

Bioequivalence Study of Two Loperamide Hydrochloride 2 mg Formulations: An Open-Label, Randomized, Single-Dose, Two-Way Crossover Study in Healthy Volunteers under Fasting Conditions

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Abstract

The objective of this study was to compare the rate and extent of drug absorption of the test product (Colodium 2 mg Capsule, Hovid Bhd.) against the reference product (Imodium® 2 mg Capsule, Janssen Cilag S.A.) in twenty-three healthy male volunteers under fasting condition in order to evaluate bioequivalence. A single dose of 8 mg (4 capsules of 2 mg each) of test and reference products were given to volunteers during two periods of the study respectively. There was a 7 day washout period between the two study periods. Blood samples were taken at pre-dose and at 13 time points up till 48 h post dosing. Plasma levels of loperamide were determined by liquid chromatography-tandem mass spectrometry. The plasma concentration-time data was used to estimate the pharmacokinetic parameters, namely, C_{max} , T_{max} , AUC_{0-4} and $AUC_{0-\infty}$. Analysis of variance (ANOVA) procedure was used to analyze the values of C_{max} , AUC_{0-4} , $AUC_{0-\infty}$ and k_e obtained from the two preparations. For the analysis of T_{max} values, the Wilcoxon Signed Rank Test for paired samples was used. In this study, the 90% confidence interval for the ratio of the AUC_{0-4} , $AUC_{0-\infty}$ and C_{max} were calculated to be between 0.8730-1.0181, 0.8852-0.9891 and 0.8023-0.9559 respectively. All of the values were within the acceptable bioequivalence requirement of 0.8000-1.2500. No drug-related adverse event was reported throughout the study. Thus, the two preparations could be concluded to be bioequivalent and interchangeable.

Keywords: Bioequivalence; Generic; Loperamide; Antidiarrheal

Introduction

Loperamide, a phenylpiperidine derivative [1], is an anti-diarrhoeal medication which acts selectively on peripheral μ -opioid receptors [2]. It is widely used for acute and chronic diarrhoea. Its efficacy is also proven in treating patients with irritable bowel syndrome who suffer predominantly from diarrhea [2].

By binding to the μ -opioid receptors in the gut wall, loperamide decreases peristalsis and fluid secretion, resulting in longer gastrointestinal transit time and increased absorption of fluids and electrolytes from the gastrointestinal tract [1]. This decreases the number of bowel movements and improve the consistency of the stools [2]. Loperamide has also been shown to reduce sensitivity of the recto-anal inhibitory reflex and increase internal anal sphincter tone, making it a potential candidate for treating faecal incontinence [3].

Loperamide is absorbed mainly from the gut. However, its systemic bioavailability is only about 0.3% due to significant first pass metabolism [4]. In the distribution studies in rats, loperamide has been shown to be a P-glycoprotein substrate which has a high affinity for the receptors of the longitudinal muscle layer at the gut wall. 95% of loperamide binds to plasma protein, mainly to albumin. Loperamide is metabolized mainly via oxidative N-demethylation. It is metabolized, conjugated and excreted predominantly via the bile. The metabolic pathway is mediated by CYP3A4 and CYP2C8. In man, loperamide's half-life is about 11 h [4].

All Malaysian citizens have access to public healthcare. The expenses are largely covered by general revenue and taxation collected by the federal government. The well-established and extensive health care services are beneficial especially to poor patients who cannot afford

private healthcare services [5]. Thus, it is vital to maintain health care expenses at an affordable level to ensure that the federal government can continue to provide healthcare services to the public in the long run.

Antimotility drugs such as loperamide are the agent of choice recommended by World Gastroenterology Organization in acute and chronic diarrhea for adults to ease symptoms. It is also one of the over-the-counter drug which shows efficacy and safety as an anti-diarrheal [6]. Despite that, the innovator product's relatively high cost does not favour its wide usage. Therefore, generic product can provide a more affordable treatment alternative to healthcare providers and patients.

The aim of this study was to compare the rate and extent of drug absorption of a generic formulation of loperamide 2 mg capsule (Colodium 2 mg Capsule, manufactured by Hovid Bhd., Perak, Malaysia) against the reference formulation (Imodium® 2 mg Capsule, manufactured by Janssen Cilag S.A., France) under fasting condition in order to evaluate bioequivalence.

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Methods

Study protocol

The Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia approved the study protocol. All procedures were conducted in accordance to the Malaysian Good Clinical Practice (GCP) Guideline and informed consents were obtained prior to study enrolment. The clinical facility for this study was situated at the Clinical Research Centre, Seberang Jaya Hospital (Penang, Malaysia) while the bioanalytical facility was located at the BA/BE Laboratory, Universiti Sains Malaysia (Penang, Malaysia).

Participants

Healthy Malaysian male volunteers aged between 21 to 55 years old who had body mass index between 18.5 to 29.9 or within 20% of ideal body weight for height and build according to the Metropolitan Life Insurance Company Standard, were recruited at the clinical research centre by GCP-certified investigators. Clinical evaluations conducted during screening include detailed medical history, physical examination, 12 lead electrocardiogram and laboratory tests namely liver function test, renal function test, full blood count and fasting blood glucose level.

Subjects with a history or suspicion of drug dependence and/or alcohol abuse, significant clinical deviation from normal as determined by investigators, requirement of tranquilizers, sedatives or medications for chronic diseases will be excluded. Other exclusion criteria included hypersensitivity to loperamide, subjects had donated blood or participated in any bioequivalence study for the past 8 weeks, heavy smoker (i.e., more than 10 cigarettes a day), or unable to demonstrate ability to read, understand and/or comply to the study protocol or to give consent.

Study design

This was a single center, single-dose, randomized, open-label, two-way crossover study under fasting condition. In the Summary of Product Characteristics of Loperamide, it was recommended to take the capsules with liquid, irrespective of food intake [4].

The subjects were equally divided into two groups randomly; where the first group received the products in the sequence of reference-test (RT) while the second group had the sequence of test-reference (TR). There was a period of at least seven days as washout between the two phases.

All volunteers were required to fast for at least 10 h prior to dosing. A single dose of 8 mg (4 capsules of 2 mg each) of either test or the reference formulation was administered with 240 ml of plain water under the supervision of a qualified pharmacist. Besides the water used for drug administration, participants were not allowed to drink water for one hour before and one hour after dosing. Post dosing, food was withheld for at least 4 h. Then, standardised, calorie-counted meals were served at 4 h and 10 h after dosing while standardised snacks were given at 7 h and 13 h after dosing.

By referring to the T_{max} and $t_{1/2}$ of loperamide, blood samples were collected in blood collection tubes (containing sodium heparin as anticoagulant) at 0 (pre-dose), 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 16, 24 and 48 h after dosing. 5% deviation from the scheduled blood sampling time was allowed before being considered as protocol deviation. At the end of the 24 h sampling period, subjects were discharged from the clinical facility. They were required to return for blood sampling at 48 h. A

total of approximately 217 ml of blood were taken from each subject for the entire study (105 ml during each phase and 7 ml for blood chemistry analysis during screening). The collected blood samples were immediately centrifuged for 15 min at 3500 rpm and the plasma samples were transferred to a separate glass tube where they were kept frozen at -20°C until analysis.

The sample size was estimated using the intra-subject coefficient of variation (CV). Based on previous bioequivalence study on loperamide capsule, the CV values for AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} ranged from approximately 18.4% to 23.2%. Therefore, according to the nomogram published by Diletti et al. [7], 24 volunteers would be required for the present study in order to achieve a statistical power of 80% by assuming that the μ_T/μ_R does not deviate by more than 5%.

Randomization and blinding

The volunteers were randomised equally into the TR or RT group by using randomisation software. This study was an open-label trial, where the investigators and subjects were not blinded as only objective measurements (plasma concentrations) were collected and no subjective data were obtained. Nevertheless, the randomisation list was only available to the bioanalytical team after the analysis was completed.

Drug analysis

The analysis of the plasma levels of loperamide was done using a liquid chromatography-tandem mass spectrometry (LC-MS-MS) in accordance with the Good Laboratory Practice. The liquid chromatography system was made up of an Agilent 1200 Series binary pump (Agilent, Waldbronn, Germany), a Agilent 1200 Series degasser (Agilent, Waldbronn, Germany), a Agilent 1200 Series thermostatted column compartment (Agilent, Waldbronn, Germany) and a Agilent 1200 Series instant pilot (Agilent, Waldbronn, Germany). Applied Biosystems API 3200 triple quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Ontario, Canada) in positive electrospray ionization (ESI) mode was used to perform MS/MS analyses. Data acquisition and analysis were conducted using Analyst version 1.4.2 (Applied Biosystems/MDS SCIEX, Ontario, Canada).

The chromatographic separation was performed at 25°C using a C18 analytical column. The mobile phase was a mixture of 30% of 5 mM ammonium acetate and 70% acetonitrile, adjusted to pH 5.0. The separation was run isocratically at a flow rate of 0.2 ml/min. The injection volume was 10 μ l and the samples were quantified using peak area.

The detector response for loperamide showed linearity over a concentration range of 0.08-40.00 ng/ml (correlation coefficient \geq 0.99). The limit of quantification was set at 0.08 ng/ml while the limit of detection was set at 0.04 ng/ml. The extraction recovery of loperamide was all above 80%.

Tolerability

At pre-dose, 4 h, 10 h and 24 h post dose, subjects' vital signs i.e. blood pressure, pulse rate; respiratory rate and temperature were recorded. Subjects were also asked to report any discomfort or adverse events at any time during the study period.

Pharmacokinetics analysis

The plasma concentration-time data was used to estimate the pharmacokinetic parameters, namely, maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), area under the plasma concentration-time curve from time zero to the last

measurable concentration (AUC_{0-t}) and total area under the plasma concentration-time curve ($AUC_{0-\infty}$). The values of C_{max} and T_{max} were obtained directly from the plasma values [8]. The value $AUC_{0-\infty}$ was obtained by adding the values of AUC_{0-t} and $AUC_{t-\infty}$. AUC_{0-t} was calculated by adding the area from time zero to last sampling time using the trapezoidal formula while $AUC_{t-\infty}$ was obtained by adding the area from time t to infinity and dividing the last measurable plasma drug concentration with the elimination rate constant (k_e).

The k_e of loperamide was estimated from the terminal slope of the individual plasma concentration-time curves after the logarithmic (ln) transformation of at least three plasma concentration values and application of linear regression [9]. The half-life ($t_{1/2}$) was derived from the equation: $t_{1/2} = \ln 2 / k_e$.

Statistical analysis

The statistical analysis was performed using the commercial software, EquivTestPK from Statistical Solution (Cork, Ireland). To distinguish effects due to subjects, periods, and treatments, analysis of variance (ANOVA) procedure was used to analyze the values of C_{max} , AUC_{0-t} , $AUC_{0-\infty}$ and k_e obtained from the two preparations [10]. The values of C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ were logarithmic transformed (natural log) before analysis. For the analysis of T_{max} values, the Wilcoxon Signed Rank Test for paired samples was used.

Bioequivalence was determined based on the 90% confidence intervals for the ratio of the C_{max} , AUC_{0-t} , $AUC_{0-\infty}$ values of test over reference formulations. The 90% confidence intervals were calculated by using the two one-sided test procedure at the $\alpha=5\%$ level of significance [11]. The 90% confidence interval of the ratio of C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ should fall between 80.00-125.00% (transformed values) [12]. The Malaysian Guideline for Conduct of Bioavailability and Bioequivalence Studies stipulated a similar range for AUC_{0-t} and $AUC_{0-\infty}$, but allowed a wider range for C_{max} when it was appropriately justified.

Results

The basic demographic characteristics of 24 subjects are as shown in Table 1.

There was one subject who withdrew from the study due to personal reasons. The remaining 23 participants successfully completed both phases of the study and the plasma samples collected were used for pharmacokinetic analysis. There was no significant deviation from the protocol arisen throughout the study.

Figure 1 showed the mean plasma loperamide concentration versus time profiles for both test and reference formulations while Table 2 showed the pharmacokinetic parameters for both formulations. As

Variable		n (%)	Median (IQR)	Mean (SD)
Age (year)			26.0 (10.00)	
Race				
	Malay	17 (70.8)		
	Chinese	5 (20.8)		
	Indian	1 (4.2)		
	Indonesian	1 (4.2)		
Height (cm)				169.1 (73.61)
Weight (kg)				70.2 (11.35)
Body Mass Index (kg/m ²)				24.5 (3.21)

Legends: SD=Standard Deviation; IQR=Interquartile Range

Table 1: Basic demographic characteristics of 24 subjects.

Pharmacokinetic Parameters	Test Formulation, Mean (SD)	Reference formulation, Mean (SD)
AUC_{0-t} (hr.ng/ml)	23.4 (9.43)	24.8 (9.64)
$AUC_{0-\infty}$ (hr.ng/ml)	26.8 (10.04)	28.7(10.20)
C_{max} (ng/ml)	1.9 (0.65)	2.2 (0.84)
T_{max} (hr)	4.0 (1.80)	2.8 (1.44)
k_e (hr ⁻¹)	0.07 (0.036)	0.07 (0.062)
$t_{1/2}$ (hr)	12.5 (3.87)	12.5 (4.22)

SD: Standard Deviation, AUC_{0-t} : Area under the Plasma-Concentration Curve from Dosing to Last Quantifiable Time Point; $AUC_{0-\infty}$: Area under the Plasma-Concentration Curve from Dosing to Infinity; C_{max} : Peak Plasma Concentration; T_{max} : Time to reach Peak Plasma Concentration; k_e : Elimination Rate Constant; $t_{1/2}$: Half-life

Table 2: Pharmacokinetic parameters of the test Loperamide formulation vs. the reference formulation after oral administration under fasting condition.

shown in Figure 1, both test and reference profiles were superimposable and demonstrated comparable C_{max} values. Both formulations reached peak plasma concentrations at approximately 2.0-5.0 h after administration.

The logarithmic transformed values of AUC_{0-t} ($p=0.28$) and $AUC_{0-\infty}$ ($p=0.11$) of both preparations were not significantly different based on ANOVA analysis. However, the logarithmic transformed values of C_{max} ($p=0.037$) showed a statistically significant difference between the two preparations. For the T_{max} value ($p=0.004$), there was a statistically significant difference too between two products as shown by Wilcoxon Signed Rank Test.

Bioequivalence can be concluded based on the 90% confidence interval for the ratio of the AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} of the test formulation (Colodium 2 mg capsule) over reference formulation (Imodium 2 mg capsule). In this study, the 90% confidence interval for the ratio of the AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} were calculated to be between 0.8730-1.0181, 0.8852-0.9891 and 0.8023-0.9559 respectively. All of the values were within the acceptable bioequivalence requirement of 0.8000-1.2500.

The analysis of the plasma samples was completed within the predetermined one month long term stability period.

The intra-subject coefficient of variation values estimated using the mean square error of the ANOVA analysis for AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} are 17.18%, 12.54% and 17.18% respectively [7]. Based on these values, the 23 subjects used in the study were found to be sufficient to provide a power ($1-\beta$) of 80% to conclude that the two formulations are equivalent where type 1 error (α) is 0.05 [7].

The inter-subject coefficient of variation values for AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} were 50.6%, 49.2% and 38.1% respectively by estimating the values using the mean square error of the ANOVA analysis.

Tolerability analysis

There was no drug-related adverse reaction observed or reported throughout the study.

Discussion

The half-lives (mean, standard deviation) found in this study were also agreeable with the previous literature reports of 11.2 (0.8) h [13]. The half-life of test formulation was 12.5 (4.22) h while the reference formulation was 12.5 (3.87) h. Thus, the washout period determined in this study (>5 half-life) was enough to allow the loperamide concentration in all subjects to fall below the lower limit of bioanalytical quantification before the second phase of the study resumed.

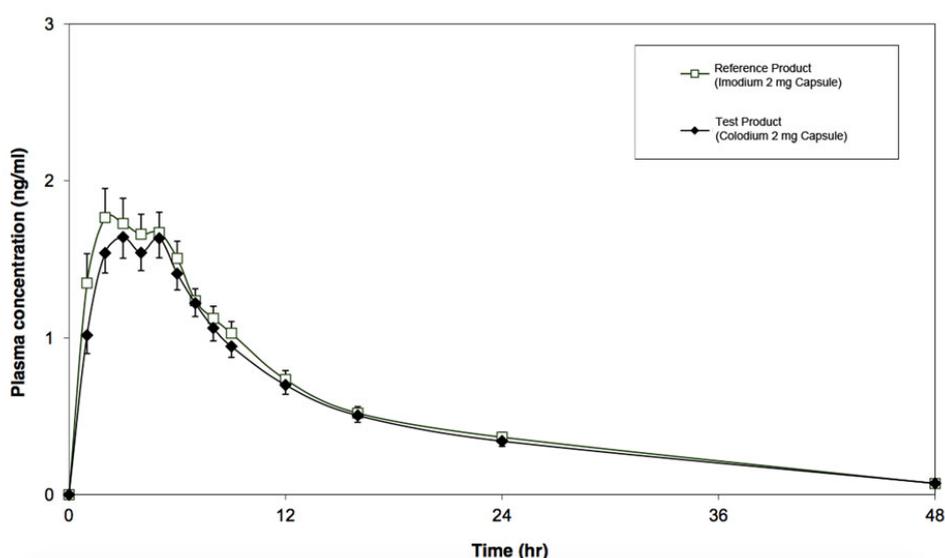


Figure 1: Mean plasma loperamide concentration versus time profiles of both reference formulation (Imodium 2 mg Capsule, manufactured by Janssen Cilag S.A., France) and the test formulation (Colodium 2 mg Capsule, manufactured by Hovid Berhad, Perak, Malaysia) after oral administration under fasting condition in healthy volunteers. Mean \pm SEM. N=23

A study by Yu et al. [14] which used the same dose of loperamide (4×2 mg) was evaluated in 8 healthy male volunteers. The pharmacokinetic values of C_{max} were 1.18 ± 0.37 ng/ml, T_{max} 5.38 ± 0.74 h and half-life $T_{1/2}$ 11.35 ± 2.06 h. The values were comparable with the pharmacokinetic values of the test products in our study. We obtained pharmacokinetic values (mean, standard deviation) of C_{max} $1.9 (0.65)$ ng/ml, T_{max} $4.0 (1.8)$ h and half-life $T_{1/2}$ $12.5 (3.87)$ h [14].

Conclusion

This study concludes that the test formulation (Colodium 2 mg capsule) is bioequivalent to the reference formulation (Imodium[®] 2 mg capsule).

Acknowledgement

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Declaration of Personal Interest

Kah Hay Yuen was the advisor to the R&D department of Hovid Bhd., the manufacturer of the test formulation. Siew Siew Tan, Jia Woei Wong, Siaw Kuen Chin, Ai Boey Lim and Ean Peng Soon are employees of Attest Research Sdn Bhd, an independent research company. Wen Yao Mak, Yi Lin Lee and Irene Looi did not have any conflict of interest to disclose.

Declaration of Funding Interest

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