( \text{AUC}_{0,t} \). However, the differences between the effects of sequence of administration were significant for \( C_{\text{max}} \) indicating normal biological variation.

**Table 1.** Pharmacokinetic Parameters of Trimetazidine After a Single Oral Dose Administration With the Test and Reference Products (Mean ± SD, \( n = 24 \)) and Statistical Analysis of log Transformed Data Within the 90% CI

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vastor (Mean ± SD)</th>
<th>Vastarel (Mean ± SD)</th>
<th>90% Confidence Interval, Point Estimate (Lower Limit–Upper Limit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{max}} ) (( \mu \text{g/mL} ))</td>
<td>148.2 ± 36.6</td>
<td>152.5 ± 29.1</td>
<td>0.98 (0.9–1.03)</td>
</tr>
<tr>
<td>( t_{\text{max}} ) (h)(^a)</td>
<td>3.0 (1.5–5.0)</td>
<td>2.5 (1.0–5.0)</td>
<td></td>
</tr>
<tr>
<td>( \text{AUC}_{0,\infty} ) (ng h/mL)</td>
<td>1,305.0 ± 356.7</td>
<td>1,207.0 ± 272.8</td>
<td>1.01 (0.99–1.08)</td>
</tr>
<tr>
<td>( \text{AUC}_{0,\infty} ) (ng h/mL)</td>
<td>163.2 ± 72.12</td>
<td>152.3 ± 41.5</td>
<td></td>
</tr>
<tr>
<td>( \text{AUC}_{0,\infty} ) (ng h/mL)</td>
<td>1,468 ± 402.5</td>
<td>1,359.0 ± 292.1</td>
<td>1.03 (0.98–1.08)</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>6.9 ± 1.3</td>
<td>7.1 ± 0.8</td>
<td></td>
</tr>
</tbody>
</table>

\( C_{\text{max}} \) peak plasma concentration; \( t_{\text{max}} \) time to reach peak plasma concentration; \( \text{AUC}_{0,\infty} \) area under the concentration-time curve from zero to infinity; \( \text{AUC}_{0,t} \) area under the concentration-time curve from zero to the last measurable plasma concentration; \( \text{AUC}_{t,\infty} \) area under the concentration-time curve from the last measurable concentration to infinity; \( t_{1/2} \), elimination half-life.

\( ^a \) \( t_{\text{max}} \) (h) was presented as median (range).
Conclusions
It could be concluded that both products tested in this study comply with regulatory requirements to be claimed bioequivalent. According to the above, the test product can be considered interchangeable in medical practice with the reference based on its PK effect and biopharmaceutical performance.

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References