

A Bioequivalence Study of Two Formulations of Rosuvastatin

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Abstract

Aim of this study was to compare the bioavailability of two tablet formulations containing Rosuvastatin: Rosuvastatin tablets 40 mg manufactured by MacLeods Pharmaceuticals Ltd. (marketed in Italy with the brand name Exorta®) and CRESTOR® 40 mg tablets marketed by Astra Zeneca.

Thirty-nine (39) healthy subjects were enrolled in an open label, balanced, analyst blind, randomized, two-treatment, two-period, two-sequence, single dose, crossover bioequivalence study, with a minimum washout period of 8 days. Thirty-six (36) out of 39 subjects completed the study. Plasma samples were collected up to 72 hours post-dosing. Rosuvastatin levels were determined using a validated LC/MS/MS method. Primary pharmacokinetic parameters used for bioequivalence assessment through a non-compartmental analysis were: area under the plasma concentration-time curve from time zero to time t (AUC_{0-t}) and maximum observed concentration (C_{max}). The 90% confidence intervals of ln-transformed parameters obtained by analysis of variance resulted within the predefined ranges: 102.85 – 124.85% for C_{max} (Test to reference ratio of geometric least squares means, LS means, 113.32%) and 100.47 – 115.28% for AUC_{0-t} (Test to reference ratio of geometric least squares means, LS means, 107.62%).

Bioequivalence between formulations was achieved considering both rate and extent of absorption.

Keywords: Oral absorption; Pharmacokinetics; Rosuvastatin; Tablet; Urinary excretion

Introduction

Rosuvastatin calcium is a novel member in the statin class of compounds, which acts as a competitive and selective inhibitor of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme decreasing hepatic cholesterol synthesis. Statin therapy substantially lowers total cholesterol and low-density lipoprotein cholesterol (LDL-C) concentrations, modestly lowers triglyceride and increases high-density lipoprotein cholesterol (HDL-C) levels [1]. Rosuvastatin has shown to be extremely efficacious in improving serum lipid profile and in achieving the LDL-C treatment goals with a safety profile comparable with that of other statins. Rosuvastatin also improves triglyceride, LDL-C, and HDL-C levels to produce a more favorable lipid profile [2,3].

From the clinical point of view, different international guidelines include the use of statins in primary and secondary cardiovascular prevention. Rosuvastatin has demonstrated its efficacy in the primary cardiovascular prevention of normo-lipidemic patients with high C-reactive protein [4-6].

The oral absolute bioavailability of Rosuvastatin is approximately 20%. Peak plasma concentration after oral administration occurs approximately 3-5 hours post dose, with a circulating plasma half-life of approximately 19 hours. Rosuvastatin is tightly bound in a reversible manner to plasma proteins (88%) [7].

The majority of Rosuvastatin is excreted via the faecal route unchanged (approximately 90%), with the remaining portion excreted in urine. Rosuvastatin is not extensively metabolized in humans, the principal isoenzyme involved is CYP2C9 [3].

The chemical structure of Rosuvastatin is shown in (Figure 1).

The present trial was planned to evaluate and compare the relative bioavailability and, therefore, the bioequivalence of two formulations of Rosuvastatin in healthy, adult, human subjects under fasting conditions: Rosuvastatin tablets 40 mg manufactured by MacLeods Pharmaceuticals Ltd. (marketed in Italy with the brand name Exorta®) and CRESTOR® 40 mg tablets marketed by Astra Zeneca.

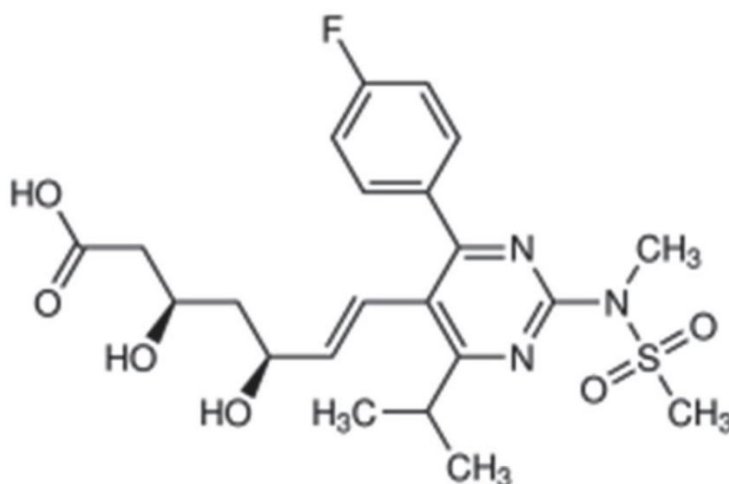


Figure 1: Chemical structure of Rosuvastatin

Materials and methods

Population

Forty (40) Italian healthy, adult, human male volunteers aged between 19 and 42 years old were screened for the study. Thirty-nine (39) volunteers, fulfilling all the inclusion and exclusion criteria defined in the study protocol, were enrolled in the study. All volunteers, after assessment of medical history, verification of vital signs, physical examination, electrocardiogram, chest X-ray and routine laboratory tests, showed good health conditions or the absence of significant diseases. Furthermore, they had negative tests for hepatitis B (HBsAg and Anti-HBc IgM), hepatitis C, HIV and routine urine examination. The protocol and the informed consent were approved by the Connoisseur Independent Ethics Committee on 15th January 2015.

The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki (ICH GCP, Schedule-Y) and other regulatory provisions under the Drug and Cosmetics Rules, with the GCP Guidelines issued by Central Drugs Standard Control Organization (CDSCO), with the “Ethical Guidelines for Biomedical Research on Human Subjects” published by Indian Council of Medical Research (ICMR) and in accordance with the European guidelines (EMA) requirement [8].

After explaining the nature and purpose of the study, all volunteers provided their written informed consent for participation.

Study Treatments

The following treatments were administered under fasting conditions: Test, one Rosuvastatin tablets 40 mg (manufactured by MacLeods Pharmaceuticals Ltd. and marketed in Italy with the brand name Exorta[®]) and Reference, one CRESTOR[®] 40 mg tablets marketed by Astra Zeneca. The products were planned to be administered to the 40 healthy male Caucasian subjects according to the following design: Sequence 1 (n=20) Period 1/Test- Period 2/Reference; Sequence 2 (n=20) Period 1/Reference – Period 2/Test.

Study Design

This was an open label, balanced, analyst blind, randomized, two-treatment, two-period, two-sequence, single dose, crossover bioequivalence study designed to be conducted on 40 Italian healthy, adult, human subjects under fasting conditions.

In each period, subjects were asked to arrive at the clinical site at least 10 hours before dosing. After a supervised overnight fast, a single oral dose of the assigned formulation was administered orally with 240 mL of water at ambient temperature, starting at 9:00 a.m. in batch of two subjects during each period. The dosing interval between successive subjects was 3 minutes. The subjects fasted for at least 10 hours prior to administration of the study drug. Fasting was continued for 4 hours post-dose; meals were then provided at specified intervals. Drinking water was disallowed for 1 hour pre-dose and 1 hour post-dose administration, except 240 mL during administration of the drug dose. Thereafter, drinking water was provided *ad libitum*. Subjects were allowed to leave the clinical site after the 24-hour post-dose blood drawn.

Subjects were asked to visit the clinical facility for ambulatory blood sample collection at 34, 48 and 72 hours post-dose. During each ambulatory visit, the breath of the subjects was analyzed to check the consumption of alcohol. Compared to other studies of bioequivalence of Rosuvastatin in our study we considered more time points; hence, vital signs and subject questionnaire was done at 34, 48 and 72 hours post-dose, and medical examinations were carried out at 72 hours post-dose [9-11]. The wash-out period had to be at least 8 days; the duration of the study was expected to be approximately 12 days. Study participants were aware that they were receiving different formulations of a same drug, without being informed on which product (Test or Reference) was administered. Receipt of any other prescription drug or over the counter (OTC) drugs (including vitamins and medicinal products

from natural origin) within two weeks prior to receiving the first dose of study medication or repeated use of drugs within the last four weeks were exclusion criterion. Furthermore, the subjects were supposed not to consume any medication during the conduct of the study. All the subjects who checked-in the study confirmed that they did not consume any medication within the 2 weeks before the start of first period, or during the study.

Blood samples for pharmacokinetic measurements (1 x 5 mL) were collected in 5 mL blood collection tubes containing K₂EDTA as anticoagulant, prior to and up to 72 hours after each drug administration. The venous blood samples were withdrawn pre-dose and at 0.17, 0.33, 0.50, 0.75, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.33, 3.67, 4.00, 4.33, 4.67, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 14.00, 18.00, 24.00, 34.00, 48.00 and 72.00 hours post-dose.

The blood samples collected at each time point were centrifuged, within 30 minutes, between 4 to 8 °C and at 4000 rpm for 10 minutes to separate plasma. In case on any delay in centrifugation sample was kept in cold condition. The separated plasma was divided in duplicate aliquots in pre-labeled polypropylene tubes during each period; tubes were labeled with study number, period number, subject number, sample number, time point (hours) and aliquot number.

These tubes were then transferred into a deep freezer maintained at -50 °C or colder for storage, and further analyzed by the Bioanalytical Department. Statistical analysis of the study was performed by MacLeods Pharmaceuticals Ltd., Bioequivalence Department located in the city of Mumbai, India.

Method of measurement

For the estimation of rosuvastatin in plasma, the plasma samples were analyzed by a validated LC-MS/MS method.

During estimation of rosuvastatin in plasma, quality control samples were distributed throughout each batch of study samples. Whenever possible, samples from each subject were analysed on the same standard curve. Samples with drug concentration greater than the upper limit of the validated range of the analysis were re-analysed, as per the standard test procedure based on method validation report.

The analysts concerned were blinded with respect to the randomization code, and as a result to the order of administration of the study medication.

The limit of quantification of 2.05 ng/mL for Rosuvastatin was enough to quantify the analyte from the plasma samples collected up to 72 hours after drug administration. The linearity range of 2.05 ng/mL to 199.33 ng/mL for Rosuvastatin was enough to quantify the expected concentration range of rosuvastatin from subjects' plasma with the proposed dose of Rosuvastatin 40 mg.

Pharmacokinetic and Statistical analysis

The Primary pharmacokinetic parameters of Rosuvastatin estimated after drug administration under fasting conditions were C_{max} and AUC_{0-t}.

Secondary Variables were AUC_{0-∞}, T_{1/2}, K_{el}, T_{max}, and Residual Area; npoints, K_e_first and K_e_last were also calculated.

These parameters were derived individually for each subject from their Rosuvastatin concentration in plasma. Actual time of blood collection was considered for pharmacokinetic calculations.

For the estimation of pharmacokinetic parameters, concentrations that were below level of quantification (BLQ) were assigned a value of zero if they preceded quantifiable samples in the initial portion of the profile. A BLQ that occurred at the end of the profile was set to zero. A BLQ or zero concentration that was embedded between two quantifiable points was assigned a value of missing. If consecutive BLQs in the terminal portion of the profile were followed by quantifiable determinations, these quantified values were excluded from pharmacokinetic analysis by assigning them a value of missing. In the calculations of pharmacokinetic parameters, missing values were ignored. The pharmacokinetic parameters were calculated by non-compartmental methods using SAS® version 9.4.

The calculations of the Primary pharmacokinetic parameters were carried out as follows: C_{max}: Maximum measured plasma concentration following each treatment; AUC_{0-t}: The area under the plasma concentration versus time curve from time zero to the last measurable concentration, as calculated by the linear trapezoidal method.

The log-transformed pharmacokinetic parameters (C_{max} and AUC_{0-t}) of Rosuvastatin were analyzed using an ANOVA model with main effects of sequence, subject nested within sequence, period and formulation. The 90% confidence interval for C_{max} and AUC_{0-t} of Rosuvastatin formed the basis to conclude the equivalence of Rosuvastatin between the Test and the Reference products. If the confidence intervals were entirely included in the range of 80 – 125 % for C_{max} and AUC_{0-t} log-transformed, the treatments would be considered as to be bioequivalent. Ratio Test/Reference for each subject at C_{max} and AUC_{0-t} bioavailability rates and power test were performed using SAS® version 9.4. The sequence, period and treatment effects were assessed at the two-sided 5% level.

The formula used to estimate the intra-subject coefficient of variation was: $100 * \sqrt{e^{\overline{MSE}} - 1}$, where \overline{MSE} is the Mean Square Error obtained from Analysis of Variance model.

Determination of Sample Size

Sample size was calculated using SAS*. The highest intra subject coefficient of variation for Rosuvastatin was observed to be 24.71% for C_{max} (ng/mL) in a previous bioequivalence study (BEQ-1100-ROSU-2013). Then, in order to achieve 80% statistical power, a sample size of 27 observations was determined sufficient to reach bioequivalence. Thus, taking into account dropouts or withdrawals of subjects during the conduct of the study, and to better characterize our study compared with other bioequivalence studies, 40 healthy subjects were considered enough to achieve the desired sample size to demonstrate bioequivalence in a crossover study design [9-11].

Results

Out of 40 participant volunteers, 39 were enrolled and 36 completed the study. Two subjects had withdrawn their consent for personal reasons, one subject had withdrawn from the study due to an adverse event (fever with mild headache). The 36 volunteers who completed the study had mean age of 28.9 years, (ranging from 19 to 42 years); mean weight of 62.4 kg (51.3 to 78.3 kg); mean height of 1.66 m (1.58 to 1.78 m) and mean BMI of 22.47 kg/m² (18.64 to 26.78 kg/m²). Apart from one subject described above, no other adverse events occurred during the study.

The mean curves for plasma concentration vs. time obtained for Test and Reference drugs and comparative semi log plot are shown in Figure 2a and b; the curves show a similar pharmacokinetic profile between the drugs.

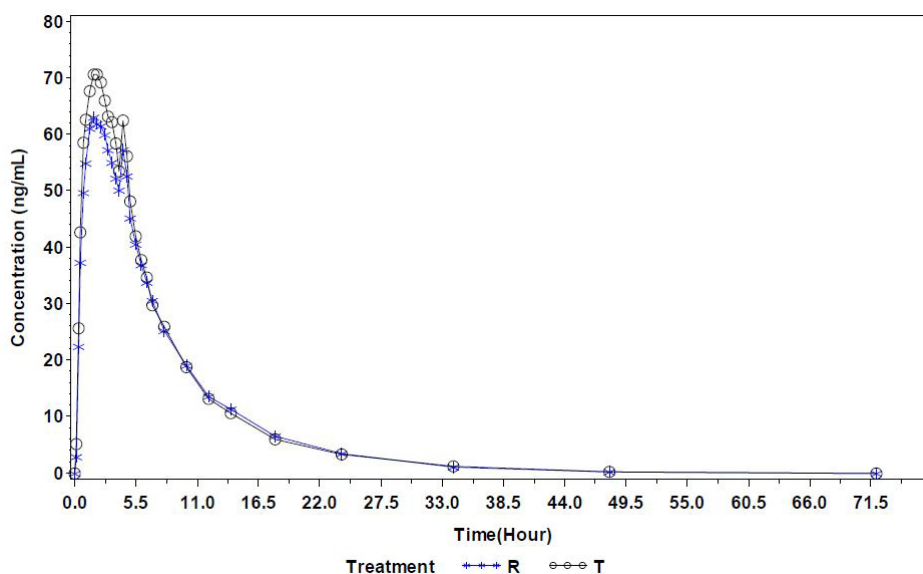


Figure 2a: Comparative Linear Plot of Rosuvastatin Mean Plasma Concentration (ng/mL) Vs Time (Hour)(N=36) subject=MEAN

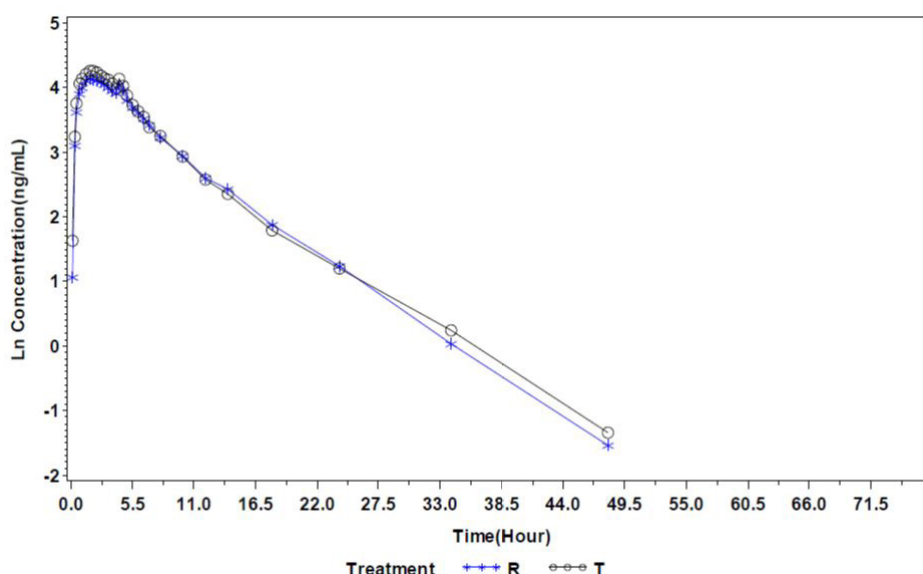


Figure 2b: Comparative Semi Log Plot of Rosuvastatin Mean Plasma Concentration (ng/mL) Vs Time (Hour)(N=36) subject=MEAN

Table 1 shows the main study results for Test and Reference; Table 2 shows the comparison of results with standards for bioequivalence.

a) Test					
Pharmacokinetic Parameters	Test Product (n=36)				
	Arithmetic Mean	S.D.	C.V. (%)	Median	Range
C_{max} (ng/mL)	81.092	37.5728	46.33	79.22	20.94-180.88
AUC_{0-t} (ng*hrs/mL)	572.48622	248.291351	43.37	530.8315	173.4253-1358.5524
$AUC_{0-\infty}$ (ng*hrs/mL)	601.60872	257.273532	42.76	558.2177	190.5193-1424.1105
T_{max} (hrs)	2.093	1.2541	59.92	2.00	0.33-4.67
$T_{1/2}$ (hrs)	7.47196	3.888538	52.04	6.0893	3.6211-18.4250
K_{el} (hr ⁻¹)	0.11270	0.043545	38.64	0.1138	0.0376-0.1914
Residual Area	0.05210	0.022198	42.61	0.0453	0.0208-0.1158
b) Reference					
Pharmacokinetic Parameters	Reference Product (n=36)				
	Arithmetic Mean	S.D.	C.V. (%)	Median	Range
C_{max} (ng/mL)	73.776	42.1808	57.17	63.80	16.41-212.93
AUC_{0-t} (ng*hrs/mL)	543.91544	286.613860	52.69	483.7370	107.9773-1518.7585
$AUC_{0-\infty}$ (ng*hrs/mL)	570.28651	293.949250	51.54	510.2939	120.5084-1569.2983
T_{max} (hrs)	2.551	1.4243	55.83	2.33	0.50-5.00
$T_{1/2}$ (hrs)	6.11034	2.694168	44.09	5.3486	2.6084-14.5016
K_{el} (hr ⁻¹)	0.13003	0.044311	34.08	0.1296	0.0478-0.2657
Residual Area	0.05188	0.018503	35.66	0.0523	0.0205-0.1040

Table 1: Main study results for (a) Test and (b) Reference

Geometric Mean, Ratio, Intra Subject C.V., Power and 90% Confidence Interval for Rosuvastatin						
Pharmacokinetic Parameters	Geometric Mean		Ratio (T/R) (%)	Intra Subject C.V. (%)	Power (%)	90% Confidence Interval (%)
	Test (T)	Reference (R)				
C_{max} (ng/mL)	73.114	64.522	113.32	24.52	96.43	102.85-124.85
AUC_{0-t} (ng*hrs/mL)	520.455	483.589	107.62	17.27	99.93	100.47-115.28

Table 2: Comparison of results with standards for bioequivalence

Discussion

The use of generic preparation of a therapeutically well-established active drug principle has to be justified by an appropriate bioequivalence study; if pharmaceutical forms are bioequivalent it follows that the clinical efficacy and safety of these pharmaceutical forms are similar and can be used indiscriminately from a therapeutic point of view [12].

This study was a standard two-formulation, two-period, two-sequence crossover trial where the volunteers received both the Test and Reference products in each period. An adequate washout period was necessary to distinguish the formulation effects from other effects. The EMA guideline recommends a minimum of 5 elimination half-lives between both periods [8]. The $T_{1/2}$ of Rosuvastatin is approximately 19 hours and does not increase at higher doses [3]. The study team had taken into consideration the reported value range, and concluded that an 8-day separation between two periods was sufficient for the drug concentration to fall below the lower limit of quantification.

Table 1 shows the main study results for Test and Reference. After oral administration of the reference product under fasting condition the drug was absorbed with median t_{max} of 2.093 hrs. Where for other PK parameters mean C_{max} was 81.092 ng/mL (range 20,94 – 180.88 ng/mL) and AUC_{0-t} was 572.48622 ng*hrs/mL (range 173.4253 - 1358.5524 ng*hrs/mL).

After oral administration of the Test product under fasting condition, the drug was absorbed with median t_{max} of 2.00 hrs. Where for other PK parameters mean C_{max} was 81.092 ng/mL (range 20.94 -180.88 ng/mL) and AUC_{0-t} was 572.48622 ng*hrs/mL (range 173.4253 - 1358.5524 ng*hrs/mL).

A validated LC-MS method was utilized for the quantification of Rosuvastatin in plasma samples. Analysis was successfully applied, providing the appropriate accuracy, sensitivity, linearity, precision, repeatability, and selectivity with high sample throughput as required for pharmacokinetic studies.

Table 2 shows the comparison of results with standards for bioequivalence for Test and Reference. With regards to the efficacy of the test product, statistical comparison of the main pharmacokinetic parameters, C_{max} and AUC_{0-t} clearly indicated no significant difference between the Test and the Reference tablets. The obtained values were compliant with FDA and EMA requirements for the bioequivalence of generic drugs, since the 90% CI for AUC_{0-t} and C_{max} mean ratios fall within the 80%–125% interval [8,13].

ANOVA analysis was conducted on the pharmacokinetic parameters of C_{max} and AUC_{0-t} to evaluate potential difference between separate effects such as formulation, sequence and period effect. None of these effects was found to be statistically significant ($p>0.05$).

Conclusion

This bioequivalence study was well designed, demonstrating bioequivalence between Rosuvastatin tablets 40 mg (manufactured by MacLeods Pharmaceuticals Ltd. and marketed in Italy with the brand name Exorta®) and CRESTOR® 40 mg tablets marketed by Astra Zeneca, in terms of both rate and extent of absorption. 90% confidence intervals of the main pharmacokinetic parameters, C_{max} and AUC_{0-t} comply with the 80 and 125 % acceptance interval.

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Dr. Dario Zava is an employee of Istituto Lusofarmaco D'Italia, dealer of Exorta® in Italy.

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