Original Article

Mahidol Univ J Pharm Sci 2015; 42 (3), 118-125

Bioequivalence study of Ribavirin 400 mg tablets in healthy Thai male volunteers under fed conditions

*J.Vattanarongkup[1], E. Yoosakul[1], C. Manamuti[1], P. Teerawonganan[1], C. Seeduang[1], P. Ratnatilaka Na Bhuket[1], A. Rojanawiwat[2], B. Karachot[1], B. Chuasuwan[1], I. Techatanawat[1]**

[1] Research and Development Institute, The Government Pharmaceutical Organization, Bangkok, Thailand

[2] Clinical Research Center, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand

Abstract

Ribavirin is a nucleoside analogue that exerts an antiviral activity. It mostly uses as a combination with interferon alfa-2b for hepatitis C treatment. Development of a generic product of ribavirin would be benefit for hepatitis C patient in Thailand. The purpose of this study is to investigate the bioequivalence of the generic formulation, Ribavirin GPO 400 mg tablets compared to an originator, Copegus® 400 mg tablets. A randomized, single dose, two-way crossover, open-label bioequivalence study in healthy Thai male volunteers under fed conditions with 6 weeks washout period was conducted. The plasma samples were collected and the ribavirin concentration was analyzed using validated liquid chromatography tandem mass spectrometry method. The pharmacokinetic parameters including of area under the plasma concentration time curve from time 0 hour to 72 hours (AUC_{0-72}), the peak plasma concentration (C_{max}) and time to achieve the C_{max} (T_{max}) were determined by using non-compartmental model. The 90% parametric confidence intervals (90% CI) values for the ratio of AUC_{0-72} and C_{max} of test/reference product were 101.3 (95.73-107.17) and 99.6 (92.50-107.32), respectively. These values were within the acceptable range (80.00 – 125.00%). No adverse effect was observed during the study. The results of this study indicated that both formulation of ribavirin, Ribavirin GPO 400 mg tablets and Copegus® 400 mg tablet were bioequivalent in term of rate and extent of drug absorption.

Keyword: Ribavirin, Pharmacokinetics, Bioequivalence, Liquid chromatography tandem mass spectrometry

1. INTRODUCTION

Ribavirin is a nucleoside analogue with exert an antiviral activity. The chemical name of ribavirin is 1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide and chemical structure is shown in figure 1. A broad spectrum antiviral activity of ribavirin have been suggested in several mechanism such as direct inhibition of viral RNA replication, inhibition of enzyme inosine-monophosphate-dehydrogenase (IMPDH), immunodulation, and mutagenesis[1, 2].

Ribavirin is absorbed rapidly following oral administration of a single dose of ribavirin (median Tmax = 1-2 hours). The absolute bioavailability is around 50%. Bioavailability of a single oral dose of ribavirin is increased to approximately 70% when co-administration with a high-fat meal. The mean half-life is 43.6 hours. The total apparent clearance following administration of a single oral dose is about 26 L/h [3]. In a single dose, randomized, open label, 2-way crossover bioequivalence study of Ribavirin 200 mg capsule in healthy subjects under fed condition, it revealed that C_{max} of Ribavirin of test and reference were 582.133±17.044 ng/mL and 538.343±139.018 ng/mL, AUC_{0-t} were 6048.902±1343.191 ng.hr/mL and 6407.033± 1478.900 ng.hr/mL and T_{max} were 1.783±0.709 hr and 2.325±0.496 hr, respectively[4].

***Corresponding author:** E-mail address: isariya_t@gpo.or.th

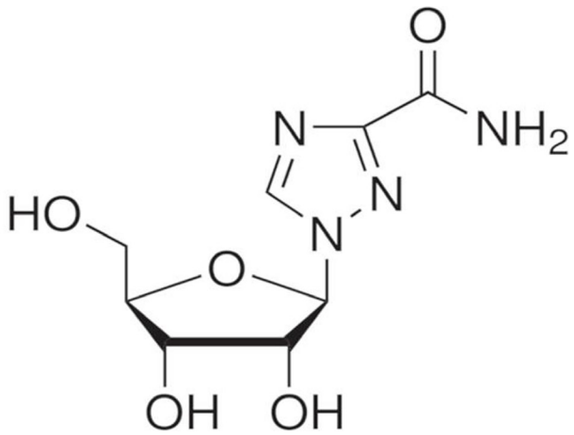

Figure 1. Chemical structure of Ribavirin[2]

Ribavirin combination with interferon alfa-2b is the most effective treatment for the hepatitis C patient [3,5,6]. The Government Pharmaceutical Organization (GPO) has been developed the generic product of ribavirin with effective and lower cost that would be benefit for patients. Therefore, the bioequivalence study is performed to establish the similarity and interchangeability between the two formulations of ribavirin in healthy Thai male.

2. MATERIALS AND METHODS

2.1. Study drug

The reference product of Ribavirin 400 mg tablets is Copegus® manufactured by Roche Pharma AG, Germany (batch no. N0005, Exp Apr 2016). The test product is Ribavirin GPO 400 mg tablets manufactured by The Government Pharmaceutical Organization, Thailand (batch no. S550454, Mfd 12 Aug 2012, Exp 16 Aug 2014).

2.2. Clinical study design

A randomized, open label, two-treatment, two-period, two-sequence, single dose, crossover, bioequivalence study of generic Ribavirin 400 mg tablets of the Government Pharmaceutical Organization, Thailand and the reference product of Roche Pharma AG, Germany in healthy human male adult subjects, under fed conditions with 6 weeks washout period between treatments was conducted. The study protocol was approved by the Institute for the Development of Human Research Protections (IHRP) before study initiation. Informed consent from each study volunteers was obtained before any study related procedures were initiated for each volunteer.

Forty-four Thai healthy male volunteers, aged between 18-55 years, having a Body Mass Index between 18-25 kg/m^2 were enrolled for the study. All subjects having no significant diseases or clinically significant abnormal laboratory values such as complete blood count, hematocrit, hemoglobin, fasting blood sugar, blood urea nitrogen (BUN), serum creatinine, alkaline phosphatase, ALT, AST, total bilirubin, total protein, albumin, hepatitis B and C test, urine analysis and ECG were included. The participants who have any history of hypersensitivity to ribavirin or any of the excipients, positive hepatitis B test, cigarette smoking, alcohol dependence, consumption of xanthine containing products more than 3 cups/day, or consumption of any medication before the study for 14 days were excluded from the study.

The order of receiving the test and reference product for each subject during both periods of the study was determined by a randomization schedule which generated by PK and statistical investigator with SAS® software. The subjects were allocated number 1 to 44 on the day of period I. The study medicine was administered to the subjects in sitting posture

and they would remain in sitting or ambulatory posture for the first 3 hours after administration of drug in each period. Each subject received a single oral dose of the test or reference products with 240±2 mL of water at 30 minutes after the start of a high fat breakfast (about 800-1000 cal). The high fat breakfast menu derived calories approximately 15%, 25% and 50-60% from protein, carbohydrate and fat, respectively. Hot drink or juice was provided after 3 hours of drug administration. Meal, snack and meal were served at 4, 7 and 10 hours after dosing, respectively. Thereafter, the subjects will be allowed to engage only in normal activities while avoiding severe physical exertion during confinement period of the study. Subjects were not received any medication and alcohol during 14 days prior to the beginning of the study and during the study. Subjects were restricted from tea, coffee or xanthine products at least 24.0 hours prior to the first dose of study medicine or during the study. The subjects were discharged in day 3 after blood collection (24 hr), vitals sign measurement and given breakfast (standard meal). The adverse events were monitored throughout the study period and recorded in case report forms.

Approximate 5 mL for post dose and 7 mL for pre-dose venous blood samples were collected into K_2EDTA vacutainers by the indwelling catheter for twenty-two sampling times (0.000, 0.250, 0.500, 0.750, 1.000, 1.250, 1.500, 1.750, 2.000, 2.250, 2.500, 2.750, 3.000, 3.500,4.000, 6.000, 8.000, 10.000, 12.000, 24.000, 48.000 and 72.000 hours). After collection, the blood samples were centrifuged at 3000±100 rcf for 5 minutes below 10 ºC. All plasma samples were divided into two aliquots as per protocol (back up lot is for reanalysis) and transferred to suitably labeled polypropylene tubes and stored at a temperature -65±10 ºC, until bioanalysis.

2.3. Determination of Ribavirin concentration in plasma

The plasma concentrations of ribavirin in study samples were determined by a validated LC-MS/MS method using Ribavirin-$^{13}C_5$ as an internal standard. The analyte and internal standard were extracted from plasma using protein precipitation method. Then samples were centrifuged to separate the precipitates. The supernatants were transferred into appropriate vials for analysis. The analyte and internal standard were monitored in the positive ion mode using ESI probe at MRM transitions of m/z 245.090→113.070 and m/z 250.100→113.100 for analyte and internal standard, respectively. The chromatographic system consisted of ACE 5 C18 150 x 4.6 mm column. The mobile phase was a mixture of 0.1% formic acid solution (V/V) and acetonitrile. The US FDA guidance for industry, bioanalytical method validation and the European Medicines Agency guideline on bioanalytical method validation were followed. The summary of validation results are shown in Table 1.

2.4. Pharmacokinetic analysis

The pharmacokinetic parameters including AUC, C_{max} and T_{max} were determined by non-compartmental model using Phoenix WinNonlin Software Version 6.3. The area under the plasma concentration versus time curve from time zero to the truncated time of 72 h was calculated by linear trapezoidal method (AUC_{0-72}) due to its long half-life. Maximum measured plasma concentration (C_{max}) and time to achieve maximal plasma concentration (T_{max}) were obtained directly from each subject's plasma concentration versus time profile.

2.5. Statistical analysis

The pharmacokinetic parameters were analysis using PROC GLM of SAS® Version 9.3 (SAS Institute Inc., USA) for both untransformed and ln-transformed pharmacokinetic parameters AUC_{0-72} and C_{max} for ribavirin. ANOVA model included Sequence, Formulation and Period as fixed effects and Subject (Sequence) as a random effect. Sequence effect was tested using Subject (Sequence) as error term. An F-test was performed to determine the statistical significance of the effects involved in the model at a significance level of 5% (α=0.05). Bioequivalence of test and reference product

Table 1. The summary of validation results

Information requested		Data
Analyte (drug)		Ribavirin
Internal standard (IS)		Ribavirin-$^{13}C_5$
Extraction Method		Protein precipitation extraction
Biological Matrix		Human plasma
Anticoagulant (only human plasma)		K_2EDTA
MRM transitions (m/z)		245.090 → 113.070 for Ribavirin, 250.100 → 113.100 for Ribavirin-13C5
Linearity (Range)		5.020 to 1008.273 ng/mL
Coefficient of determination (r^2)		Greater than 0.98
Lower limit of quantification		5.020 ng/mL
Precision	Within-batch (Intra-day precision)	0.4% to 6.2%
	Between-batch (Inter-day precision)	0.8% to 5.1%
Accuracy	Within-batch (Intra-day precision)	99.4% to 105.6%
	Between-batch (Inter-day precision)	100.1% to 103.4%
Robustness and Ruggedness experiment		Method is rugged and robust (up to 150 injections)
Average recovery of drug (%)		LQC, MQC and HQC 106.1%, 100.9% and 105.5%
Average recovery of internal standard (%)		105.8%
Autosampler / Wet extract stability		79.0 hours (within 2 to 8°C)
Freeze and thaw stability		4 cycles
Bench top stability		15.0 hours (at room temperature)
Wet extract bench top stability		2.0 hours (at room temperature)
Reagents stability		7 days (at room temperature)
Mobile phase stability		11 days (at room temperature)
Long term stability of drug in matrix		219 days (at -65±10 °C)

were concluded, if the 90% confidence interval of ratio of geometric least square mean fell within the acceptance range of 80.00-125.00% for ln-transformed pharmacokinetic parameters AUC and C_{max} for ribavirin.

3. RESULTS AND DISCUSSION

The number of subjects was calculated by using SAS® Software Version 9.3 and based on the literature data, the estimate sample size of 36 subjects would be sufficient to establish bioequivalence with adequate power. Considering dropouts and withdrawals, 44 subjects would be sufficient for this study. This study was determined in Thai healthy male in fed condition because the bioavailability of Ribavirin was increased to approximately 70% when co-administration with high fat meal and gender have no clinically significant between male and female subject and Ribavirin may cause birth defects and/or death of the exposed fetus, Hence male subject is suitable for this study [7]. Wash out period between period I and period II was 6 weeks because Ribavirin has long half-life, about 2 days, hence 6 weeks are enough for elimination.

Forty-four subjects were enrolled for the study but only forty subjects completed the study. The demographic data of dosed subjects and completed subjects is shown in table 2. Four of volunteers were withdrawn

in period 2 due to personal reason. In case of those subjects could not to complete the provided breakfast on dosing day, we calculated the remaining amount of calories. If consumed breakfast by particular subjects having < 800 Kcal, consumed for 500-799 Kcal then we justify as moderate fat meal, then we recorded as protocol deviations. However, we did not withdraw the subjects from the study because all the subjects try to consume as much as they can but some of them had full stomach and another of the rest were not familiar with high fat menu and feel nausea after intake it. The clinical investigator had made a decision to continue the study due to respect to subject's human right. Hence, thirteen subjects who consumed less than 800 Kcal of high-calorie breakfast were excluded from pharmacokinetic analysis. Only twenty-seven subjects were included in pharmacokinetics and statistical analysis. The blood samples were divided into two aliquots, first for analysis and second for repeat analysis, and stored at the clinical site until transferred to the bioequivalence center for sample analysis. The blood samples were separated transfer, one for first aliquot and second for second aliquot to prevent all samples loss when car accident. At the bioanalysis site the sample was separately stored in different freezer to prevent all samples damage when freezer malfunctions.

Table 2. The demographic data of dosed subjects (N=44) and completed subjects (N=40)

	Dosed subjects	Completed subjects
	Mean ±SD	
Ages (years)	33.14 ± 8.02	33.98 ± 7.85
Height (cm)	172.16 ± 6.95	172.13 ± 7.17
Weight (kg)	66.44 ± 7.75	66.44 ± 7.69
BMI (kg/m^2)	22.38 ± 1.84	22.39 ± 1.83

Safety assessment included the incidence of adverse events were monitored. Two adverse events were observed from the study. One was a lacerated wound from accidental injury before dosing and received only supportive care such as cold compression without any medicine. The other adverse event was dizziness which developed on subject, it occurred since 2 hours after administration and relieved within 5 hours later without any medicine and the justified as probable related to drug. Safety evaluation at the end of the study was found to be safe for all study subjects.

The mean of plasma concentration of Ribavirin versus time curves after administration of test and reference is showed in figure 2. The primary pharmacokinetic parameters (AUC_{0-72} and C_{max}), secondary pharmacokinetic parameters (T_{max}) of test and reference are showed in table 3, respectively. The ANOVA results of pharmacokinetic parameters of Ribavirin for ln-transformed data is showed in table 4. The 90% parametric confidence intervals were calculated for the ln-transformed primary pharmacokinetic parameters, AUC_{0-72} and C_{max} of ribavirin are within the bioequivalence range of 80.00–125.00% as demonstrated in Table 5. The power of primary pharmacokinetic parameters are 100.0% for AUC_{0-72} and 99.9% for C_{max}. The $AUC_{0-\infty}$ parameter is unnecessary in this study because Ribavirin is a long elimination half-life drug (>24 hr), therefore an AUC truncated at 72 hours (AUC_{0-72}) can use in place of AUC_{0-t} or $AUC_{0-\infty}$ [8]. When compared the pharmacokinetic results with a previous study it demonstrated that the result were similar and both studies using the validated LC-MS/MS method for determination the concentration of Ribavirin[4]. These results indicated that the test product was bioequivalent to the reference product.

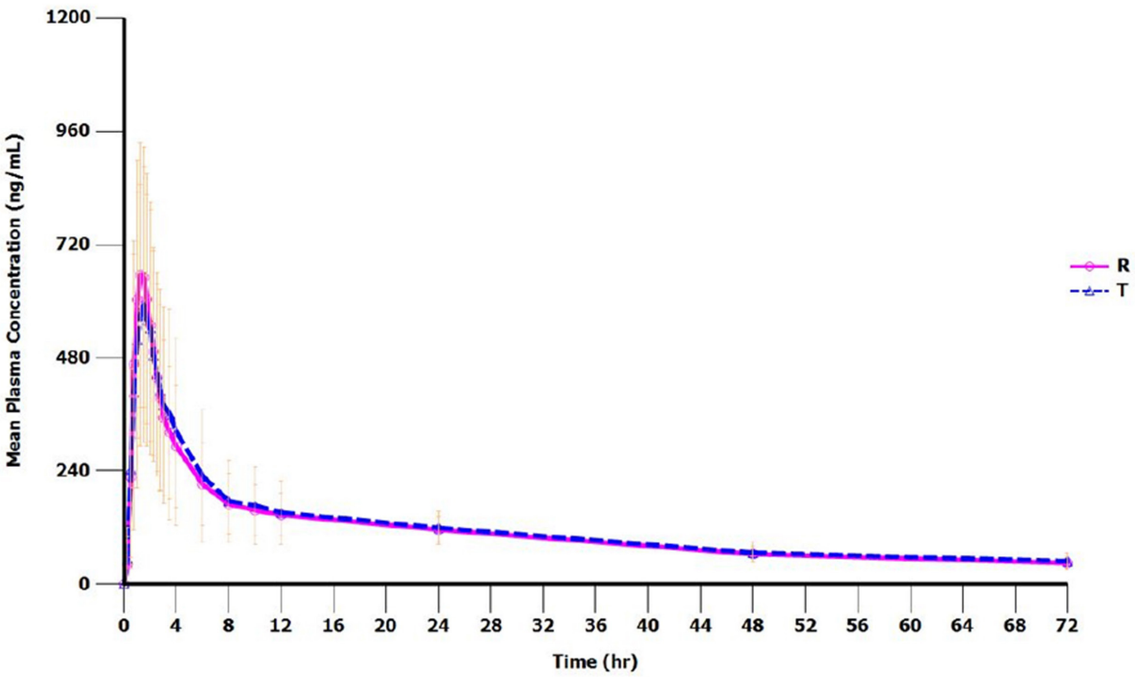

Figure 2. Linear Plot of Mean (±SD) Plasma Concentration of Ribavirin versus Time Curves after Administration of Test Product-T and Reference Product-R in Healthy Thai Male Volunteers under Fed Conditions (N = 27)

Table 3. The pharmacokinetic parameters of test and reference products of Ribavirin (Un-transformed)

Product/Statistic	AUC_{0-72} (ng.hr/mL)	C_{max} (ng/mL)	T_{max} (hr)
Test product			
Mean	8478.685	749.206	1.657
CV (%)	35.7	39.4	47.7
N	27	27	27
Reference product			
Mean	8197.546	743.558	1.454
CV (%)	28.6	38.8	34.7
N	27	27	27

Table 4. ANOVA of Pharmacokinetic Parameters of Ribavirin for Ln-Transformed Data (N=27)

Source	AUC_{0-72} (ln-transformed data)				
	D.F.	SS	MS	F	p-values
Period	1	0.08297245	0.08297245	5.64	0.0255
Subject (Sequence)	25	3.85669756	0.15426790	10.49	<.0001
Formulation	1	0.00221045	0.00221045	0.15	0.7015
Sequence	1	0.21946897	0.21946897	1.42	0.2442
Error	25	0.36765079	0.01470603	-	-
Total	53	4.53012178	-	-	-
Source	C_{max} (ln-transformed data)				
Period	1	0.01349533	0.01349533	0.53	0.4735
Subject (Sequence)	25	8.16140827	0.32645633	12.81	<.0001
Formulation	1	0.00018014	0.00018014	0.01	0.9337
Sequence	1	0.00141486	0.00141486	0.00	0.9480
Error	25	0.63692324	0.02547693	-	-
Total	53	8.81332497	-	-	-

Table 5. 90% Confident intervals of the ln-transformed primary pharmacokinetic parameters

Parameters	Ratios	90% CI	Power (%)
ln AUC_{0-72}	101.3	95.73 – 107.17	100.0
ln Cmax	99.6	92.50 – 107.32	99.9

The one limitation of this study was the subject were not familiar a high-fat diet which lead to the subject exclusion. However, the result of this study was not impacted by this subject removal.

4. CONCLUSION

According to the results of pharmacokinetic parameters in this study it can concluded that the test product (Ribavirin GPO 400 mg tablet) and reference product (Copegus® 400 mg tablet) were bioequivalent with respect to the rate and extent of absorption.

5. ACKNOWLEDGEMENTS

This study was sponsored by the Government Pharmaceutical Organization, Thailand. The authors would like to thank Ms.Wilak Vangkanonta and Ms.Valaimon Vimolprasan for their assistance.

REFERENCES

1. Dixit N, Perelson A. The metabolism, pharmacokinetics and mechanisms of antiviral activity of ribavirin against hepatitis C virus. Cell Mol Life Sci. 2006;63(7-8): 832-42.
2. Parker W. Metabolism and antiviral activity of ribavirin. Virus Res. 2005;107(2):165-71.
3. Thomas E, Ghany M, Liang T. The application and mechanism of action of ribavirin in therapy of hepatitis C. Antivir Chem Chemother. 2013;23(1):1-12.

4. European Medicines Agency. CHMP assessment report for Ribavirin TEVA Pharma B.V. [document on the Internet]. London, United Kingdom; 2009 [updated 2013 July 26; cited 2015 October 10]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/001064/WC500056848.pdf.
5. Te H, Randall G, Jensen D. Mechanism of action of ribavirin in the treatment of chronic hepatitis C. Gastroenterol hepatol. 2007;3 (3):218-25.
6. Feld J, Hoofnagle J. Mechanism of action of interferon and ribavirin in treatment of hepatitis C. Nature. 2005;436(7053):967-72.
7. United States Food and Drug Administration. Full prescribing information of COPEGUS (Ribavirin) [document on the Internet]. Maryland, United State; 2002 [updated 2011 August; cited 2015 October 10]. Available from:http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021511s023lbl.pdf
8. United States Food and Drug Administration. Draft Guidance for industry. Bioequivalence studies with pharmacokinetic endpoints for drugs submitted under and ANDA [document on the Internet]. Maryland, United State; 2013 [updated 2013 December 13; cited 2015 October 10]. Available from: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm377465.pdf