Comparative Bioavailability Study of Two Imidapril Tablet Formulations in Indonesian Healthy Subjects

Yahdiana Harahap1,2, Vincentia Prasetyo1, Monika Sandra3, Tri Rahayu4, Windy Lusthom5, Budi Prasaja6

1Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia
2Indonesia Defense University, Sentul, Bogor, Indonesia
3PT. Novell Pharmaceutical Laboratories, Jakarta, Indonesia
4PT. Clinisindo Laboratories, Jakarta, Indonesia

ABSTRACT
This study aimed to compare the bioavailability of two 10-mg imidapril HCl tablet formulations using TENSIMID® as the test formulation and TANAPRESS® as the reference formulation. Twenty-seven healthy subjects completed a single-dosed, open-label, randomized, two-way crossover bioequivalence study under overnight fasting condition with one week wash-out period. The blood samples were collected from the subjects prior to administration and up to 12 hours after dosing. Plasma concentrations of imidapril were determined using LC-MS/MS method with TurboIon Spray mode. Pharmacokinetic parameters of AUC0-t, AUC∞, and Cmax were tested for bioequivalence after log-transformation of data and ratios of tmax was evaluated non-parametrically. The estimated points and 90% confidence interval (CI) for AUC0-t, AUC∞, and Cmax of imidapril were 93.04% (82.84-104.67%), 93.12% (82.84-104.67%), and 94.00% (80.96-109.14%), respectively. There was no statistically significant difference of tmax and Cmax detected in both formulations (p>0.05). The result indicated that the two formulations of imidapril were bioequivalent and thus may be prescribed interchangeably.

INTRODUCTION
Treatment of hypertension has been associated with reductions in the incidence of stroke and coronary heart disease, risk of major cardiovascular event, cardiovascular death, and total mortality which are apparently dependent on the magnitude of blood pressure (BP) reduction, particularly systolic BP reduction [1, 2, 3, 4]. For many years, a variety of angiotensin-converting enzyme (ACE) inhibitors have been effective in reducing BP by inhibiting the renin-angiotensin-aldosterone system [5].

Imidapril is indicated for the treatment of essential hypertension as well as for the treatment of renal parenchymal hypertension, diabetic nephropathy associated with type 1 diabetes mellitus and/or chronic heart failure in some countries[7]. As a prodrug, imidapril is converted by hydrolysis in the liver into its active metabolite imidaprilat. Imidaprilat competitively binds to and inhibits ACE, thereby blocking the conversion of angiotensin I to angiotensin II. The potent vasoconstrictive actions of angiotensin II are subsequently prevented and results in vasodilation. The secretion of angiotensin II-induced aldosterone by adrenal cortex is also decreased by Imidaprilat leading to an increase in sodium excretion and subsequently to an increase in water outflow. Imidapril does not affect heart rate and contractility [6].

The most commonly reported adverse events during clinical trials and post-marketing surveillance were cough, hypotension, dizziness, and pharyngeal discomfort [5]. The persistent dry cough which is a common adverse event associated with the use of ACE inhibitor drugs is sometimes severe enough to require drug withdrawal. However, among other ACE inhibitors, imidapril is generally well tolerated, with a lower incidence of dry
cough compared to enalapril or benazepril. In patients
whom treatment with an ACE inhibitor, imidapril is
considered as a first choice option in the treatment of
mild to moderate essential hypertension based on its
efficacy and tolerability[5]. In the essential hypertension
treatment, once-daily oral imidapril is initiated at
5mg/day. The dosage may be titrated to 10 mg/day or 20
mg/day in some cases depending on efficacy. Dosage
adjustment is needed in elderly patients, those with
impaired renal or hepatic function, those at an increased
risk of first-dose hypotension, and in patients with renal
parenchymal hypertension[7]. Like other ACE inhibitor
drugs, imidapril may inhibit aldosterone production and
decrease secretion of potassium. Therefore, concomitant
use of any medications that may increase the potassium
levels, like potassium sparing diuretics or potassium
supplements, is not recommended. Co-administration
imidapril with lithium preparations may increase
reabsorption of lithium in the renal tubules and
subsequently may become toxic[5].

The absorption of imidapril is significantly reduced when
it is administered with high-fat meal. Imidapril is
recommended to be taken ≈15 minutes before meal at
about the same time of a day[5]. After single oral dose
administration of imidapril 2.5-20 mg, Imidapril and
imidaprilat reach its peak plasma concentrations (C\text{\text{max}}) of
5.8-54.9 ng/mL and 1.2-20.8 ng/mL after median time
(t\text{\text{max}}) of 2 hours and 5 hours, respectively[5,7]. The
respective areas under the imidapril and imidaprilat
plasma concentration-time curve from 0 to 24
hours (AUC0-24h) were 30.2-238.2 ng/h/mL and 18-304.1
ng/h/mL, respectively. It increased linearly in a dose-
related manner[7]. The plasma protein binding of
imidapril and imidaprilat is 85% and 53%, respectively.

Imidapril is absorbed from the gastrointestinal tract in
proportionally to dose and is rapidly converted to the
active metabolite, imidaprilat. It is also metabolized into
three inactive metabolites in the liver. Single dose of
imidapril is primarily eliminated through excretion in the
urine and faeces with terminal elimination half-life of
imidaprilat is ≈24 hours. The absolute bioavailability of
imidapril is ≈42%[7].

The present study was conducted to investigate the
pharmacokinetic and bioavailability of two imidapril
tablet formulations in order to prove the bioequivalence
between both formulations.

MATERIALS AND METHODS
Subjects and Study Design
A single dose, open-label, randomized, two-way
crossover study with an overnight fasting and one-week
wash-out period was conducted in compliance with the
ethical principles of the Declaration of Helsinki for
biomedical research involving human volunteers and
Good Clinical Practice (GCP). The study protocol was
reviewed by the Committee of the Faculty of Medicine,
Ethics of the Faculty of Medicine, University of Indonesia
(Jakarta, Indonesia) and was approved by National
Agency of Drug and Food Control (Jakarta, Indonesia).
All participants signed a written informed consent after they
had been informed of the nature and details of the study
in accordance with Indonesian Guidelines for
Bioequivalence Studies[8]. Twenty-seven selected Indonesian subjects (16 males, 11
females) participated in this study. Demographic of the
subjects are shown in Table 1. Demographic data for
imidapril bioequivalence study in 27 volunteers. Subjects
were selected after passing clinical screening procedures
which included physical examination, hematology test
(hemoglobin, hematocrit, erythrocyte, leukocyte, mean
corporuscular (MC) values, leukocyte differential, platelets
count, and Erythrocyte Sedimentation Rate(ESR)),
laboratory test (serum Glutamic Oxaloacetic
Transaminase (SGOT), serum Glutamic Pyruvic
Transaminase (GPT), alkaline phosphatase, total
bilirubin, total protein, albumin, globulin, fasting glucose,
total cholesterol, blood urea nitrogen, ureum, and
creatinine), urine analysis, serological test (Hepatitis B
antigen (HBsAg), Hepatitis C (anti HCV), and HIV (anti-
HIV)), drug abuse test, and additional pregnancy test for
female subjects. Subjects were excluded if they were
smoker, pregnant, had history or condition of hepatic,
cardiovacular, cerebrovascular, renal, or gastrointestinal
disease, potentially hypersensitive to Imidapril, had
history of drug abuse, and donated or lost more than 300
mL of blood within 3 months prior to screening of the
study. All subjects were required not to take any drugs for
at least two weeks prior to the study until completion of
the study. They also refrained from consuming alcohol,
caffeine, chocolate, tea or coke-containing beverages at
least 48 h before each dosing and until the last blood
sampling. Subjects were randomized to one of the two
sequences to receive the formulations according to the
randomization scheme. The test formulation was
TENSIMID® (10-mg Imidapril HCl tablet) manufactured by
PT. Novell Pharmaceutical Laboratories (Jakarta,
Indonesia) and the reference formulation was
TANAPRESS® (10-mg Imidapril HCl tablet)
manufactured by PT. Tanabe Indonesia (under license from
Mitsubishi Tanabe Pharma Corporation, Japan).

Subjects were confined to clinical unit of Clínisindo
Laboratories for one night prior to the study to assure the
fasting condition (10 h before drug administration)
day of the study, and 24 h after dosing. On the study day,
subjects were given one tablet of either product with 240
mL of water. No food was allowed until 4 h after dose
administration. Water intake was allowed 1 h before and
after dosing. Standard meals were served at 4 h
(+1163.04kcal) and 11 h (+1226.07kcal), snacks were
served at 8 h (+290.83kcal) after drug administration.
Total calories were calculated by a nutritionist. Strenuous
exercise was not allowed during the sampling days. Blood
pressure, heart rate, body temperature and adverse
events were monitored during blood sampling.
Approximately 5 mL blood samples were collected prior
to dosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8,
10 and 12 h after drug administration in the Li-Heparin
collection tubes. The blood plasma was separated and
kept frozen at -20°C until analysis. After one-week wash-
out period, subjects returned to Clínisindo Laboratories
and the study was repeated in the same manner to
complete the crossover design.

| Table 1. Demographic data for Imidapril bioequivalence study in 27 volunteers. |
|----------------|--------------------------|-----------------|
|                | Mean (±SD) | Range |
| Age (years)   | 34.9 ± 9.0 | 19-51 |
| Weight (kg)   | 59.6 ± 8.6 | 45-70 |
| Height (cm)   | 162.3 ± 8.4 | 148-181 |
| BMI (kg/m²)   | 22.5 ± 2.0 | 18.0-24.9 |
Analytical Method
The plasma concentration of imidapril was determined using LC-MS/MS method with Turbolon Spray mode. Perindopril (CAS 107133-36-8, MW 368.468) was used as the internal standard. The assay method has been already validated in terms of selectivity, sensitivity, linearity, precision and accuracy, recovery, stability, matrix effect, and carry-over; and has been also verified before being used in the study according to Guideline on Bioanalytical Method Validation, European Medicines Agency 2011[9]. Briefly, the plasma samples (300 µL) were added with the internal standard. After mixing, 600 µL of methanol was added. Subsequently, the mixture was vortex-mixed for 30 seconds and centrifuged at 3000 rpm for 10 minutes. The supernatant (200 µL) was transferred into vial and added with 800 µL of methanol-water (1:1) and vortex-mixed for 5 seconds. The aliquot (5 µL) was injected into the LC MS/MS system.

The analytical separation was performed on a Synergi™ 4µm, Polar-RP-80Å, 50 x 2.0 mm, 4 µm column (Phenomenex®, Torrance, CA, USA) preceded by a AQ C18, 4 x 2.0 mm guard column (Phenomenex®, Torrance, CA, USA). Mobile phase was 0.1% formic acid in water and 0.1% formic acid in acetonitrile set as gradient. Flow rate used was 0.6 mL/min. Column temperature was maintained at 40°C. Multiple Reaction Monitoring (MRM) in positive ion mode was used to monitor transitions at m/z 406.140 → 234.100 and m/z 369.172 → 172.100 for Imidapril and the IS, respectively.

Safety Evaluation
Analysis of safety-related data was considered using common adverse events, which occurred after initiation of the study and supported by detailed tabulation and analysis.

Pharmacokinetic and Statistical Analysis
The bioequivalence evaluation was determined using parameters of AUC0-T, AUC0-∞, and Cmax. The AUC0-∞ and Cmax were calculated using the trapezoidal rule as the extent of absorption. The AUC0-∞ values were calculated by adding the quotient of C0 (estimated last plasma concentration) and the appropriate Kel (elimination rate constant) to the corresponding AUC0-T. The elimination half-life (t1/2) was calculated by using the equation of t1/2 = (ln2)/Kel. 90% Confidence Interval (CI) of the geometric mean ratio Test/Reference (T/R) for AUC0-T, AUC0-∞, and Cmax were calculated assuming a multiplicative model and Analysis of Variance (ANOVA) was applied using the respective logarithmic-transformed data. The accepted bioequivalence range for the parameters was 80.00-125.00%[8, 10]. For t1/2, data would be analyzed for normality of data distribution prior to statistical calculation. t1/2 would be calculated without logarithmic-transformed data using paired t-test (parametric method) if it was normally distributed or using Wilcoxon Signed rank test (non-parametric method) if it was not normally distributed. T max was analyzed by non-parametric method using Wilcoxon signed rank test without logarithmic transformation. Statistical analysis was performed using EquivTest® version 2.0 (Statistical Solution, Cork, Ireland) and SAS® version 9.1 (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION
Bioanalytical Method Validation
The best linear fit and least-squares residual for the calibration curve were achieved with 1/x weighing factor. The peak-area ratios between imidapril and the internal standard were linear over the within the concentration range of 0.2002-80.08 ng/mL and was created at the following concentration of 0.2002, 0.4004, 1.001, 2.002, 5.005, 10.01, 20.02, 40.04, and 80.08 ng/mL. The limit of quantification (LOQ) of imidapril was established at 0.2002 ng/mL and the precision and accuracy at the LOQ were 11.38% and (~17.49)-11.49% respectively. Table 2. Precision and accuracy of the method. summarizes the precision and accuracy of the quality control samples during pre-study validation.

### Table 2. Precision and accuracy of the method.

<table>
<thead>
<tr>
<th>Concentrations (ng/mL)</th>
<th>Intra-batch CV</th>
<th>Inter-batch CV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (ng/mL)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>0.60</td>
<td>0.59 ± 0.05</td>
<td>8.40</td>
</tr>
<tr>
<td>32.06</td>
<td>31.20 ± 0.72</td>
<td>2.30</td>
</tr>
<tr>
<td>64.13</td>
<td>60.87 ± 3.31</td>
<td>5.44</td>
</tr>
</tbody>
</table>

The mean recoveries of imidapril at low, medium, and high concentration were 92.40%, 94.42%, and 93.45%, respectively. The mean recovery of the IS was 99.87%. Matrix effect of human plasma on ionization efficiency was assessed by comparing the peak response of 6 determinations in low and high concentrations spiked in extracted drug-free human plasma samples with that of neat standards at corresponding concentration. The same evaluation was also performed for the IS. The coefficient variation (CV) of the IS-normalized matrix factor calculated from the 6 lots of matrix of low and high concentrations were 8.84% and 3.97%, respectively. Percent interference in the blank sample was found not more than 20% and 5% for imidapril and the IS, respectively, indicating that there was no carry-over effect during validation. The stability study showed that imidapril in human plasma was stable at room temperature for 6 hours, at -20°C for 61 days, and after 3 freeze-thaw cycles. The autosampler stability showed that imidapril was stable for 26 hours.

Clinical Observation
Both formulations of imidapril were well tolerated at the administered dose and no significant adverse clinical events were observed. All subjects experienced in total of 31 adverse events during the study. All the adverse events were mild, and the subjects recovered without using any medications. There were no serious adverse
events. The disposition of adverse events is shown in Table 3. Disposition of adverse events.

Table 3. Disposition of adverse events

<table>
<thead>
<tr>
<th>Casual relation to study drug</th>
<th>Events</th>
<th>Test Formulation</th>
<th>Reference Formulation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Related</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somnolence</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Hypotension</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Probable</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bradycardia</td>
<td>7</td>
<td>4</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Unrelated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Bradycardia</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>16</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

Pharmacokinetic and Statistical Evaluation
A total of twenty-eight subjects were enrolled in the study. One subject withdrew the consent before period I due to personal reasons. Thus, a total of twenty-seven subjects were available for pharmacokinetic evaluation. The imidapril mean plasma concentration-time profiles are presented in Figure 2. Mean plasma concentration-time profiles of imidapril after a single dose of 10-mg imidapril tablet formulations in 27 subjects.

Figure 2. Mean plasma concentration-time profiles of imidapril after a single dose of 10-mg imidapril tablet formulations in 27 subjects.

Descriptive statistics of the pharmacokinetic parameters of imidapril test and reference formulations are summarized in Table 4. Pharmacokinetic parameters of imidapril after administration of two tablet formulations in 27 subjects, where geometric and range values for the parameters of AUC_{0-12h}, AUC_{0-∞}, C_{max} and t_{1/2} and median and range value for the parameter of t_{max} obtained for both formulations are presented.

Table 4. Pharmacokinetic parameters of imidapril after administration of two tablet formulations in 27 subjects.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Test Formulation</th>
<th>Reference Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{0-12h} (ng×h/mL)</td>
<td>156.23 ± 59.11</td>
<td>58.36-293.24</td>
</tr>
<tr>
<td>AUC_{0-∞} (ng×h/mL)</td>
<td>157.81 ± 59.32</td>
<td>58.90-294.27</td>
</tr>
<tr>
<td>C_{max} (ng/mL)</td>
<td>44.31 ± 21.04</td>
<td>12.20-102.77</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>1.66 ± 0.32</td>
<td>1.22-2.58</td>
</tr>
<tr>
<td>t_{max} (h)</td>
<td>2.00 ± 0.51</td>
<td>1.00-3.00</td>
</tr>
</tbody>
</table>

The obtained mean values for test and reference formulations were 156.23 ng×h/mL and 169.61 ng×h/mL for AUC_{0-12h}, 157.81 ng×h/mL and 171.20 ng×h/mL for AUC_{0-∞}, 44.31 ng/mL and 48.08 ng/mL for C_{max} and 1.66 hours and 1.60 hours for t_{1/2}, respectively. The t_{max} median value for both formulations was 2 hours.

In this study, AUC_{0-12h}, AUC_{0-∞} and C_{max} were defined as the primary parameters in order to assess bioequivalence conclusion between both formulations. The results of bioequivalence statistical analysis of imidapril are presented in Table 5. Statistical evaluation and intra-subject CV of imidapril. The 90% CI for the ratio of T/R ranged from 83.63-104.76% with 93.04%-point estimate for AUC_{0-12h}, 82.84-104.67% with 93.12%-point estimate for AUC_{0-∞} and 80.96-109.14% with 94.00-point estimate for C_{max}. The result of the statistical evaluation for the 90% CI of the primary parameters were all within the bioequivalence acceptance limit of 80-125%.
Additionally, for both $t_{\text{max}}$ and $t_{1/2}$, as the secondary pharmacokinetic parameters, there were not statistically differences detected between test and reference formulations (p<0.05) as calculated using Wilcoxon signed rank test.

Table 5. Statistical evaluation and intra-subject CV of imidapril.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AU C₀–12h (%)</th>
<th>AU C₀–∞ (%)</th>
<th>Cₘₐₓ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio (T/R point estimate)</td>
<td>93.04</td>
<td>93.12</td>
<td>94.00</td>
</tr>
<tr>
<td>90% geometric CI</td>
<td>83.63-104.76</td>
<td>82.84-104.67</td>
<td>80.96-109.14</td>
</tr>
<tr>
<td>Intra-subject CV</td>
<td>25.50</td>
<td>25.14</td>
<td>32.09</td>
</tr>
</tbody>
</table>

The mean ratio of AU C₀–12h/AU C₀–∞ for all individuals on test and reference formulations were around 98%, indicating that the sampling time was adequate since the extrapolated portion of the total AUC was less than 20%. The intra-subject CV of imidapril for pharmacokinetic parameters of AU C₀–12h, AU C₀–∞, and Cₘₐₓ as determined by ANOVA were 25.50%, 25.14% and 32.09%, respectively. Considering on the intra-subject CV of AU C₀–12h parameter which was 25.05%, the sample size in this study (n=27) was sufficient in order to conclude bioequivalence with nominal power of 80% at significance level of 5%[11, 12, 13].

CONCLUSION
In conclusion, the two imidapril formulations were equivalent with respect to the rate and extent of absorption and it can be assumed to be therapeutically equivalent and exchangeable in clinical practice.

FUNDING
This study was supported by PT. Novell Pharmaceutical Laboratories, Jakarta, Indonesia.

CONFLICT OF INTEREST
The authors have declared that no competing interests exist.

REFERENCES