Formulation and In Vitro, In Vivo Correlation Between Two Candesartan Cilexetil Tablets

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Abstract
In this study, the in vitro and in vivo interchangeability between generic candesartan 16 mg and the branded formulation was assessed. The in vitro release of these products was conducted in 3 pH media (1.2, 5.0, and 6.8), and similarity factors (f²) were calculated. This bioequivalence study was a randomized, 2-period crossover study that included 42 healthy adult male subjects under fasting conditions with a 9-day washout. The pharmacokinetic (PK) parameters AUC₀-last, AUC₀-∞, and Cmax, tmax, and the elimination half-life time were assessed based on the plasma concentrations of candesartan, using a newly developed and validated liquid chromatography-tandem mass spectrometry bioanalytical method with acceptable degrees of linearity, sensitivity, precision, and accuracy. The geometric mean (ng·h/mL) of the AUC₀-∞ for the test and brand was 1595.49 and 1620.54, respectively, and the Cmax (ng/mL) was 160.91 and 160.88, respectively. The 90%CIs of geometric mean ratios (test-to-reference ratios) were 98.26%, 98.45%, and 99.86% for AUC₀-last, AUC₀-∞, and Cmax respectively. These PK parameters lie within the US Food and Drug Administration– and European Medicines Agency–specified bioequivalence limit (80%-125%). Both products were well tolerated by all the subjects. The tested drug product was bioequivalent to the reference drug and had the same safety profile.

Keywords
candesartan, validation, bioanalysis, bioequivalence, F₂

The use of generic drugs represents a cost-effective and technologically viable way of providing acceptable-quality drugs to a country’s population, especially in low-income countries. However, the regulatory requirements behind the approval and registration of new generic drugs represent a big barrier for the rapid development and market spreading of these products.¹ Demonstration of bioequivalence in a bioequivalence study (BES) between the generic product and the reference is required for the approval process. Therefore, waiving the need for these BESs can significantly speed the development process of new generic drugs. This could be achieved when the bioavailability of drugs can instead be assessed by in vitro estimation of parameters such as solubility, permeability, and dissolution. Recently, a Biopharmaceutics Classification System has been introduced by Amidon et al. This system has been approved by many regulatory authorities including the US Food and Drug Administration (FDA), World Health organization, and European Medicines Agency (EMA).²–⁴ According to these guidelines, waiving of bioavailability and BESs for the immediate-release solid oral dosage forms is now possible and could be recommended for class I drugs (highly soluble and permeable drugs). In addition, this decision was extended to classes II and III under certain conditions.³–⁵–⁷ In the case of class II drugs, dissolution is considered the rate-limiting step for drug absorption, whereas for class III drugs, the rate-limiting step in absorption is intestinal permeability. Accordingly, to safely reduce the cost and time to develop a new generic drug, the distinct pharmacokinetic properties of the drug can be simulated

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Submitted for publication 4 May 2017; accepted 15 February 2018.

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Clinical Pharmacology in Drug Development 2018, 7(6)

Figure 1. Chemical structure of candesartan cilexetil.

by in vitro and in vivo correlation (IVIVC), which can be used in combination with computational simulation tools such as GastroPlus software. A level A IVIVC demonstrates that the conditions of the dissolution test are able to simulate in vivo performance. In fact, this has been employed in the assessment of waiving BESs and the determination of IVIVC for classes II and III drugs, resulting in great savings of both time and cost of the development of a new generic drug.

Candesartan cilexetil (CaC) is an angiotensin receptor blocker that is used in the treatment of diseases such as hypertension, heart failure, and myocardial infarction. Its International Union of Pure and Applied Chemistry name is (±)-1-Hydroxyethyl 2-ethoxy-1-(p-[o-1H-tetrazol-5ylphenyl]benzyl)-7-benzimidazolecarboxylate, cyclohexyl carbonate, as reported in Figure 1.

Candesartan cilexetil is a prodrug that undergoes ester hydrolysis in the intestinal wall to form the active metabolite candesartan. Candesartan can lower blood pressure (BP) by antagonizing the renin-angiotensin-aldosterone system. It selectively blocks angiotensin II for binding to the type 1 angiotensin II receptor (AT1) subtype, which results in a decrease in BP. This drug is primarily excreted unchanged in urine (33%) and in feces (67%) by the biliary route. It undergoes minor hepatic metabolism (<20%) by O-deethylation via cytochrome P450 2C9 to form an inactive metabolite. Candesartan undergoes N-glucuronidation in the tetrazole ring by uridine diphosphate glucuronosyltransferase 1A3 to form candesartan N2-glucuronide.

CaC was classified as class II because it was found to have high permeability (more than 90% permeation) but still have low water solubility.

Commercially this drug is available as oral tablets with several strengths such as 4, 8, 16, and 32 mg of CaC. It is indicated for the treatment of high blood pressure in adults and children younger than 17 years of age. It may be used alone or in combination with other antihypertensive drugs.

The blood concentrations of angiotensin I, angiotensin II, and renin activity are increased in a dose-dependent pattern after single and repeated dosing of CaC to healthy subjects, as well as to patients with high BP and heart failure. The BP response of CaC is dose dependent over the range of 2 to 32 mg; the usual recommended initial dose is 16 mg once daily. Most of the antihypertensive effect of CaC was demonstrated within 2 weeks, and the full-effect generally occurred after 4 to 6 weeks of continuous treatment. In heart failure, treatment with CaC decreases mortality and hospitalization. Bioavailability of CaC is not affected following ingestion of food with a high-fat content. After single and repeated administration, the pharmacokinetic profile is linear for oral doses of up to 32 mg of CaC, and its inactive metabolites are not accumulated in serum after repeated once-daily dosing. Its absolute bioavailability is close to 15%, and maximum plasma concentrations \( C_{\text{max}} \) were achieved within 4 to 8 hours after oral tablet dosing. More than 99% of the drug is bound to plasma proteins, and the volume of distribution in healthy individuals is 0.13 L/kg. The total plasma clearance of CaC is 0.37 mL/min/kg, with renal clearance of 0.19 mL/min/kg.

It is mainly excreted unchanged in urine and stool. Its elimination half-life \( t_{1/2} \) is close to 9.7 hours, and important drug-food or drug-drug interactions are not reported. Various CaC generic tablet formulations are currently available in the marketplace around the world. These generic products are clinically of great help because of their low price. However, there has been growing concern about the safety and efficacy of these generic products. The BESs of CaC from different oral tablet formulations are important parameters to compare the clinical efficacy and safety of these generic products. These parameters will be very helpful for clinicians and pharmacists in difficult situations to choose among alternatives. In this study a new CaC generic tablet formulation was developed, and in vitro dissolution studies were used to predict its BE with the original brand. Moreover, a BES was conducted using a new high-pressure liquid chromatography–mass spectrometry (HPLC-MS) bi-analytical method that was previously validated. A comparative study between two candesartan products (16-mg tablets of Avalon Pharma, Middle East Pharmaceutical Industries Co. Ltd., Kingdom of Saudi Arabia, versus 16-mg tablets of Arab Pharmaceutical Manufacturing Co. Ltd., Sult-Jordan [under license of Takeda Japan]) was designed and the key pharmacokinetic parameters (PKPs) for both drugs were assessed.

Materials and Methods

The study was a comparative, randomized, 2-period, 2-treatment, 2-sequence, single-dose, open-label, crossover bioequivalence study of a generic 16-mg
tablet versus the brand-name drug Blopress 16-mg tablet in healthy subjects under fasting conditions.

**Volunteers and Clinical Protocol**

The study was conducted by Arab Pharmaceutical Industry Consulting Co. Ltd./Pharmaceutical Research Unit, Amman, Jordan, in accordance with the requirements of the Declarations of Helsinki, the current Good Clinical Practice guidelines, and the International Conference on Harmonization (ICH) guidelines. The study was approved by the Institutional Review Board (IRB) of IPRC, Amman, Jordan, which operates in accordance with the principles and requirements described in “Guidelines on Research Involving Human Subjects.” The study protocols and the informed consent forms were reviewed by the IRB of IPRC, approved, and given the code CAND 466/PRO-00.

Forty-two adult male volunteers were recruited to participate in the study. Healthy mixed-skin Arab and Mediterranean subjects aged between 18 and 50 years with a body mass index between 18.5 and 30.0 kg/m² inclusive (minimum weight of 50 kg), who were non-smokers or light smokers (smokers of not more than 10 cigarettes per day) were included in the study. The volunteers were subjected to a full medical and physical exam to confirm their healthy status and were not on any medication during the study period. A written informed consent letter, which explained in detail the nature of the study, was given to each volunteer. The volunteers were instructed to abstain from taking drugs 1 month before starting the study and caffeine and alcohol-containing beverages for at least 16 hours prior to drug administration. Consumption of beverages was not allowed during the study. Drinking of water was allowed except for 1 hour prior to administration of the study drug.

The study was open label, randomized, 2 treatments, and 2 periods (each lasting 48 hours and separated by a washout period of 9 days). Forty-two healthy male subjects agreed to be enrolled in the protocol, 42 subjects completed the study, and 42 subjects were evaluated for PKPs. The volunteers were randomly divided into 2 equal groups of 21 subjects. The first group was given the reference brand, and the second group was given the test formulation, with a crossover after a 9-day washout.

The study was conducted at a single center, and subjects were confined until the last sampling time. During the 9 days of the washout period, they were asked to leave, and they returned to the clinical site 16 hours before the dosing of the second period. On the morning of the study, a blood sample to serve as a blank for the drug assay was taken from each volunteer. Each volunteer received an oral dose of the assigned formulation, given with 240 mL of water in the sitting position. During each period 20 blood samples were taken: an 8-mL sample 1 hour before dosing and 8-mL samples 1.0, 1.5, 2.0, 2.50, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0, 10.0, 12.0, 16.0, 24.0, 36.0, and 48.0 hours after dosing. A volume of 8 mL of blood was placed in a 10-mL heparin blood tube, and the tubes were centrifuged at 4000 rpm for 5 minutes within a maximum of 15 minutes. Each plasma sample was transferred using disposable polypropylene droppers into 2 labeled polypropylene tubes and then capped and stored in an Ultra freezer (-70°C) until analysis. Four hours after drug administration, a standard lunch meal containing soup, a half chicken, and rice with mixed vegetables was served. Carrots, which are a potassium-rich food, were avoided to restrict the potassium level, especially because CaC can make the body retain potassium. The subjects had free access to water 1 hour after drug administration.

**Chemicals**

HPLC-grade methanol, isopropanol, and formic acid (FA) were from Scharlau. Ammonium acetate was from Merck. HPLC-grade water was supplied by the Carbon group. Acetonitrile (ACN) was from Romil. Control human plasma (0.2 mL) was harvested from donors.

**Instruments and Chromatographic Separations**

The analysis was performed using an HPLC system (Agilent autosampler model 1100) coupled with an MS detector (Applied Biosystem API Sciex 5000).

Four hours after drug administration, several chromatographic conditions were optimized during the method validation. The stationary phase was a Gemini 3-μm C18 50 × 3.00 mm (Phenomenex), the mobile phase was ACN:H2O:FA 90:10:0.1 (v/v/v), and pH was adjusted at 3.11 ± 0.20. Injection volume was 10 μL, and flow rate was 0.500 mL/min, whereas the flushing solution (ACN:isopropanol:H2O:FA) was set at a volume ratio of 45:45:10:0.1 (v/v/v/v). Regarding the detection settings, m/z 441.269-263.100 was selected for CaC, and m/z 446.182-268.100 was selected for the internal stock (IS) solution.

**Preparation of Standard Solutions**

Stock solutions were prepared by dissolving 0.010097 g of the drug in methanol up to a 25-mL final volume to make up a stock solution containing 403.88 μg/mL. Then a serial dilution was carried out to prepare diluted solutions in the range of 1.43-237.48 ng/mL. The IS solution was prepared by dissolving 0.001125 g of candesartan-d5 using methanol up to
a 100-mL final volume to make up a stock solution containing 11.25 μg/mL.

**Sample Preparations for LC Injection**
The extraction method was protein precipitation extraction using cold acetonitrile. Before the extraction, each sample including calibrators, quality-control (QC) samples, and authentic samples were spiked with the candesartan-d5 IS solution. The IS was employed to ensure the accuracy of measurements corresponding to the analyte.

**Validation Procedures**
The used analytical method was validated according to ICH Guidelines using parameters such as linearity, precision, sensitivity, and recovery (Supplementary Table 1). Moreover, part of the validation protocol was to assess some concomitant over-the-counter drugs such as ascorbic acid, acetaminophen, and caffeine. For candesartan, the linearity study was carried out in the range of concentrations from 1.425 to 237.481 ng/mL. The lower limit of quantitation (LLOQ) was determined as the lowest concentration that could be detected by the HPLC system producing a signal-to-noise ratio of 10. The LLOQ was estimated by analyzing known samples of candesartan at different drug concentrations starting from the lower concentrations of the calibration curves. In addition, the values below the limit of quantitation were entered as zero and included as such in the calculation of mean values. Assay precision was assessed using accuracy and coefficient of variation. Stock solution stability in the mobile phase was evaluated using 2 standard mixtures that were equivalent to the LLOQ and the upper limit of quantitation. The IS concentration was 11.250 ng/mL. Short-term matrix-based solution stability was assessed using plasma samples at QC concentration of Low and high. Samples were left at room temperature for 24 hours. Long-term matrix-based stability was assessed using 2 quality-control samples, low (QCL) and high (QCH). Candesartan concentrations were stored at -70°C and -20°C. Autosampler stability (injection phase) was assessed using 6 replicates of QCL and QCH. Stability after freeze-and-thaw cycles was also assessed using 2 sets of QC samples. Recovery for the drug and internal standard was assessed using 6 extracts at 3 concentrations. Matrix effect was investigated for candesartan and the IS.

**Pharmacokinetic and Statistical Analysis**
The PKPs were calculated using standard noncompartamental methods. The peak plasma concentration (C_{max}) and the corresponding time (T_{max}) were estimated directly from the data. The elimination rate constant (K_e) was estimated from the slope of the semilogarithmic plot of the terminal elimination phase of the plasma concentration-time curve. The elimination half-life time (t_{1/2}) was calculated using the equation $t_{1/2} = \ln 2/K_e$. The areas under the plasma concentration-time curve from time zero to last (AUC_{0-last}) and from time zero infinity (AUC_{0-∞}) were calculated using the linear trapezoidal method. Two-way analysis of variance (ANOVA) was used to calculate the effect of tablet formulations, periods, sequences (fixed effects), and subjects (random effect) on AUC_{0-last}, AUC_{0-∞}, and C_{max} using Thermo Scientific Kinetica (version 5.1), a commercially available software package. SAS was used to generate the statistical report including the 90% CIs for all PKPs. As per the FDA and the EMA guidelines for conducting bioequivalence studies, the 90% CIs for PKPs, C_{max} and AUC should fall within 80%-125%.

**Results**

**Results of Validation Procedures**
Results of the validation parameters for the assay used are summarized in Supplementary Tables 1 and 2. In fact, the relationship between concentration and peak area ratio was found to be linear within the range of 1.425-237.481 ng/mL for CaC, with a correlation coefficient (r) of 0.998. The method was found to be sensitive to the LLOQ, recovery of the drug, and recovery of IS, as reported in Supplementary Tables 2 and 3. Short- and long-term plasma-based stability proved that the drug was at -20°C and -70°C, respectively, as reported in Supplementary Table 2. A postpreparative stability study showed that the drug is stable for 78 hours in the dry and injection phases. No carryover or matrix effect was found.

**Results of Pharmacokinetic Study**
Both the reference and the test formulations were well tolerated by all subjects, and the subjects were discharged in good health. Figure 2 shows plasma concentrations of both brands, indicating that mean profiles after administration of the 2 formulations are superimposable. As per the statistical report, there was no significant difference in the effect of period, sequence, and formulation. All estimated PKPs were in agreement with reported values. Table 1 shows a summary of the PKPs for the 2 formulations of candesartan 16 mg. As per the guidelines and the protocol of the clinical study, the 90% CI was calculated for all pharmacokinetic parameters. The 90% CIs of geometric mean ratios (test-to-reference ratios) were 98.26%, 98.45%, and 99.86% for AUC_{0-last}, AUC_{0-∞}, and C_{max}, respectively. No statistically significant difference between the 2 formulations was found. These PK parameter values lie within the FDA- and European Medicines Agency–specified bioequivalence limit (80%-125%).
Our results in this part of the study suggest equivalent clinical efficacy of the 2 brands, as can be seen in Table 1 and Figure 2.

**Discussion**

The FDA defines a brand-name drug as a drug marketed under a proprietary, trademark-protected name, and a generic drug is the same as a brand-name drug in active ingredient, dosage form, safety, strength, route of administration, quality, performance, and intended use and contains the same salt, ester, or chemical form. If all these criteria are proven, then the 2 drugs are considered therapeutical equivalents and therefore can be interchangeable. During the development phase of an oral immediate-release tablet, several preformulation and formulation trials and tests are carried out to achieve a product that can be interchangeable with the original brand in terms of efficacy and safety. In this study, the bioavailability of a candesartan 16-mg generic tablet was assessed versus the reference candesartan 16 mg produced by Arab Pharmaceutical Manufacturing Co. Ltd., Jordan (under license of Takeda Japan). The 2 dosage forms were administered to 42 fasting Mediterranean male volunteers to eliminate the influence of food on drug absorption. The validated analytical method described above was used to quantify the plasma concentrations of candesartan after administration of 16 mg of this drug as immediate-release tablets. Analysis was successfully applied without interference with the excipients used in the tablet formulation. They provided the appropriate accuracy, sensitivity, and selectivity, with high sample throughput and an economically convenient procedure required for PK studies. In this study several PK were tested using a validated HPLC-MS method. In fact, all validation parameters were conducted according to the international guidelines, and they were within the accepted limits as summarized in Supplementary Table 1. Regarding the efficacy of our generic product, statistical comparison of the main PKPs, AUC$_\text{0-last}$, AUC$_\text{0-\infty}$, C$_\text{max}$, and T$_\text{max}$, indicated no existence of any significant differences between test and reference tables in any of the calculated PK parameters. The obtained values were compliant with the FDA and EMEA BE study for bioequivalence of generic drugs because the AUC$_\text{0-\infty}$, AUC$_\text{0-last}$, and C$_\text{max}$ mean ratios were within the 80%-125% interval.

**Table 1. Summary of Calculated Pharmacokinetic Parameters of Candesartan in the Bioequivalence Study (n = 42)**

| Parameters | Test Candesartan | Reference BLOPRESS |
|------------|------------------|--------------------|
| C$_\text{max}$ (ng/mL) | 171.219 (64.103) | 170.302 (59.890) |
| AUC$_\text{0-last}$ (ng·h/mL) | 1600.355 (461.192) | 1634.279 (484.584) |
| AUC$_\text{0-\infty}$ (ng·h/mL) | 1654.247 (473.201) | 1684.980 (490.838) |
| T$_\text{max}$ (h) | 3.74 (1.13) | 3.90 (1.13) |
| t$_{1/2}$ (h) | 5.49 | 5.33 |

| Bioequivalence Results Summary | Parameter | Point Estimate (Ratio of Arithmetic Means) | Lower Limit (%) | Upper Limit (%) | CV% |
|-------------------------------|----------|-------------------------------------------|-----------------|-----------------|-----|
| C$_\text{max}$ | 100.54 | 92.07 | 108.30 | 22.36 |
| AUC$_\text{0-last}$ | 97.92 | 93.63 | 103.13 | 13.21 |
| AUC$_\text{0-\infty}$ | 98.18 | 93.79 | 103.35 | 13.26 |

CV, coefficient of variation.
It was concluded that the generic test tablets were bioequivalent to the commercial brand for both extent and rate of absorption after administration of a single oral dose of each to healthy male adults under fasting conditions (Figure 2).

Safety was also important in our study. All the recruited volunteers completed the study without showing any sign of adverse effect, and they left the study in good health. Moreover, the dissolution studies using similarity and nonsimilarity factors at different pH media were of great help and had a positive role in reducing the cost of the final product. These dissolution studies can be conducted on other products to see if there are similar developments.

Conclusion
The developed bioanalytical method proved to be effective in quantifying candesartan in blood plasma. The dissolution studies were very helpful in predicting the in vivo behavior of CaC. The statistical analysis of the results performed on AUC0-last, AUC0-∞, and Cmax using the ANOVA method showed that both generic test tablets and reference tablets are bioequivalent, as they deliver equivalent quantities of active ingredient to systemic circulation at equivalent rates for both AUC0-last and Cmax ratios within the 80%-125% interval proposed by the FDA and the EMA. The tested candesartan was bioequivalent to the reference brand and had the same safety profile. This is important for achieving good therapeutic benefits and avoiding potential problems that may arise because of bad formulation or production.

Declaration of Conflicting Interests
The authors report no conflicts of interest in this article.

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