Bioequivalence study of 300 mg irbesartan film-coated tablets preparations in healthy Thai volunteers

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Abstract:

Irbesartan, an angiotensin II receptor antagonist, is indicated for the treatment of essential hypertension alone or in combination with other antihypertensive agents. The purpose of this study was to compare the bioavailability of two formulations of irbesartan 300 mg film-coated tablets administered orally in Thai healthy volunteers. One formulation was a generic product named “irbesartan 300 mg film-coated tablet” which was a representative of a “test drug” in this study. Another was an innovator product of irbesartan 300 mg tablet commercially available in the market which was a representative of a “reference drug”. An open-label, single dose, randomized crossover study was designed and conducted in 26 healthy Thai volunteers. Each subject received both formulations of irbesartan 300 mg tablet with respect to either reference drug or test drug in each period. Blood samples were collected prior to dosing and at various blood collection time points after dosing up to 72 hours and irbesartan concentrations in plasma samples were determined using a validated High Performance Liquid Chromatography (HPLC) method with fluorescence. The pharmacokinetic parameters including Cmax, AUC0-tlast, and AUC0-inf were statistically compared in order to evaluate the bioequivalence of the two formulations. Drug safety and tolerability were assessed. Twenty-six volunteers completed both treatment periods. It was found that 90% confidence intervals for the log-transformed test/reference ratios of irbesartan were 103.71% (90% CI, 94.86-113.39%) for Cmax, 92.91% (90% CI, 86.27-100.08%) for AUC0-tlast, and 92.81% (90% CI, 86.11-100.03%) for AUC0-inf. The results of statistical analysis showed that two formulations were bioequivalent in terms of both rate and extent of absorption according to the guideline of the Food and Drug Administration of Thailand.

Keywords: Irbesartan; Bioequivalence; Pharmacokinetic; HPLC
**Introduction**

Irbesartan, 2-butyl-3-[[29-(1H-tetrazole-5-yl)[1,19-biphenyl]-4-yl[methyl]-1,3-diazaspiro-[4,4]non-1-en-4-one (Figure 1), is an angiotensin II (AII) receptor antagonist indicated for the treatment of essential hypertension alone or in combination with other antihypertensive agents. Clinical trials conducted in hypertensive patients have demonstrated that irbesartan is well tolerated and effective in reducing blood pressure in a dose-dependent manner [1-5].

Irbesartan is an orally active agent that does not require biotransformation into an active form. The oral absorption of Irbesartan is rapid and complete with an average absolute bioavailability of 60-80% with no food effect. Following oral administration, peak plasma concentrations of irbesartan are attained at 1.5-2 hours after dosing. Irbesartan is 90% bound to serum proteins with negligible binding to cellular components of blood. Irbesartan has the longest half-life of all AII receptor antagonists (11-15 h) [6-9]. Irbesartan is primarily metabolized by the cytochrome P450, 2C9 isoenzyme. Its metabolites do not have pharmacological activity [10]. Irbesartan and its metabolites are excreted by both biliary and renal routes. Total plasma and renal clearances are approximately 157 mL/min and 3.0 mL/min, respectively [11].

Although the pharmacokinetic (PK) characteristics of Irbesartan have been studied previously, the studies were not performed in a Thai population. The purpose of this study is to compare the bioavailability of two formulations of irbesartan 300 mg film-coated tablets administered orally in Thai healthy volunteers. The bioequivalence study was already approved by the Food and Drug Administration of Thailand.

**Material and Methods**

**Test and reference drugs**

Irbesartan 300 mg film-coated tablets of batch number IRB 019 with expiration date of 05/2012 were used as the test formulation and Innovator irbesartan 300 mg tablets of batch number 2891 with expiration date of 02/2013 were used as the reference formulation.

**Participants and study design**

The present study was designed as an open-label, single dose, randomized, two-way crossover study with a 7-day washout period between periods 1 and 2. Both formulations of irbesartan 300 mg tablets were administered orally to each subject with one formulation in period 1 and another in period 2 depending on the randomization code of administration of each period. The study protocol, informed consent form, and consent methods were approved by the ethics committee of the Institute for Development of Human Research Protection (IHRP), Thailand. All clinical work has been carried out in compliance with GCP [12] and guidelines according to the ethical standards for studies in humans of the Declaration of Helsinki [13]. The clinical part of the study was conducted at the Clinical Research Department, International Bio Service Co., Ltd., Nakhonpathom, Thailand.

During the study, twenty-six healthy Thai volunteers were confined for both periods 1 and 2. The participants in this study had the age range of 18-55 years (27.08 ± 5.04 years) with BMI range of 18-25 kg/m² (the mean body weight of 59.69 ± 8.60 kg and the mean height of 1.64 ± 0.09 meters). All participants in the study had to be in compliance with the inclusion/exclusion criteria.

![Figure 1 The chemical structure of irbesartan](image-url)
defined in the study protocol and were considered eligible based on the screening process including completion of the informed consent form with each volunteer’s signature, demographic data, medical histories, physical examination, concomitant medication checking, vital signs and blood pressure measurements, clinical laboratory tests with respect to haematology and blood biochemistry. The consumption of alcohol was not permitted at least 14 days prior to the study until the last blood sample collection in each period. In addition, the consumption of grapefruit juice or grapefruit-related citrus fruits (e.g. seville orange, pomelo) was not allowed at least 7 days prior to the study until the last blood sample collection in each period. Subjects were also instructed to abstain from taking any medication for at least 1 week prior to and during the study period. All participants were informed consent prior to the study. For each period, subjects were confined to the Clinical Research Department from at least 10 hours prior to drug administration and were fasted overnight. After drug administration with drinking water of 240 mL, water intake was allowed from 1 hour after dosing onward. Consequently, a controlled meal was served at 4 hours post-dosing and standard meals were given to all volunteers according to the time schedule. Data obtained from subjects who completed the study for both periods were used for pharmacokinetic and statistical analysis.

**Inclusion and exclusion criteria**

The study recruited male or female aged between 18-55 years with body mass index (BMI) range of 18-25 kg/m². The volunteers were determined to be healthy by assessment of physical examination, drug abuse, medical history, and vital signs. The screening tests composed of complete blood count (CBC), fasting blood sugar (FBS), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, blood urea nitrogen (BUN), serum creatinine (Cr), HBsAg test and EKG. The female must be non-pregnant woman (negative pregnancy test) or using appropriate contraceptive method or must not currently breast feeding. In addition, the subjects must be willingly to participate and sign the informed consent form.

Exclusion criteria included history of allergic reaction to irbesartan and/or related molecular structure materials and/or any of the components of the product as well as history of concurrent symptoms of allergy, cardiovascular, liver, kidney, gastro-intestinal, hematological disorders and/or any diseases that may affect the bioavailability of drug. The exclusion criteria also meant regularly alcohol consumption (more than 1 times/week), drug addict, ex-smoker less than 30 days prior to study. They also excluded ones who used any medications within 14 days, used food supplements, vitamins, mineral, herbal remedies and/or contraceptives hormonal within 14 days prior to drug administration. The subjects who participated in other clinical studies within the last 30 days were excluded. In addition, pregnant women (positive pregnancy test) or women in breast feeding period, as well as ones with history of hypotension were also excluded.

**Drug administration and sample collection**

After an overnight fasting of phase I, volunteers were given a single dose of either test or reference formulation in accordance with a web-base randomization scheme with 240 mL of water and a mouth check was performed to ensure consumption of the drug. The volunteers were continuously monitored by the Clinical Research Department staffs throughout the confinement period of the study. Approximately 6 mL of blood sample for irbesartan assay was drawn into heparinized tube through an indwelling cannula before (0 hour baseline) and at 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12, 24, 48 and 72 hours after dosing. Blood samples were centrifuged at 3,000 rpm at 4°C for 10 min. The plasma was separated and kept frozen at -70 ± 5°C until assayed. After a washout period of 7 days the study was repeated in the same manner to complete the cross-over design.

**Safety evaluation**

Adverse events (AEs) were monitored and recorded in case report forms (CRF) based on volunteer interview and physical examination. The safety of patients, adverse events and concomitant drug assessment were performed.
throughout the study. Vital signs were measured at screening and during the entire study period. No abnormalities were observed in terms of blood pressure, heart rate, respiratory rate and body temperature.

All of the 26 healthy adult volunteers enrolled in the study completed the study. No death or serious adverse events occurred during the conduct of the study. Both test and reference formulations were generally well tolerated by the study volunteers. A total of 4 post-dose adverse events were reported by 3 out of 26 volunteers. The adverse events were reported in 2 (7.7%) out of 26 subjects receiving the test formulation compared with 1 (3.85%) out of 26 subjects receiving the reference formulation. The most frequent adverse events reported were nausea and vomiting, dizziness and hypotension. All of the adverse events were assessed to be mild in intensity and most of them were possibly related to the study drugs.

Chromatographic condition

The analytical method of irbesartan in human plasma was applied from previous study with some modification by the analytical investigator [14]. The analysis of irbesartan was successful by using validated HPLC with fluorescence measurement at 259 nm (excitation) and 385 nm (emission). Chromatograms were obtained on a Zorbax Eclipse XDB C18 column (1.6 × 150 mm, 5 micron) with the column temperature of 25°C. The mobile phase composed of 62% of acetic acid (0.2% v/v) containing 0.06% triethylamine (v/v) and 38% of acetonitrile delivered at the flow rate 1 mL/min with isocratic elution system. The injection volume was 10 microliters with the total run time was 12 minutes. Retention time of Irbesartan and internal standard were shown at 8.9-9.9 minutes and at 4.4-5.4 minutes, respectively.

Plasma sample preparation

Extraction of irbesartan and losartan (internal standard, I.S.) from human plasma was performed by employing optimized protein precipitation using acetonitrile plus saturated NaCl [14, 15]. Briefly, 180 microliters of human blank plasma were added into a microcentrifuge tube containing either 20 microliters of irbesartan calibration standards at concentration of 20-8,000 ng/mL or QC samples at 60 ng/mL (LQC), 4,000 ng/mL (MQC) and 6,000 ng/mL (HQC) and 100 µL internal standard (150,000 ng/mL losartan). The plasma protein was precipitated with 500 microliters of acetonitrile plus 200 microliters of saturated NaCl and then vortex-mixed for 30 seconds. After centrifugation at 13,000 rpm for 12 minutes, 200 microliters of upper phase were transferred to insert-HPLC vial. The 10 microliters of the resulting solution was injected into the HPLC system.

Calculation of pharmacokinetic parameter and statistical analysis

Irbesartan bioequivalence between the two treatments were compared with respect to $AUC_{0-\infty}$, $AUC_{0-t_{\text{last}}}$, $C_{\text{max}}$, $T_{\text{max}}$, $t_{1/2}$ and $\lambda_z$. The definition of each is shown below:

1. $AUC_{0-t_{\text{last}}}$ is the areas under the plasma concentration-time curves ($AUC$) from 0 to the last quantifiable concentration. The $AUC_{0-t_{\text{last}}}$ is calculated by taking the average of two subsequent plasma concentrations ($C_i$ and $C_{i-1}$) and multiplying that average by the time difference between the consecutive measuring points ($t_i$ and $t_{i-1}$). All these outcomes are then summed to render the $AUC$ from 0 to the last quantifiable concentration. This approach is called the linear-log trapezoidal approach. The formulation is:

$$AUC_{0-t_{\text{last}}} = \sum_{i=1}^{t} \left( \frac{C_i + C_{i-1}}{\ln(C_i/C_{i-1})} \right) (t_i - t_{i-1}) \quad (1)$$

2. $\lambda_z$ is the terminal rate constant or the slope of the regression line. To obtain $\lambda_z$ the log (ln) transformation is applied to make it possible to draw a straight line through the elimination phase.

3. $AUC_{0-\infty}$ is an extrapolation of $AUC_{0-t_{\text{last}}}$ to extend the plasma concentration-time profile to infinity. This parameter is an estimate of the total mass of...
drug present in the blood. To obtain $AUC_{0-\infty}$, $\lambda_z$ is of the most logical parameter. The formulation is:

$$AUC_{0-\infty} = AUC_{0-t_{last}} + \frac{C_{last}}{\lambda_z} \tag{2}$$

4. $C_{\text{max}}$ is the maximum observed plasma concentration that is presented on the curves.

5. $T_{\text{max}}$ is the time taken to achieve maximum concentration that can be detected directly from the curves.

6. $t_{1/2}$ is the plasma concentration half-life. The $t_{1/2}$ can be calculated by simply divided 0.693 by the $\lambda_z$.

The term 0.693 is derived from $\ln(2) = 0.693$. The formulation is:

$$t_{1/2} = \frac{0.693}{\lambda_z} \tag{3}$$

For the purpose of bioequivalence analysis, $AUC_{0-t_{last}}$, $AUC_{0-\infty}$, and $C_{\text{max}}$ were considered as the primary variables. The difference between two related parameters was considered statistically significant for $p$-value equal to or less than 0.05. The 90% confidence intervals (CI) for the ratios of geometric mean of Test to Reference (T/R) for $AUC$ and $C_{\text{max}}$ were calculated based on least squares means from the ANOVA of log-transformed data.

**Results**

Irbesartan in human plasma was successfully determined by a validated HPLC using the standard calibration curve in the range of 20-8,000 ng/mL. The mean plasma concentration-time profile over the whole 72-hour pharmacokinetic study is illustrated in Figure 2. The pharmacokinetic parameters were calculated by noncompartmental methods using WinNonlin® software version 6.1.0.173 (Pharsight®, North Carolina, USA) as shown in Table 1. The pharmacokinetic parameters are subjected to a comparative statistical evaluation by determining the position of the 90% confidence intervals for the individual ratios “test/reference” by least square means of ANOVA of logarithmically transformed data for $C_{\text{max}}$, $AUC_{0-t_{last}}$, and $AUC_{0-\infty}$ to obtain the residual error. The statistical evaluation of primary pharmacokinetic parameters for irbesartan after administration of test and reference formulations is presented in Table 2.

**Table 1** Pharmacokinetic parameters of irbesartan following administration of test and reference formulations from 26 healthy Thai volunteers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Test (Mean ± SD)</th>
<th>Reference (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>3324.93 ± 1288.19</td>
<td>3300.21 ± 1659.86</td>
</tr>
<tr>
<td>$AUC_{0-t_{last}}$ (ng.h/mL)</td>
<td>14047.88 ± 6473.19</td>
<td>15264.56 ± 7482.53</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (ng.h/mL)</td>
<td>14993.47 ± 6562.97</td>
<td>16290.89 ± 7587.70</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>1.77 ± 0.91</td>
<td>1.40 ± 0.97</td>
</tr>
<tr>
<td>$t_{1/2}$ (h) (median)</td>
<td>8.38 ± 4.39 (2.00)</td>
<td>7.60 ± 3.60 (1.00)</td>
</tr>
<tr>
<td>$K_{el}$ (h⁻¹)</td>
<td>0.1015 ± 0.0426</td>
<td>0.1125 ± 0.0510</td>
</tr>
</tbody>
</table>

**Table 2** Statistical evaluation of primary pharmacokinetic parameters for irbesartan following administration of test and reference formulations from 26 healthy Thai volunteers

<table>
<thead>
<tr>
<th>Parametric analysis</th>
<th>LSM ratio (%)</th>
<th>90% CIs</th>
<th>Power (%)</th>
<th>Intra-subject CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>103.71</td>
<td>94.86-113.39</td>
<td>99.16</td>
<td>18.96</td>
</tr>
<tr>
<td>$AUC_{0-t_{last}}$ (ng.h/mL)</td>
<td>92.91</td>
<td>86.27-100.08</td>
<td>99.89</td>
<td>15.74</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (ng.h/mL)</td>
<td>92.81</td>
<td>86.11-100.03</td>
<td>99.88</td>
<td>15.88</td>
</tr>
</tbody>
</table>
The analytical method of Irbesartan with the use of losartan as an internal standard was successfully developed and validated according to the acceptance criteria of the guideline on bioanalytical method validation issued by EMEA. In this method, the plasma concentrations of irbesartan were determined by using a validated High Performance Liquid Chromatography (HPLC) method with fluorescence measurement at 259 nm (excitation) and 385 nm (emission) with an accuracy range of 85-115% and a precision range of not more than ±15% of coefficient of variation (CV). This method was found to be selective for irbesartan analysis with no interfering peaks in blank plasma samples with the limit of quantification of 20 ng/mL of irbesartan. The calibration curve obtained from irbesartan standard concentration range of 20-8,000 ng/mL was constructed using 1/concentration as a weighting factor for regression analysis. The stock solution stability was evaluated to ensure the stability of the standard stock solution used and it was found to be 6 hours at room temperature and 88 days at -20°C with %variations of -2.41% and 0.94% at room temperature and -0.65% and -0.67% at -20°C for irbesartan and losartan, respectively. In addition, the dilution process was occurred.
during the analysis of plasma samples; therefore, the dilution integrity was performed to ensure the integrity of the dilution and the results obtained were found to be 101.16% and 97.71% for two-fold and four-fold dilution, respectively. The average %recovery of irbesartan and losartan were found to be 96.47% and 94.20%, respectively. The plasma samples were well-tolerated to 3 cycles of freeze-thaw with the changes of -0.76% and 0.41% for low and high concentration, respectively. The short-term stability of plasma samples was found to be 8 hours at room temperature with %change of -4.07% and 7.67% for low and high concentration, respectively. The long-term stability was found to be 88 days with %changes of -8.37% and -11.49% for low and high concentration, respectively. During the analysis, samples were stayed in autosampler for some period of time; hence, the autosampler stability was also performed and the results indicated that these samples were stable for 59 hours in autosampler at room temperature with %changes of -3.05% and 0.51% for low and high concentration, respectively.

The statistical analysis obtained from this study showed that the points estimated with 90% confidence intervals of the geometric mean ratios of test and reference (T/R) of $C_{\text{max}}$, $\text{AUC}_{0-\text{tlast}}$, and $\text{AUC}_{0-\infty}$ were entirely within the equivalence criteria of 80.00-125.00%. As can be seen in Table 1, $C_{\text{max}}$ for the test and reference formulations were 3,324.93 and 3,300.21 ng/mL with standard deviations of $\pm$ 1,288.19 and $\pm$ 1,659.86 ng/mL, respectively. The means of $\text{AUC}_{0-\text{tlast}}$ for the test and reference formulations were 14,047.88 and 15,264.56 ng⋅h/mL with standard deviations of $\pm$ 6,473.19 and $\pm$ 7,482.53 ng⋅h/mL, respectively while those of $\text{AUC}_{0-\infty}$ for the test and reference formulations were 14,993.47 and 16,290.89 ng⋅h/mL with standard deviations of $\pm$ 6,562.97 and $\pm$ 7,587.70 ng⋅h/mL, respectively. The point estimate with 90% confidence intervals of the geometric mean ratios of test and reference (T/R) in the study were found to be 103.71% (94.86%-113.39%) for $C_{\text{max}}$ with the power of 99.16%, 92.91% (86.27%-100.08%) for $\text{AUC}_{0-\text{tlast}}$ with the power of 99.89% and 92.81% (86.11%-100.03%) for $\text{AUC}_{0-\infty}$ with the power of 99.88%. From plasma samples obtained from 26 subjects, irbesartan showed average maximum concentration ($T_{\text{max}}$) at 1.77 ± 0.91 and 1.40 ± 0.97 hours for test and reference drug, respectively. The median drug plasma concentration half-life ($t_{1/2}$) of the test drug was found to be 8.38 ± 4.39 hours with average terminal rate constant of 0.1015 ± 0.0426 hour⁻¹ while that of the reference drug was 7.60 ± 3.60 hours with average terminal rate constant of 0.1125 ± 0.0510 hour⁻¹.

Discussion

Two drug products are considered to be bioequivalent if they exhibit a comparable rate and extent of absorption when administered in the same molar dose and under similar experimental conditions. Bioequivalent formulations are usually considered to be therapeutically equivalent. AUC is accepted as a good indicator of the extent of absorption, whereas $C_{\text{max}}$ and $T_{\text{max}}$ are considered estimators of the rate of absorption. US FDA generally accepts that the AUC and $C_{\text{max}}$ of a test formulation should lie within 20% deviation of the reference formulation, so that the ratio of AUC and $C_{\text{max}}$ should be between 0.8000 and 1.2500 for logarithm-transformed data.

The single dose irbesartan $C_{\text{max}}$ in the present study was consistent with the previous published data [7, 16]. For all volunteers, the average $\text{AUC}_{0-\text{tlast}}$ was a good representatives of the extent of absorption since the average %AUC_{0-72} obtained were found to be greater than 80% of the average %AUC_{0-\infty} for both test and reference formulations. The $C_{\text{max}}$ was not observed in the first sampling time, therefore, the $C_{\text{max}}$ is considered to be estimated correctly due to the adequate frequency of the sampling around $T_{\text{max}}$. The 90% confidence interval of the logarithmic transformed of $C_{\text{max}}$, $\text{AUC}_{0-\text{tlast}}$ and $\text{AUC}_{0-\infty}$ were within 80.00-125.00% with the power of more than 80% indicating that the study was designed with sufficient population to acquire the reliable data. Based on the pharmacokinetic parameters of irbesartan, the test and reference formulations are considered bioequivalent with respect to the extent and rate of absorption. In addition, the $T_{\text{max}}$ and $t_{1/2}$ values obtained from this study were well in line with those previously published [11-18].
Conclusion

The statistical analysis obtained from the study showed that the points estimated with 90% confidence intervals of the geometric mean ratios of test and reference (T/R) of C\text{max}, AUC\text{0-tlast} and AUC\text{0-inf} were entirely within the equivalence criteria (80.00-125.00%). The study was designed properly to have sufficient blood collection time point to obtain AUC\text{0-tlast} more than 80% of AUC\text{inf} with enough number of subjects to acquire the power of more than 80% for critical pharmacokinetic parameters such as C\text{max}, AUC\text{0-tlast} and AUC\text{0-inf}.

In summary, it can be concluded that these two irbesartan tablet formulations established bioequivalence in terms of rate and extent of absorption.

References


