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Original article

Bioequivalence of indinavir capsules in healthy volunteers

Suvatna Chulavatnatol[a], Kumthorn Malathum[b], Sasisopin Kiertiburanakul[b], Kittisak Sripha[c], Pojawon Lawanprasert[d]

[a]Department of Pharmacy, Faculty of Pharmacy, [b]Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, [c]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, [d]Department of Manufacturing Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand

Background: Indinavir, one component in the HAART regimen, plays an important role in the current treatment of HIV-infection and AIDS. Availability and accessibility of qualified generic indinavir to patients may be the keys for the success of treatment.

Objective: Compare the rate and extent of absorption of a generic indinavir formulation with those of an original formulation in healthy Thai volunteers.

Method: A randomized, two-period, two-treatment, two-sequence, crossover study with a two-week washout period was performed. A single dose of 2x400 mg indinavir capsules of each formulation was administered to 24 volunteers after an overnight fast. Indinavir plasma concentrations up to 10 hours postdose were determined using high-performance liquid chromatography. Relevant pharmacokinetic parameters were derived and tested for statistically significant differences using ANOVA and criteria of bioequivalence determination were applied.

Results: No statistically significant differences were demonstrated for pharmacokinetic parameters including C_{max}, T_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ derived from the two formulations (n=23, p>0.05). The criteria of bioequivalence determination i.e., the 90% confidence intervals on the mean ratio (generic/original formulation) of natural logarithm-transformed values of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were 86.3-106.5%, 94.0-108.5%, and 93.9-108.5%, respectively.

Conclusion: As the mean ratios of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of the generic and original formulations were entirely within the guideline range of bioequivalence (80.0-125.0%), the two formulations were considered bioequivalent in terms of rate and extent of absorption.

Keywords: Bioequivalence, indinavir, pharmacokinetics, protease inhibitors, Thai volunteers

Availability of anti-HIV agents and patients' accessibility as well as patients' adherence may be the keys for the success of treatment and quality of patients' lives. However, accessibility to these agents is still a burden. According to Wainberg [1]. One strategy to overcome such problems may be availability of qualified generic anti-HIV agents [2].

Indinavir, a protease inhibitor, plays an important role in the current treatment of HIV infection and AIDS. It is employed as one component in the HAART (highly active antiretroviral therapy) regimen. After oral administration, indinavir is absorbed rapidly. It has a peak plasma concentration appears within 0.5 to 2.4 hours [3-5]. Hepatic metabolism by cytochrome P450 3A4 is the major pathway of its elimination with a mean elimination half-life of 1.8 hours [3, 4].

In Thailand, only original indinavir is available presently. This might not serve all patients with HIV-infections because of their financial constraints. The Research and Development Institute of the Government Pharmaceutical Organization (GPO), Thailand decided to develop generic indinavir formulation to provide more accessibility. However, this generic formulation must be proved to have comparable rate and extent of absorption, in other words, bioequivalence to the original formulation. We conducted this study to determine the bioequivalence between the generic indinavir formulation (GPO, Thailand) and the original formulation (Merck, Elkton, USA) in Thai healthy volunteers.

Correspondence to: Suvatna Chulavatnatol, PhD, Department of Pharmacy, Faculty of Pharmacy, Mahidol University, 447 Sri Ayudhaya Rd, Ratchathevee, Bangkok 10400, Thailand. E-mail: pyscl@mahidol.ac.th

Materials and methods

Subjects

Healthy, male and female volunteers aged between 18-45 years were recruited to the study. Their body mass indices (BMI) were between 18-23 kg/m^2. All volunteers were determined to be healthy based on medical history, physical examination, and routine laboratory tests (complete blood count, fasting blood glucose, blood urea nitrogen, serum creatinine, alanine aminotransferase, aspartate aminotransferase, total and direct bilirubin, alkaline phosphatase, and gamma glutamyl transferase) and be negative for hepatitis B surface antigen, hepatitis C viral antibody, and anti-HIV. All female volunteers were negative for pregnancy test. Volunteers were excluded if they smoked, had allergic history to indinavir, history of alcohol and/or substance abuse, had participated in any clinical trial or were using any medications within at least four weeks prior to this study, and/or had any clinically significant abnormality based on medical history, physical examination and laboratory analysis. In addition, the volunteers were asked to refrain from taking any medication or drinking alcohol for at least two weeks prior to and throughout the study period. All volunteers had been informed about the details, risks, and benefits of this study, and their unconditional right to withdraw from the study at any time and for any reason without penalty of any kind. Written informed consent was provided from all volunteers prior to participation in the study.

Study drugs

The generic formulation was indinavir 400 mg capsules (batch number R48017, developed by the Research and Development Institute, GPO, Thailand) and the original formulation was Crixivan® 400 mg capsules (Merck, Elkton, USA). Both formulations were needed to meet standard requirements of the US Pharmacopoeia (USP) 26/National Formulary (NF) 21 [6] in terms of content of active ingredient, content uniformity, and dissolution tests. To determine the content of active ingredient and content uniformity, the content of 20 combined capsules and 10 individual capsules, respectively, were used in accordance with the USP 26/NF 21 requirements. The content (mean$\pm$SD) of indinavir was 101.24$\pm$1.29% of the labeled amount for the generic formulation, while this was 100.74$\pm$1.35% for the original formulation. The content uniformity as determined from relative standard deviation (RSD) of the generic and the original formulations were 3.90% and 2.36%, respectively. Dissolution profiles were conducted in three media: 0.1 N HCl, acetate buffer pH 4.5, and phosphate buffer pH 6.8. Twelve capsules of each formulation were tested using USP Dissolution Test Apparatus II at 50 rpm. The dissolution profiles of the two formulations were similar in all three media even though both formulations did not dissolve well in phosphate buffer pH 6.8. The amount of indinavir in both formulations which dissolved within 15 minutes were more than 85% of the labeled amount both in 0.1 N HCl, and acetate buffer pH 4.5. Based on these data, the two formulations met the standard requirements [6], and the difference of the content of the generic formulation was less than 5% of that of the original formulation as required for the product specification in bioequivalence study by the regulation of the Thailand FDA [7].

Study design

A randomized, two-period, two-treatment, two-sequence, crossover study with a two-week washout period was conducted. The study protocol and the consent form were approved by the Thailand Food and Drug Administration (FDA) and the Ethics Committee of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand.

The volunteers were randomly assigned into two groups as follows:

Group A: received the generic formulation in period 1 and the original formulation in period 2.

Group B: received the original formulation in period 1 and the generic formulation in period 2.

On the study day of each period, each volunteer received a single oral dose of 2x400 mg indinavir capsules with 240 mL of water in the morning following with an at least eight-hour overnight fast. Food was abstained until four hours after drug administration. Adverse events were observed during the study period.

Blood sampling

Blood samples were drawn from the forearm vein through an indwelling catheter, which was left in place until the 10-hour blood sampling was complete. Five milliliters of blood were collected in EDTA tubes at pre-dose for blank plasma and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 10 hours after indinavir administration. The blood samples were centrifuged at 3,000 rpm for 10 minutes. Plasma was separated and kept at -20°C until analysis.

Quantitative drug analysis

The analytical method used for the determination of plasma indinavir concentrations in this study was modified from the report by Poirier et al. [8]. A solid phase extraction (SPE) was used to process the plasma samples as follows. The SPE column (Oasis HLB®, Waters, Milford, USA) was sequentially conditioned with one mL of methanol, followed by the two times washing with one mL of 50% methanol and one mL of water. Then, the column was loaded with 700 mL of plasma sample and was eluted with one mL of 5% methanol followed by 700 mL of mobile phase. Ten mL of the eluent was injected directly to high-performance liquid chromatography (HPLC) system (Thermoseparation Products, San Jose, USA) using an autosampler. A reversed phase column (Hypersil Gold®, Thermo Electron, Bellesonte, USA), C18, 5 μm-particle size, 250x4.6 mm internal diameter connected with a guard column (C18, 5 μm-particle size, 10x4.6 mm internal diameter) was used as separating column. A mixture of 50 mM phosphate buffer pH 6.0 and acetonitrile (60:40v/v) was used as a mobile phase, and was delivered at a flow rate of 1.0 mL/min. The indinavir peak was detected at 210 nm using UV variable-wavelength detector (Thermoseparation Products, San Jose, USA).

The analytical method was validated according to the guidance for industry, bioanalytical method validation [9]. The standard calibration plot was constructed by least-square linear regression of peak area on indinavir concentration in plasma. The relationship was linear (R^2= 0.9998) over a concentration range of 100-20,000 ng/mL. The unknown indinavir concentration in plasma sample was determined by inverse prediction from the standard calibration plot weighted by the reciprocal of the variance [10]. The intra- and inter-day coefficient of variations on the assay were less than or equal to 6.09% and less than or equal to 6.58%, respectively. The lower quantification limit of indinavir concentration in plasma was 100 ng/mL. The recovery of the analytic method was greater than 94% while the accuracy was greater than 95%. The processed indinavir plasma samples could be left in the autosampler at ambient temperature for 8 hours and indinavir in plasma was stable for at least four months when stored at -20°C.

Pharmacokinetic analysis

Maximum plasma concentration (C_{max}) and time to maximum concentration (T_{max}) were directly determined from the concentration-time data of individual volunteer. Based on the standard non-compartmental method, we derived other pharmacokinetic parameters from individual volunteer data as follows: area under the plasma concentration-time curve (AUC), elimination rate constant (k_e), and elimination half-life ($t_{1/2}$). The AUC from time zero to time of last quantifiable concentration (AUC_{0-t}) was determined by the linear trapezoidal rule. The AUC extrapolated to infinity ($AUC_{0-\infty}$) was the sum of AUC_{0-t} and C_t/k_e, where C_t was the last quantifiable plasma concentration, and k_e was determined from the slope of the terminal logarithm-linear portion of the plasma concentration-time curve by the least-square regression analysis. This k_e was used to calculate the $t_{1/2}$ by the formula $0.693/k_e$.

In the above calculation, we used the WinNonlin Professional program version 5.1 (Pharsight, Mountain View, USA).

Statistical analysis

Pharmacokinetic parameters (C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$) of the two formulations were compared by analysis of variance (ANOVA) using natural logarithmic-transformed data to evaluate the effects of formulation, period, sequence, and subject (within sequence) at the significance level (α) of 0.05. The 90% confidence intervals (CIs) of the mean ratio of the generic to the original formulation for the natural logarithmic-transformed values of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were calculated. The generic formulation was accepted to be bioequivalent to the original formulation if the 90% CIs of those ratios were within 80.0-125.0% [7, 11, 12].

Results

Twenty-four healthy volunteers, 13 males and 11 females, were enrolled. One volunteer needed to delay his second period of drug administration for another two weeks. Accordingly, the data of 23 volunteers were used in the data analysis. With 23 volunteers, the mean age (± SD) was 25.17 (± 3.85) years (range 21-34 years), and the mean weight (±SD) was 54.28 (5.81) kg (range 46-67 kg), while the mean BMI (±SD) was 20.48 (±1.60) kg/m^2. The plasma concentration-time profiles of each formulation are demonstrated in **Fig. 1**.

Table 1 presents all pharmacokinetic parameters derived from the two formulations. The ANOVA tests of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ between the two formulations show no statistically significant

differences (**Table 2**). The 90% CIs of the ratio of natural logarithmic-transformed data of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were shown in **Table 3**.

During the study, bitter taste was reported from four volunteers as adverse events. One volunteer reported when the generic formulation was taken, two reported when taking the original formulation, and the last volunteer reported after both formulations were taken. This event occurred approximately at 0.25-1 hour after drug administration. However, the adverse event disappeared in about two hours without any management.

Discussion

The generic indinavir formulation has been developed in Thailand with the main purposes to offer more drug availability and to reduce the treatment cost of HIV-infection and AIDS. The present study was designed to compare the rate and extent of absorption of the generic formulation with those of an original formulation in order to confirm the bioequivalence of the generic product.

The present results showed that indinavir plasma concentration-time profiles obtained from the generic and original formulations were comparable. The generic indinavir capsule provided a slightly lower

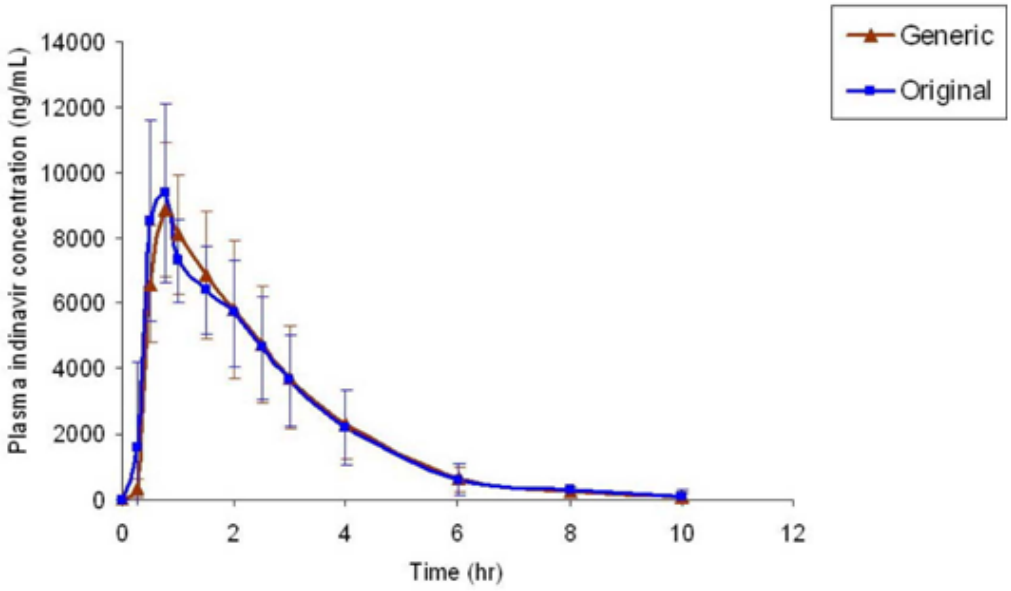

Fig. 1 Time profiles of plasma indinavir concentration (mean±SD) of the generic formulation *vs.* the original formulation after the administration of 2x400 mg capsules in healthy volunteers (n = 23).

Table 1. Pharmacokinetic parameters (mean±SD) derived from the generic and the original indinavir formulations (n=23).

Pharmacokinetic parameter	Generic indinavir formulation	Original indinavir formulation
C_{max} (ng/mL)	9487.88 ± 1428.77	10027.45 ± 2401.28
T_{max} (hour)	0.88 ± 0.26	0.83 ± 0.30
AUC_{0-t} (ng/mLx hour)	23022.82 ± 6491.13	22615.85 ± 5970.53
$AUC_{0-\infty}$ (ng/mLx hour)	23473.51 ± 6555.77	23077.98 ± 6110.53
$t_{1/2}$ (hour)	1.25 ± 0.25	1.23 ± 0.25
k_e (/hour)	0.5751 ± 0.1125	0.5837 ± 0.1070

Table 2. Analysis of variance of pharmacokinetic parameters, relevant to bioequivalence criteria, (natural logarithmic-transformed) derived from the generic *vs.* original indinavir formulations (n=23).

Pharmacokinetic parameter/Source of variation	Degree of freedom	Sum of square	Mean square	F-score	P-value
C_{max}					
Sequence	1	0.0065	0.0065	0.16	0.693
Subject (Sequence)	21	0.8485	0.0404	0.94	0.552
Period	1	0.0163	0.0163	0.38	0.544
Formulation	1	0.0186	0.0186	0.43	0.517
Error	21	0.8985	0.0428	-	-
Intra-subject CV = 20.69%					
AUC_{0-t}					
Sequence	1	0.0387	0.0387	0.32	0.579
Subject (Sequence)	21	2.5606	0.1219	6.08	0.0001
Period	1	0.0572	0.0572	2.85	0.106
Formulation	1	0.0019	0.0019	0.09	0.763
Error	21	0.4210	0.0200	-	-
Intra-subject CV = 14.14%					
$AUC_{0-\infty}$					
Sequence	1	0.0400	0.0400	0.33	0.571
Subject (Sequence)	21	2.5358	0.1208	5.97	0.0001
Period	1	0.0619	0.0619	3.06	0.095
Formulation	1	0.0018	0.0018	0.09	0.772
Error	21	0.4245	0.0202	-	-
Intra-subject CV = 14.21%					

Table 3. 90% confidence intervals of the ratios for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ and the power of test.

Pharmacokinetic parameter	90% Confidence interval	Power (%)
C_{max} (ng/mL)	86.3 -106.5	96.7
AUC_{0-t} (ng/mLx hour)	94.0 -108.5	99.9
$AUC_{0-\infty}$ (ng/mLx hour)	93.9 -108.5	99.9

mean C_{max} value than that of the original formulation (approximately 9,500 ng/mL *vs.* 10,000 ng/mL) and a slightly longer mean T_{max} (0.88 hour *vs.* 0.83 hour). This might be a result of a slight slower dissolution in the first 10 minutes of the generic formulation compared to the original formulation in 0.1 N HCl (data not shown). Nonetheless, there were no significant differences between these two parameters (C_{max} and T_{max}) of the two formulations. These values were generally in the range or higher than those reported previously in a single dose or multiple dose study. Hugen et al. [3] reported a mean C_{max} of 9,420 ng/mL in the small-scale study (n = 12) where 800 mg of indinavir oral liquid formulation for children were administered to healthy male volunteers as a single dose. While Yeh et al. [4] reported a mean C_{max} of approximately 7,170 ng/mL in a single dose (800 mg) study in healthy volunteers (n = 12). According to Kraft et al. [13], the mean C_{max} at steady state was approximately 8,864 ng/mL in healthy volunteers. The T_{max} values obtained in the present study agreed with those in previous reports [3-5], which fell at the range of 0.5-2.4 hours.

The present study showed no statistically significant differences in the AUC between the generic and original formulations. The powers of tests conducted for AUC_{0-t} and $AUC_{0-\infty}$ values were very high, i.e., 99.9% for both parameters as shown in

Table 3. This indicated that a sample size of 23 volunteers was reasonably adequate to prove bioequivalency of the products. This was also confirmed by the high power of the test conducted for C_{max} (96.7%). The mean values of AUC_{0-t} and $AUC_{0-\infty}$ obtained in our study were approximately 23,000 ng x hr/mL and 23,000 ng x hr/mL, respectively for the generic formulation and approximately 23,000 ng x hr/mL and 23,000 ng x hr/mL, respectively for the original formulation. These values appeared to be higher than those in previous reports [3, 4]. Those previous studies used a small number of healthy male volunteers and conducted for only eight hours [3] or up to 24 hours [4]. It is, therefore, difficult to compare our result with the previous results. However, the higher AUC estimated in our study might again be results of the higher C_{max}. According to the above results as well as 90% CIs of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were within 80.0-125.0%, and the two formulations were thus considered bioequivalent.

Interestingly, the mean C_{max} in the present study also agreed with the median (interquartile range) C_{max} reported by Burger et al. [14] where 800 mg indinavir was administered in HIV-infected Thai patients every eight hours and T_{max} was 1.5 hours. These demonstrated that the absorption of indinavir in healthy volunteers and patients was at least in the same range and there might have no accumulation when multiple doses were applied. The slightly higher C_{max} in this report might be a result of low body weight (54 kg at average) of Thai people as has been mentioned in previous studies [14]. It must be mentioned that with this slightly high C_{max} in the present study, no serious adverse events were observed. Only bitter taste was reported by a few volunteers. Protease inhibitors might produce taste complaints clinically [15]. Since this adverse event disappeared without any management, the two formulations were considered well tolerated.

The present results demonstrated that the generic formulation of indinavir is bioequivalent to the original formulation and hence possibly interchangeable. As it is generally agreed, in addition to qualified manufacturing, distribution, and administration of the drug, proven comparative bioavailability or bioequivalence is essential to ensure for the efficacy and safety of the generic product. The cost of care is expected to be decreased.

Conclusion

This pharmacokinetic study in healthy Thai volunteers demonstrated no statistically significant differences in C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ between the generic and original formulations of indinavir 400-mg capsules. These two formulations were considered bioequivalent. Both formulations were also well tolerated.

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