Bioequivalence study of two rosuvastatin tablet formulations in healthy Indonesian subjects

Yahdiana Harahap1*, Budi Prasaja2, Fahmi Azmi2, Windy Lusthom2, Theresia Sinandang2, Vita Felicia2, Lia Yumi Yusvita2, and Lianna Y. Panjaitan2

1Faculty of Pharmacy, Universitas Indonesia, Depok, and 2PT. Clinisindo Laboratories, Jakarta, Indonesia

Abstract. Aim: To compare the bioavailability of two 40-mg Rosuvastatin tablet formulations. Methods: 24 subjects were included in this single-dose, open-label, randomized, two-way crossover study following an overnight fast. A 2-week wash out period was applied. Blood samples were drawn up to 72 hours following drug administrations. Rosuvastatin plasma concentrations were determined by liquid chromatography-tandem mass spectrometry method with TurboIonSpray mode. Pharmacokinetic parameters AUC0–t, AUC0–∞, and Cmax were determined and used for bioequivalence evaluation after log-transformation, whereas tmax ratios were evaluated nonparametrically. Results: The estimated point and 90% confidence intervals (CI) for AUC0–t, AUC0–∞, and Cmax for rosuvastatin were 95.21% (87.56 – 103.53%), 95.76% (88.01 – 104.18%), and 99.33% (89.37 – 110.41%), respectively. Conclusion: These results indicated that the two formulations of rosuvastatin were bioequivalent; therefore, they may be prescribed interchangeably.

Introduction

Rosuvastatin is a synthetic HMG-CoA reductase inhibitor. More specifically, it is produced as monocalcium bis(+)-7-(4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methansulfonylaminopyrimidine)-5-yl)-(3R,5S)-dihydroxy-(E)-6-heptenoate (calcium salt of the active hydroxy acid). Rosuvastatin has been shown to be remarkably efficacious in improving serum lipid profile and in achieving the low-density lipoprotein-cholesterol (LDL-C) treatment goals, and its safety profile is comparable with that of other statins. Rosuvastatin also improves triglyceride, LDL-C, and high-density lipoprotein cholesterol (HDL-C) levels to produce a more favorable lipid profile [1, 2].

The absolute oral bioavailability of rosuvastatin was ~ 20%, and the hepatic extraction ratio was estimated to be 0.63. The peak plasma concentration (Cmax) was 10.3 ng/mL and occurred at 5 hours (tmax) after a single oral 40-mg dose [2, 3]. At steady state, the mean volume of distribution for rosuvastatin was 134 liters. Rosuvastatin is tightly bound in a reversible manner to plasma proteins (88%). Renal clearance accounted for ~ 28% of total plasma clearance (48.9 L/h). Recovery of rosuvastatin is primarily via the fecal route of elimination (90%) compared to renal excretion (10%). Approximately 72% of absorbed rosuvastatin is eliminated via bile secretion and 28% via renal excretion. The circulating plasma half-life of rosuvastatin is ~ 20 hours [2, 3, 4, 5]. The population pharmacokinetic study revealed that plasma exposure to rosuvastatin was significantly higher in Asian subjects than in Caucasian subjects living in the same environment [6].

Overall, the statin drug class is very safe. In a review of rosuvastatin’s pooled safety data, the most frequently reported adverse events (incidence > 5%) during rosuvastatin therapy were pharyngitis (12.2%), pain (6.7%), headache (6.6%), flu syndrome (5.3%), and myalgia (5.1%) [2].

In Indonesia, it is compulsory to show bioequivalence with an authorized originator product to ensure efficacy and safety. This study was performed to investigate the pharmacokinetics and bioavailability of two rosuvastatin tablet formulations in order to prove bioequivalence between the two formulations.
Materials and methods

Chemical and reagents

Rosuvastatin was synthesized by Synfine Research (Ontario, Canada). Irbesartan was obtained from Zhuhai Sanxin Fine Chemical Co, Ltd. (Guangdong, China) and was used as an internal standard (IS). Acetonitrile (HPLC grade), diethyl ether, and dichloromethane were purchased from PT. Merck (Jakarta, Indonesia).

Subjects and study design

A single-dose, open-label, randomized, two-way crossover study with an overnight fast and 2-week wash out period was conducted in compliance with the ethical principles of the Declaration of Helsinki for biomedical research involving human subjects and Good Clinical Practice (GCP) [7]. The study protocol was reviewed by the Committee of The Medical Research Ethics of the Faculty of Medicine, University of Indonesia (Jakarta, Indonesia) and was approved by the 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.

24 Indonesian subjects (18 males and 6 females) were selected among Indonesian and participated in this study. Subjects were selected after passing a clinical screening procedure, which included physical examination, ECG, and clinical laboratory tests: hemoglobin, hematocrit, WBC, platelets, WBC differential, blood urea nitrogen, sGPT, sGOT, alkaline phosphatase, total bilirubin, total protein, fasting glucose, albumin, total cholesterol, creatinine, urine analysis, pregnancy test (for female subjects), and negative results of HBsAg, anti-HBC and anti-HIV. Subjects were excluded if they showed any disorder of the hepatic, renal, or cardiovascular system; consumed alcohol or other medications for a long period of time; were hypersensitivity to statins; received any investigated drugs within 4 weeks (or suitable longer period for slowly eliminated drugs) of enrollment, or donated or lost > 450 mL of blood within 3 months prior to study screening. All subjects were required to not use any drugs for at least 2 weeks prior to the study until completion of the study. They were also refrained from ingesting alcohol, caffeine, chocolate, tea, or coke-containing beverages at least 48 hours before each dosing and until last blood sampling.

Subjects were randomized to 1 of the 2 sequences to receive the formulations according to randomization scheme. The test preparation was Rosantin® (40-mg rosuvastatin tablet manufactured by PT. Novell Pharmaceutical Laboratories, Jakarta, Indonesia (batch no. D11G01) and Crestor® 40-mg tablet of the originator product as reference formulation (batch no. HC452), manufactured by IPR Pharmaceutical Inc., ((Canovanas, Puerto Rico) for AstraZeneca UK Ltd (Cheshire, UK).

Subjects were confined in a clinical unit of Clinisindo Laboratories 1 night prior to the study to assure the fasting condition (10 hours before drug administration). On the study day, subjects were given 1 tablet of either product with 240 mL of water. No food was allowed until 4 hours after dose administration. Water intake was not allowed 1 hour before drug administration, but was allowed 2 hours after the dose. Standard meals were served at 4 hours (± 939 calories) and 11 hours (± 858 calories), snacks were served at 4 hours (± 165 calories) after drug administration. Total calories were calculated by a nutritionist.

Subjects were positioned (sitting or standing) for the first 4 hours. Subjects stayed at Clinisindo Laboratories clinical unit for 24 hours and were not allowed to do strenuous exercise during the sampling days. Blood pressure, heart rate, body temperature, and adverse events were monitored during blood sampling.

Blood samples (5 mL) were collected prior to dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 9, 12, 16, 24, 36, 48, and 72 hours after drug administration in heparin tubes. Plasma was separated and kept frozen at –20 °C until analysis [8].
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Bioanalytical method

Rosuvastatin plasma concentration was determined using LC-MS/MS (API 3200) method with TurboIonSpray mode. Irbesartan was used as an internal standard (IS).

Briefly, plasma samples (1 mL) were added with an internal standard and 100 µL of phosphoric acid 10%. After mixing, 5 mL of diethyl ether : dichloromethane (7 : 3) was added. The mixture was vortex-mixed for 1 minute and centrifuged at 3,000 rpm for 10 minutes. The organic phase was transferred into evaporated tube, and evaporated to dryness under vacuum for 15 minutes. The residue was reconstituted with 300 µL of acetonitrile : water (1 : 1). A 15 µL aliquot was injected into the LC-MS/MS system for analysis.

The analytical separation was performed on a Synergi 4 µ POLAR-RP-80A, 50 × 2.0 mm, 4 µm (Phenomenex®, Torrance, CA, USA) preceded by a guard column AQ C18, 4 × 2.0 mm (Phenomenex®). Mobile phase was 0.1% formic acid in acetonitrile and 0.1% formic acid in water, and was set as gradient. The flow rate was 0.6 mL/min. Column temperature was maintained at 40 °C. Multiple reaction monitoring (MRM) in negative ion mode was used to monitor transitions at m/z 482.1 → 258.1 and m/z 429.2 → 207.1 for rosuvastatin and the IS.

Safety evaluation

Analysis of safety-related data was considered using common adverse events, that occurred after initiation of the study and were supported by the following detail tabulations and analysis.

Pharmacokinetic and statistical analysis

Bioequivalence evaluation was determined using the primary parameters of AUC_{0-t}, AUC_{0-∞}, and C_{max}. The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were obtained from observing data of the individual drug plasma concentration time data and were used as a measurement of absorption rate. The AUC_{0-1} was calculated using the trapezoidal rule. The elimination rate constants (K_{el}) were calculated by least-squares regression from the data of the last 4 – 6 points of each plasma concentration data curve. The AUC_{0-∞} values were determined by adding the quotient of C_{t} (estimated last plasma concentration) and the appropriate K_{el} to the corresponding AUC_{0-1}:

\[ \text{AUC}_{0-\infty} = \text{AUC}_{0-1} + \frac{C_{t}}{K_{el}}. \]

The apparent elimination half-life (t_{1/2}) of rosuvastatin in plasma was also calculated by using the following equation: t_{1/2} = (ln 2)/ K_{el}.

A multiplicative model was assumed for the parameters of AUC_{0-1}, AUC_{0-∞}, and C_{max}, and analysis of variance (ANOVA) was applied using the respective ln-transformed data. 90% CI of the geometric mean ratio test/reference (T/R) for AUC_{0-1}, AUC_{0-∞}, and C_{max} were calculated assuming a multiplicative model. The accepted bioequivalence range for these parameters was 80.00 – 125.00% [8]. All statistical analysis were performed using EquivTest version 2.0 software (Statistical Solution, Cork, Ireland).

Results and discussion

Bioanalytical method validation

The assay has been validated in terms of selectivity, sensitivity, linearity, accuracy and precision, recovery and matrix effect,
Harahap, Prasaja, Azmi, et al. and carry-over according to the Guideline on bioanalytical validation, EMA 2011. This method was also verified before being used in this study.

The best linear fit and least-squares residual for the calibration curve were achieved with $1/x^2$ weighing factor. The standard calibration curve for rosuvastatin was ranged from 0.2 to 150 ng/mL. The lower limit of quantification was 0.2 ng/mL, and the precision and accuracy that was obtained at the lower limit of quantification (LLOQ) were 8.90% and –10.33%. Table 1 summarizes the precision and accuracy of the quality control (QC) samples during pre-study validation.

The mean recoveries of rosuvastatin at low, medium, and high concentration were 94.68%, 98.66%, and 98.53%, respectively. The mean recovery of the IS was 97.60%. The matrix effect was also investigated. The coefficient of variation (CV) of the IS-normalized matrix factor (MF) was calculated from the 6 lots of the matrix of low and high concentrations were 5.60% and 5.10%, respectively.

The stability study showed that rosuvastatin in plasma was stable at room temperature for 6 hours, at –20 °C for 67 days, and after 3 freeze-thaw cycles. The stability autosampler showed that rosuvastatin was stable after reconstitution for 24 hours.

### Clinical observation

Both formulations of rosuvastatin were well-tolerated at the administered dose, and no significant adverse clinical events were observed. A total of 17 adverse events were experienced during the study; they were all mild, and the patients recovered without any concomitant medication. There were no serious adverse events. The list of adverse events is shown in Table 2.

### Pharmacokinetic and statistical evaluation

A total of 24 subjects were available for pharmacokinetic evaluation. The mean rosuvastatin concentrations versus time profile for both formulations are shown in Figure 2. Descriptive statistics of the pharmacokinetic parameters for rosuvastatin for test and reference products are summarized in Table 3, where the geometric mean values and the range for the AUC$_{0-\infty}$, AUC$_{0-t}$, and C$_{max}$ values obtained for each formulation are shown. The pharmacokinetic characteristic $t_{max}$ was presented as mean values. The mean obtained values for test and reference products were 83.42 ng/mL and 83.98 ng/mL for C$_{max}$; 683.54 ng×h/mL and 717.93 ng×h/mL for AUC$_{0-\infty}$; 700.78 ng×h/mL and 731.83 ng×h/mL for AUC$_{0-t}$, respectively. The median $t_{max}$ for test and reference formulations

<table>
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</tr>
<tr>
<td>Total</td>
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</table>

Table 2. Disposition of adverse events.

![Figure 1. Chemical structure of rosuvastatin.](image)

![Figure 2. Geometric mean plasma concentration-time profiles of rosuvastatin after single dose of two 40-mg rosuvastatin tablet formulations in 24 subjects.](image)
were 3 hours and 2.5 hours, respectively. The terminal half-lives for test and reference formulations were 13.05 hours and 12.66 hours, respectively. The sample size n = 24 was sufficient in this study to ensure power of 80% for correctly concluding bioequivalence under the following assumption: $\alpha = 0.05$, $0.95 < \mu_T/\mu_R < 1.05 \ [10]$.

## Conclusion

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