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Bioequivalence Study of Two Minocycline Capsule Formulations in Healthy Subjects

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Abstract

The purpose of this study was to find out whether the bioavailability of 100 mg minocycline capsule manufactured by Y.S.P. Industries (M) Sdn. Bhd. was equivalent to that produced by Apotex Canada (Apo-Minocycline® 100 mg). The pharmacokinetic parameters assessed in this study were area under the plasma concentration-time curve from time zero to the last observed quantifiable concentration (AUC_t), area under the plasma concentration-time curve from time zero to infinity (AUC_{inf}), the peak plasma concentration of the drug (C_{max}), time needed to achieve the peak plasma concentration (t_{max}), and the elimination half life ($t_{1/2}$). These parameters were determined on plasma concentrations of minocycline.

This was a randomized, single blind, two-period, two-sequence crossover study which included 20 healthy adult male and female subjects under fasting conditions. In each of the two study periods (separated by a washout of one week) single dose of test or reference drug was administered. Blood samples were taken up to 60 h post dose, the plasma was separated and the concentration of minocycline were determined by HPLC-UV method.

In this study, the mean (SD) AUC_t, AUC_{inf}, C_{max}, and $t_{½}$ of minocycline from the test drug were 17272.46 (3316.80) ng.h.mL^{-1}, 19438.68 (3862.36) ng.h.mL^{-1}, 938.75 (192.92) ng/mL, and 19.46 (4.90) h, respectively, with the median (range) t_{max} of minocycline from the test drug was 2.00 (0.67 – 3.00) h. The mean (SD) AUC_t, AUC_{inf}, C_{max}, and $t_{½}$ of minocycline from the reference drug were 16999.33 (3103.27) ng.h.mL^{-1}, 19078.66 (3401.97) ng.h.mL^{-1}, 944.19 (188.56) ng/mL, and 18.90 (4.84) h, respectively, with the median (range) t_{max} of minocycline from the reference drug was 2.00 (1.00 – 3.00) h. The geometric mean ratios (90% C.I.) of the test drug/reference drug for minocycline were 101.36% (97.85 – 105.00%) for AUC_t, 101.53% (98.31 – 104.85%) for AUC_{inf}, and 99.22% (95.92 – 102.63%) for C_{max}, respectively.

Based on this study, it can be concluded that the two minocycline capsules (test and drug reference drug) were bioequivalent in term of the rate and extent of absorption.

Keywords: Minoc ycline; Minocycline hydrochloride; CAS 10118-90-8; CAS-13614-98-7; Borymycin; Bioavailability; Bioequivalence; Pharmacokinetic

Introduction

Bioavailability and bioequivalence of drug products have emerged as critical issues in pharmacy and medicine during the last three decades. Bioavailability is a pharmacokinetic term that describes the rate and extent to which the drug ingredient is absorbed from a drug product and becomes available in the systemic circulation. The area under the concentration versus time curve (AUC) serves as the extent of absorption, the time to reach the peak concentration (t_{max}) reflects the rate of absorption, while the peak concentration (C_{max}) reflects both the extent and the rate of absorption.

Minocycline hydrochloride is a semisynthetic derivate of tetracycline. Minocycline occurs as a yellow crystalline powder. It is sparingly soluble in water, slightly soluble in alcohol; practically insoluble in chloroform and in ether; soluble in solutions of alkali hydroxides and carbonates. Minocycline is chemically described as 4,7-bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12, 12a-tetrahydroxy-1,11- dioxo- 2 - naphthacenecarboxamidemono - hydrochloride with an empirical formula of $C_{23}H_{27}N_3O_7$.HCl. Minocycline has a molecular weight of 493.94. A 1% solution in water has a pH of 3.5 to 4.5. It should be stored in airtight containers and protected from lights [4].

Minocycline has the following structure (Figure 1):

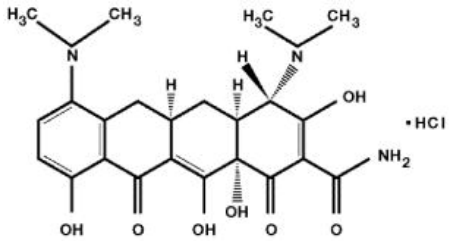

Figure 1: Chemical structure of minocycline.

Minocycline, a member of the tetracycline class of antibiotics, has a broad spectrum of activity. It is a bacteriostatic and exerts its antimicrobial activity by inhibiting protein synthesis. Minocycline hydrochloride is rapidly absorbed from the gastrointestinal tract following oral administration. When minocycline hydrochloride

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capsules were given concomitantly with a meal, which included dairy products, the extent of absorption of minocycline hydrochloride capsules was slightly decreased (6%). The peak plasma concentrations were slightly decreased (12%) and delayed by 1.09 hours when administered with food, compared to dosing under fasting conditions.

Minocycline is more lipid soluble than doxycycline and the other tetracyclines and widely distributed in body tissues and fluids, but the penetration into the CSF is relatively poor. It crosses the plasenta and is distributed into breast milk. About 75% of minocycline in the circulation is bound to plasma proteins. It has a low renal clearence: only about 5 to 10% of the dose is excreted in the urine and up to 34% is excreted in the faeces. However, in contrast to most tetracyclines it appears to undergo some metabolism in the liver, mainly to 9-hydroxyminocycline.

The pharmacokinetic parameters of minocycline following an administration of 100 mg minocycline hydrochloride capsule obtained from previous BE study based on literature [4] were attained in 1 to 3 hours (average 1.71 hours) and ranged from 491.71 to 1292.70 ng/mL (average 758.29 ng/mL) for maximum serum concentrations. The serum half life in the normal subjects ranged from 11.38 to 24.31 hours (average 17.03).

The most common adverse events reported after administration of minocycline are dizziness or vertigo, arthralgia, myalgia, pulmonary infiltration, anaphylaxis, alopecia, myocarditis, pigmentation of the skin and other tissues and vasculitis.

The objective of this study was to investigate the pharmacokinetic and bioavailability of two different oral minocycline formulations following single dosing in healthy adult subjects in order to prove the bioequivalence between both preparation.

Subjects, Materials, Methods

Subjects and study design

The study was performed at PT Equilab, Jakarta-Indonesia and was conducted according to the Declaration of Helsinki and the GCP, and GLP Guideline. The study protocol was reviewed and approved by the the committee of The Faculty of Medicine, University of Indonesia, Jakarta- Indonesia.

The test formulation was Borymycin (100 mg minocycline), capsule (batch number TI 003), manufactured by Y.S.P. Industries (M) Sdn. Bhd. Selangor Darul Ehsan, Malaysia. The reference formulation was Apo-Minocycline® (100 mg minocycline) capsule, produced by Apotex Canada. Canada was purchased at a local pharmacy in Malaysia.

Twenty (20) healthy subjects, 15 males and 5 females, aged between 20 and 46 years, body weight within normal range (BMI= 18.17 – 25 kg/m^2), blood pressure within normal range (100 – 120 mmHg for systolic, and 60 – 80 mmHg for diastolic), pulse rate between 60 and 90 bpm, that had signed the informed consent were included in this study.

At least one week before and during the study period, the subjects were not allowed to take any drug including food supplement and herbal medicine. Pregnant women, nursing mothers, women childbearing potential without adequate contraception, subjects with known contraindications or hypersensitivity to minocycline, chronic gastrointestinal problems, liver dysfunction, clinically significant hematology abnormalities, significant electrocardiogram (ECG) abnormalities, renal insufficiency, and positive test results for HBsAg, anti-HCV, and/or anti -HIV , any surgical or medical condition (present or history) which might significantly alter the absorption, distribution, metabolism or excretion of the study drug, e.g. gastrointestinal disease including gastric or duodenal ulcers or history of gastric surgery, a donation or loss of 500 mL (or more) of blood within 3 months before this study's first dosing day, history of drug or alcohol abuse within 12 months prior to this screening, participation in a previous study within 3 months of this study's first dosing day were excluded from the study, as assessed of physical examination, vital signs (blood pressure, pulse/heart rate, respiratory rate and temperature), and laboratory values of liver function (AP, ALT, AST and total/direct bilirubin); renal function (serum creatinine and ureum); routine hematology (haemoglobin, leucocyte count, platelet and leucocyte differential count); blood glucose; routine urinalysis (pH, glucose, protein, and urine sediment), and immunology test for HBsAg, anti-HCV, and anti-HIV within 14 days prior to their first dosing day.

Treatment phase and blood sampling

Subjects attended to PT Equilab International a night before drug administration and they were requested to fast from any food and drink except mineral water from 21:00 PM. In the morning (approximately 06:00 AM) of the dosing day (day 1), after an overnight fast, a pre-dose pharmacokinetic blood sample was taken. Then the study drug (one capsule of Borymycin 100 mg or one capsule of Apo-Minocycline® 100 mg) were given at 07.00 AM with 200 mL of water and swallowed whole without chewing the drug in sitting posture. Subjects remained seated for 2 hours after drug administration, and avoiding severe physical exertion.

The venous blood samples were withdrawn 10 mL immediately before taking the drug (control), and 5 mL each at 0.33, 0.67, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, and 60 hours after drug administration. One week after the first drug administration (washout period), the same procedure was repeated the alternate drug.

The date and the time of taking each sample were recorded in the CRF. Lunch and dinner were provided 4 hours and 10 hours after drug administration.

The amount of food and water intake and physical activity for each individual subject were standardized during the sampling days. Xanthine-containing food or beverages and fruit juices were not allowed for 24 hours before and during the entire sampling days.

The subjects were under direct medical supervision during the days of minocycline administration. Blood pressure, heart rate and adverse events were monitored during the blood sampling and also on follow-up study.

One physician and two nurses with sufficient qualifications and training were present at dosing time and stayed at the site until the last subject left the study unit; thereafter they were reachable by mobile telephone.

Assay Methodology

Method of analysis

The minocycline concentrations in plasma were assayed using a fully validated high performance liquid chromatography with ultra violet detection (HPLC-UV) method, with respect to adequate

sensitivity, specificity, linearity, recovery, accuracy and precision (both within and between days). The Stability of the samples under frozen conditions, at room temperature, and during freeze-thaw cycle were also determined. The following data were taken from the validation report: Calibration curve for minocycline ranged from 50.38 to 6045.83 ng/mL; linear relationship between concentration and signal intensity were obtained (r = 0.9997); the limit of quantitation (LoQ) was 50.38 ng/mL; precision: intra-assay coefficient of variation were 1.12%, 0.53%, and 0.76% at low (151.15 ng/mL), medium (1511.46 ng/mL), and high (5038.19 ng/mL) concentrations, respectively; inter-assay coefficient of variations were 6.13%, 2.09%, and 1.57% at low, medium, and high concentrations, respectively; accuracy: intra assay (% diff) were ranged from -7.16% to -4.95% for the low concentration, -2.32% to -0.92% for the medium concentration, and from +5.06% to +6.99% for the high concentration; inter assay (% diff) were ranged from -7.16% to +10.99% for the low concentration, -4.49% to +2.61% for the medium concentration, and from +4.90% to +10.84% for the high concentration.

Assay procedure

The procedure was applied for the extraction of subject samples, calibration and quality control standards. Plasma sample was dispensed in an appropriate tube, and an appropriate solvent was added. The content of the tube was vortexed and centrifuged. The organic phase was transferred to a vial and an aliquot was injected into the HPLC-UV at λ 355 nm system with a suitable condition. Calibration standards, controls, and samples were processed in batches.

Instrument and analysis conditions

A liquid chromatography with Sunfire C18 (5 µm) 3.9x150 mm (Waters, 2695) and pre column of Symmetry C18 5µm, 3.9 x 20mm were used for separation of minocycline. The mobile phase was acetonitrile and disodium hydrogen phosphate : $Na2HPO_4$ 50 mM pH 3,80 (25 : 75, v/v). The flow rate was 0.9 mL/min and the injection volume was 50 µL. Detection were performed with ultra violet at 355 nm.

Pharmacokinetic evaluation

The non-compartmental pharmacokinetic analysis method was employed to determine the pharmacokinetic parameter of minocycline. C_{max} and t_{max} were obtained directly from the observed data. The AUC_t is calculated by the trapezoidal method. The AUC_{inf} was calculated as $AUC_t + C_t/k_e$, where C_t was the last quantifiable concentration; k_e was the terminal elimination rate constant and was determined by least-squares regression analysis during the terminal log-linear phase of the concentration–time curve. The $t_{1/2}$ was calculated as $0.693/k_e$.

Statistical evaluation

EquivTest version 2.0 (Statistical Solution Ltd., Saugus, MA, USA) was used to perform the statistical analyses of AUC_t, AUC_{inf}, and C_{max} using analysis of variance (ANOVA) after transformation of the data to their logarithmic (ln) values. Using the error variance (S^2) obtained from the ANOVA, the 90% confidence intervals (CIs) were calculated from the following equation:

$$90\%\ CI = (\bar{X}_T - \bar{X}_R) \pm t_{0.1(v)} \sqrt{(s^2 \times 2/n)}$$

- $\bar{X}_T, \bar{X}_R$: the means of the ln transformed values for the test product (T) and the reference product (R)
- S^2 : the error variance obtained from the ANOVA
- n : the number of subjects
- $t_{0.1}$: the t value for 90% CI
- v : the degree of freedom of the error variance from the ANOVA

The anti ln of the above confidence intervals were the 90% Cls of the ratios of the test / the reference geometric means.The power of study would be 90 % with 0.05 alpha.The acceptance criteria for bioequivalence were that the 90% Cls of the geometric mean ratios 0.80 – 1.25 for the AUC and C_{max}. The t_{max} difference was analyzed non-parametrically on the original data using Wilcoxon matched-pairs test. The $t_{1/2}$ difference was analysed using Student's paired t-test.

Results

A total 20 subjects were invited to participate in this study (Gender and age on what basis-reference). There was no dropout case in this study. There were two adverse events, nausea and myalgia encountered during the study. Mean plasma concentrations versus time profiles of minocycline after administration of both formulation in healthy subjects (n=20) are shown in Figure 2. The mean plasma concentration-time curve of the test product and the reference drug were comparable.

In this study, the mean (SD) AUC_t , AUC_{inf}, C_{max}, and $t_{½}$ of minocycline from the test drug were 17272.46 (3316.80) $ng.h.mL^{-1}$, 19438.68 (3862.36) $ng.h.mL^{-1}$, 938.75 (192.92) ng/mL, and 19.46 (4.90) h, respectively, with the median (range) t_{max} of 2.00 (0.67– 3.00) h. The mean (SD) AUC_t, AUC_{inf} , C_{max}, and $t_{½}$ of minocycline from the reference drug were 16999.33 (3103.27) $ng.h.mL^{-1}$, 19078.66 (3401.97) $ng.h.mL^{-1}$, 944.19 (188.56) ng/mL, and 18.90 (4.84) h, respectively, with the median (range) t_{max} of 2.00 (1.00 – 3.00) h. The pharmacokinetic parameters AUC_t, AUC_{inf}, C_{max}, and t_{max} were used for bioequivalence evaluation. The reference drug are presented in Table 1.

AUC_t, AUC_{inf} and C_{max} of test drug and the reference drug are presented in Table 1. The 90% confidence intervals for geometric mean ratios of test/reference for AUCt, AUC_{inf} and C_{max} were within the acceptable limits (80 -125%) of bioequivalence which implies that the bioequivalence criteria were met. Evaluation of original data

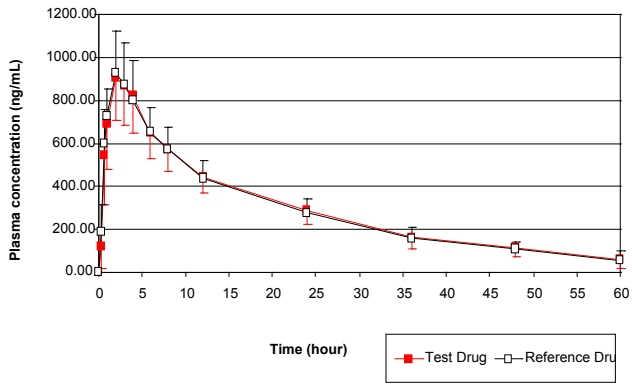

Figure 2: Mean plasma concentration- time profiles of minocycline in healthy subjects (n=20) after oral administration of 100 mg minocycline capsule (test) and that produced by the innovator (reference).

Parameter	Test product mean (SD)	Reference product mean (SD)	Mean ratio of T/R (90% CI)	Wilcoxon matched-pairs / paired - t
AUC_t (ng.h/mL)	17272.46 (3316.80)	16999.33 (3103.27)	101.36% (97.85 – 105.00)%	–
AUC_{inf} (ng.h/mL)	19438.68 (3862.36)	19078.66 (3401.97)	101.53% (98.31 – 104.85)%	–
C_{max} (ng/mL)	938.75 (192.92)	944.19 (188.56)	99.22% (95.92 – 102.63)%	–
t_{max} (h)	2.00 (0.67 – 3.00)*	2.00 (1.00 – 3.00)*	–	NS
$t_{½}$ (h)	19.46 (4.90)	18.90 (4.84)	–	NS

* Median (range)
NS = not significantly different

Table 1: Pharmacokinetic parameters and statistical comparison of minocycline after 100 mg minocycline capsule single dose administration of the test and reference drug in 20 healthy volunteers.

with Wilcoxon matched-pairs test showed that was no statistically significant difference between the two formulations for t_{max} values.

Discussion

The aim of the present randomized, single-blind, two-period, cross-over study with one week wash-out period was to compare the bioavailability of the test minocycline capsule produced by Y.S.P. Industries (M) Sdn. Bhd. (Borymycin 100 mg) with the reference minocycline capsule (Apo-Minocycline® 100 mg). The formulation was administered to overnight fast in order to eliminate the influence of food on drug absorption.

For bioequivalence study, AUC_t, C_{max} and t_{max} were the main target parameters in order to assess possible bioequivalence between both preparations. Based on bioequivalence guideline, the acceptance criteria for bioequivalence were that the 90% confidence intervals of the the test/reference geometric means ratio for both compounds were in the range 0.80 – 1.25 for the AUC and C_{max}.

T he result of this study showed that the 90% confidence intervals of the test/reference AUC ratio and C_{max} ratio were within the acceptable range for bioequivalence.

The mean (SD) elimination half-lives ($t_{½}$) of minocycline for the test drug was 19.46 (4.90) h and for the reference drug was 18.90 (4.84) h. These values were within the minocycline half-life range based on the literature, which is 11.38 – 24.31 h (mean: 17.03 hours). In each subject, the AUC_t value of minocycline was more than 80% compared to the value of AUC_{inf} (% AUC_t / AUC_{inf} ratio > 80%, 80.32 to 93.99% for test drug and 80.52 to 94.83% for reference drug), indicating that the sampling time was sufficiently long to ensure an adequate description of the absorption phase.

The median (range) of the time to reach maximum minocycline plasma concentration (t_{max}) of the test drug was 2.00 (0.67 – 3.00) h and 2.00 (1.00 – 3.00) h for the reference drug. Using Wilcoxon matched-pairs test on the original data, the difference between the two drugs (test and reference drug) t_{max} values was not significantly different. These values were also within the minocycline t_{max} range in the literature, which is 1 to 3 hours after administration of minocycline capsules.

In the present study, the intra subject coefficient of variance (% CV) obtained from the ANOVA for minocycline was 6.40%, it means that the number of subjects in this study (20 subjects) was adequate to ensure that this study has adequate power to confirm a statistical conclusion.

There was no dropout case in this study. There were two adverse events, nausea and myalgia encountered during the study. Among the 20 subjects who received the study drug, nausea was encountered in 1 subject (S 5) during period 1, and myalgia was encountered in 1 subject (S 14) during period 1.

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