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Bioequivalence Evaluation of Two Formulations of Doxazosin Tablet in Healthy Thai Male Volunteers

Pattana Sripalakit, Penporn Nermhom, and Sirada Maphanta
Bioequivalence Test Center, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand

Sangla Polnok
Faculty of Nursing, Naresuan University, Phitsanulok, Thailand

Poj Jianmongkol
Health Sciences Research Institute, Naresuan University, Phitsanulok, Thailand

Aurasorn Saraphanchotiwitthaya
Bioequivalence Test Center, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand

ABSTRACT The bioequivalence of two doxazosin 2 mg tablets was determined in 24 healthy Thai male volunteers after one single dose in a randomized cross-over study with a one week washout period. The study was conducted at Faculty of Pharmaceutical Sciences and Health Sciences Research Institute, Naresuan University, Phitsanulok, Thailand. Reference (Cardura®, Heinrich Mack Nachf. GmbH & Co. GK, Illertissen, Germany) and test (Dozozin-2®, Umeda Co., Ltd., Bangkok Thailand) were administered to volunteers after overnight fasting. Blood samples were collected at specified time intervals and plasma was separated. The validated HPLC method with fluorescence detection was used for quantification of doxazosin in plasma samples. The pharmacokinetic parameters, T_{max}, C_{max}, AUC_t, AUC_∞, $T_{1/2}$, λ_z, Cl and V_d, were determined from plasma concentration time profile of both formulations by using non-compartment analysis. The calculated pharmacokinetic parameters were compared statistically to evaluate bioequivalence between the two brands. The analysis of variance (ANOVA) using log-transformed C_{max}, AUC_t, and AUC_∞ did not show any significant difference between two formulations. The point estimates and 90% confidence intervals for C_{max}, AUC_t and AUC_∞ were within the acceptance range (0.80–1.25), satisfying the bioequivalence criteria of the Thailand Food and Drug Administration Guidelines. These results indicate that Dozozin-2® is bioequivalent to Cardura® and, thus, may be prescribed interchangeably.

KEYWORDS Bioequivalence, Doxazosin, Pharmacokinetics, Tablet

INTRODUCTION

Doxazosin mesylate (4-amino-2-[4-(1,4-benzodiaxan-2-carboxyl)-piperazin-1-yl]-6,7-dimethyloxyquinazoline mesylate), a quinazoline compound is effective and well known for treatment of hypertension and benign prostatic hyperplasia (BPH) by selectively inhibiting the $alpha_1$ subtype of alpha adrenergic receptors (Chung et al., 1999; Fulton et al., 1995; Lepor et al., 1997). After oral administration, peak plasma levels of doxazosin occur at about 2–3 h. Bioavailability is approximately 65%, reflecting first pass

Address correspondence to Pattana Sripalakit, Bioequivalence Test Center, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand; Fax: +66-55-261057; E-mail: pattana9@excite.com

metabolism by the liver. Doxazosin is extensively metabolized in the liver, mainly by *O*-demethylation of the quinazoline nucleus or hydroxylation of the benzodioxan moiety. Several metabolites of doxazosin have been identified and the most potent compound (6′-hydroxy) to the antihypertensive effect in man is probably small (Elliott et al., 1987; Pfizer Inc., 2002). Plasma elimination of doxazosin is biphasic, with a terminal elimination half-life of 22 h (Penenberg et al., 2000). At the plasma concentrations achieved by therapeutic doses, approximately 98% of the circulating drug is bound to plasma protein (Elliott et al., 1987; Pfizer Inc., 2002). The pharmacokinetics of doxazosin was linear within a range of 1–16 mg (Chung et al., 1999). The recommended doses are 1–16 mg once daily for mild to moderate stage of essential hypertension and 2–4 mg once daily for benign prostatic hyperplasia. Its common adverse drug reactions are orthostatic effects such as dizziness, light headedness, and vertigo (Pfizer Inc., 2002).

Bioequivalence of two formulations of the same drug comprises equivalence with respect to the rate and extent of their absorption while the area under concentration time curve (AUC) generally serves as the characteristic of the extent of absorption (Hauschke et al., 1990; Schulz & Steinijans, 1992). No single parameter reliably measures the rate of absorption; for instance, the maximal drug concentration (C_{max}) has been widely used, but it depends more on the fraction absorbed than of the rate of absorption; the time of the maximal concentration (T_{max}), depends on both absorption and elimination rates (Farolfi et al., 1999). Although several studies have been published regarding doxazosin pharmacokinetics, very few of them have focused on the proof of bioequivalence two formulations. The purpose of this study was to determine the pharmacokinetic parameters of two formulations of doxazosin 2 mg tablets available in Thailand and then to compare these parameters statistically to evaluate the bioequivalence between the two formulations.

MATERIALS AND METHODS

Study Products

Test product was Dozozin-2®—doxazosin 2 mg tablets; Lot No. DZ2-25; expiration date 08/2009, manufactured by Umeda Co., Ltd. (Bangkok, Thailand). Reference product was Cardura®—doxazosin 2 mg tablets; Lot No. 310435333; expiration date 09/2008 and manufactured by Heinrich Mack Nachf. GmbH & Co. GK (Illertissen, Germany). The dissolution profiles of both tablets were carried out, according to the guideline (Thailand FDA, 2001) before the bioequivalence study.

Study Design

A total number of 24 healthy Thai male volunteers was enrolled. The number of volunteer estimation was based on the previously reported pharmacokinetic study of doxazosin (Pfizer Inc., 2002; Vashi et al., 1996). Volunteers were selected after passing a clinical screening procedure including a physical examination and laboratory tests (hematology; RBC, WBC, hemoglobin, hematocrit, platelets, clinical chemistry; blood urea nitrogen, creatinine, albumin, total protein, AST (SGOT), ALT (SGPT), bilirubin, alkaline phosphatase and glucose, virology; HBs antigen, Anti-HCV and Anti-HIV; and urine examination). Upon completion of study, the physical examination was repeated. The subjects were instructed to abstain from alcoholic beverages, smoking, and medication for one week prior to and during the study period.

Drug Administration and Sample Collection

This study was based on a single-dose, randomized, two-sequence, two-period cross-over design with a one week washout interval and conducted at Health Sciences Research Institute, Naresuan University, Thailand. Informed consent was obtained form the subjects after explaining the nature and purpose of the study in accordance with Guidelines for Bioavailability and Bioequivalence Studies (Thailand FDA, 2001). The study clinical protocol was approved by Naresuan University Ethical Committee. During each period, after an overnight fasting (>10 h), volunteers were given single dose of either product (test and reference) of doxazosin 2 mg with 240 mL of water. No food was allowed until 4 h after dose administration. Water, lunch, and dinner were given to all volunteers according to a time schedule. Approximately 8 mL of blood samples were collected into lithium-heparinized tube by catheterized venipuncture at forearms before (0 h) and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, 48, and 72 h dosing. The blood samples were

centrifuged at 4,700 rpm for 15 min at 4°C. The plasma samples were separated and kept in the cryovial tube at −80°C until assayed.

Chromatographic Analysis

The HPLC method was validated by following the local guidelines (Thailand FDA, 2001) for doxazosin assay in plasma samples. The reference standards, doxazosin mesylate, and prazosin hydrochloride as internal standard were obtained from the United States Pharmacopeial Convention, Inc. (Rockville, MD). Briefly, 50 µl aliquot of internal standard (50 ng/ml) and 200 µl aliquot of 1 M NaOH were added to a 500 µl aliquot of plasma sample in glass test tube. After vigorously mixing, 2 mL aliquot of ethyl acetate was added and mixed for 20 s using rotating mixer. After centrifugation at 3,000 rpm for 5 min, organic layer was transferred to a new glass test tube. The residue was twice extracted with 1 mL of ethyl acetate. All portions of organic layer were combined and evaporated to dryness using speed evaporator for 2 h at 45°C. Then, the dried extract was reconstituted in 400 µl of mobile phase, filtered through 0.45-µm nylon disposable filter, and a 100 µl aliquot was injected into HPLC. Samples were quantified using the peak area ratio of doxazosin over the internal standard.

The HPLC system comprised of a pump and fluorescence detector equipped with system controller (Shimadzu, Kyoto, Japan) and a Rheodyne sample injector (Rohnert Park, CA) fitted with a 100 µl sample loop. The separation was performed by using an Apollo C18 column (250 × 4.6 mm i.d., 5 µm, 250°A, Alltech, Deerfield, IL). The mobile phase consisted of 22% methanol, 22% acetonitrile, and 56% 0.04 M disodium hydrogen orthophosphate (pH 4.9), and was isocratically pumped at a flow rate of 1.2 mL/min. The detector was operated at 246 nm for excitation and 389 nm for emission, and corresponding peak areas were recorded.

Pharmacokinetic and Statistical Analysis

A non-compartment pharmacokinetic method was employed to determine the pharmacokinetic parameters of doxazosin. The time to peak plasma

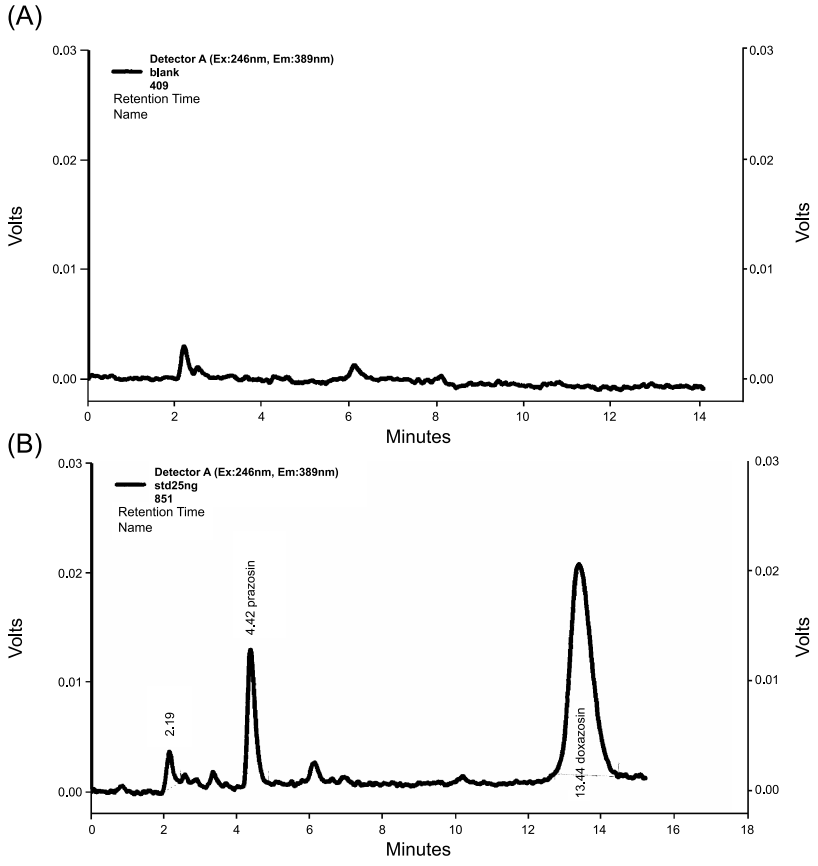

FIGURE 1 **Chromatograms for the Analysis of Doxazosin in Drug-Free Human Plasma. (A) Blank Plasma. (B) Human Plasma Spiked with 50 ng/mL Prazosin and 25 ng/mL Doxazosin.**

concentration (T_{max}) and peak concentration (C_{max}) were determined by the inspection of the individual drug concentration-time profiles. The area under the concentration-time curve from the time of dosing to the last measurable concentration (AUC_t, calculated using the linear-log trapezoidal rule), the area under the curve extrapolated to infinity (AUC_∞), the terminal half-life ($T_{1/2}$), the terminal rate constant (λ_z), the total body clearance (Cl), and the volume of distribution (V_d) were determined by WinNonlin Professional version 4.0.1 (Pharsight Corporation, Mountain View, CA).

An analysis of variance (ANOVA) was performed by SPSS for Windows standard version 10.0.7 (SPSS, Inc., Chicago, IL), on the pharmacokinetic parameters C_{max} and AUCs, using general linear models procedures, in which sources of variation were subject, period, and formulation. The two formulations were bioequivalent if the point estimates for mean of test product/reference product of C_{max} and AUCs and their 90% confidence intervals were within 0.80–1.25 for log-transformed data.

RESULTS

Chromatographic Analysis

In this HPLC method, no interferences were observed in human plasma (Fig. 1). The retention time for prazosin (internal standard) and doxazosin were 4.4 and 13.4 min, respectively. The quantification limit of doxazosin in plasma was 1 ng/mL based on a signal-to-noise ratio of 5.0. The calibration curve of doxazosin was ranged from 1 to 25 ng/mL. The validation results for analysis of doxazosin in human plasma were accepted by the guideline (Thailand FDA, 2001).

Pharmacokinetic Studies

This bioequivalence study was conducted in 24 healthy Thai adult males who ranged from 18 to 23 years of age (mean±SD; 20.9±1.26 years), weighed 57.7±6.64 kg, averaged 168.0±5.47 cm in height, and 20.5±1.83 kg/m^2 of body mass index. The tolerability of both doxazosin products was good. No volunteer was withdrawn from the bioequivalence study. No serious adverse events were found during this study.

The mean plasma concentrations of test (Dozozin-2®) and reference (Cardura®) products of 24 healthy male volunteers at various time points are summarized in Table 1. The average concentration-time curves of both products of doxazosin tablets are presented in Fig. 2. Pharmacokinetic parameters (T_{max}, C_{max}, AUC_t, AUC_∞, $T_{1/2}$, λ_z, Cl, and V_d) were calculated individually on the basis of concentration-time data. From individual pharmacokinetic parameters, their

TABLE 1 **Mean Plasma Concentrations of Doxazosin After Administration of Test (Dozozin-2®) and Reference (Cardura®) Products to 24 Healthy Thai Male Volunteers**

	Plasma concentrations (ng/ml)					
	Test product			Reference product		
Time (h)	Mean	S.D.	C.V. (%)	Mean	S.D.	C.V. (%)
0	0.00	0.00	0.00	0.00	0.00	0.00
0.5	7.67	6.55	85.5	8.61	6.10	70.83
1	13.53	5.97	44.1	13.77	5.94	43.15
1.5	15.55	5.70	36.7	15.59	4.80	30.80
2	15.51	3.97	25.6	15.31	4.03	26.31
2.5	15.58	4.09	26.2	15.47	4.10	26.50
3	15.29	4.01	26.2	14.89	3.70	24.86
4	14.27	3.49	24.5	13.18	3.39	25.76
6	11.91	5.18	43.5	10.86	2.81	25.88
8	10.09	3.49	34.6	9.23	3.42	37.06
12	6.29	2.29	36.3	6.54	2.01	32.10
18	4.89	2.90	59.4	4.52	1.79	39.53
24	2.83	2.50	88.4	2.53	1.62	64.30
48	ND	—	—	1.04	3.13	301.45
72	ND	—	—	ND	—	—

ND: not detectable.

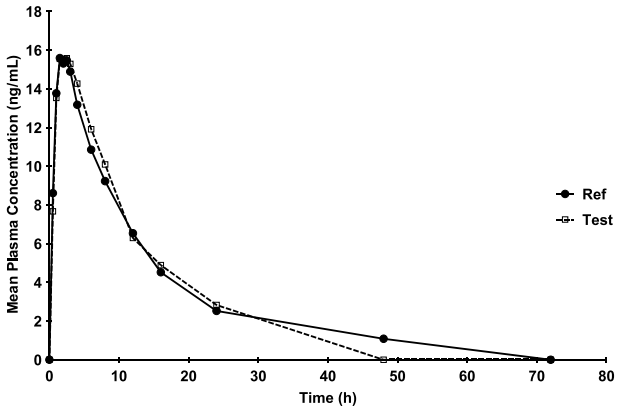

FIGURE 2 **Mean Plasma Concentration-Time Profiles of Doxazosin After Administration of Test (Dozozin-2®, Test) and Reference (Cardura®, Ref) Products in 24 Healthy Thai Male Volunteers.**

mean values (±S.D.) were obtained and are provided in Table 2 for both test and reference products. According to the mean plasma levels of the 24 subjects completing the study, the relative bioavailability values of test product/reference product were found to be 1.04±0.23, 0.99±0.27, and 1.10±0.59 on the basis of C_{max}, AUC_t, and AUC_∞, respectively.

Bioequivalence Evaluation

The results of the analysis pf variance (ANOVA) for the assessment of subject, period, and formulation effects, and the 90% confidence intervals (90% C.I.) for the ratio of C_{max}, AUC_t, and AUC_∞ values for the test and reference products, using logarithmic transformed data, are shown in Table 3.

DISCUSSION

Almost identical plasma doxazosin concentration profiles were obtained from both formulations. The mean C_{max} and T_{max} of both products were related to other studies (Elliott et al., 1987; Pfizer Inc., 2002). The mean percentage ratios of AUC_t/AUC_∞ for test product (75.83%) and reference product (78.36%) were lower than 80% and thus, this error might affect to evaluate terminal half-life. Additionally, since limitation of the determination method, the terminal phase for doxazosin could not be analyzed at low plasma levels (<1 ng/mL). The total mean terminal half-life for both products were 13.2±9.3 h, which were lower

TABLE 2 **Pharmacokinetic Parameters After Administration of Test (Dozozin-2®) and Reference (Cardura®) Products to 24 Healthy Thai Male Volunteers**

	C_{max} (ng/mL)	T_{max} (h)	AUC_t (ng·h/mL)	AUC_∞ (ng·h/mL)	$T_{1/2}$ (h)	λ_z (1/h)	Cl (mL/min/kg)	V_d (l/kg)
Test product								
Mean	18.39	2.1	184.23	264.87	12.7	0.0670	2.70	2.53
S.D.	5.16	1.2	66.35	163.09	7.4	0.0243	0.98	0.86
C.V. (%)	28.1	55.6	36.01	61.6	58.4	36.3	36.5	34.0
Reference product								
Mean	17.97	1.9	198.50	289.76	13.8	0.0670	2.65	2.59
S.D.	4.44	0.8	97.18	295.27	11.1	0.0261	0.95	1.33
C.V. (%)	24.7	43.3	49.0	101.9	80.4	38.9	35.7	51.3

TABLE 3 **Analysis of Variance (ANOVA) for the Assessment of the Subject, Period, and Formulations Effects, and T/R Point Estimate and 90% Confidence Intervals (90% C.I.) for the ratio of C_{max}, AUC_t, and AUC_∞ Values for the Test and Reference Products Using Logarithmic Transformed Data, After Administration of Test and Reference Products to 24 Healthy Thai Male Volunteers (α=0.05)**

	ANOVA (*P* value)				
	Source of variation				
Pharmacokinetic parameters	Subject	Period	Formulation	T/R point estimate	90% C.I.
C_{max}	0.000	0.566	0.632	0.98	0.91–1.05
AUC_t	0.000	0.657	0.416	1.05	0.95–1.15
AUC_∞	0.004	0.511	0.865	1.02	0.86–1.20

than the previous report (Penenberg et al., 2000), because the true terminal phase was not observed. The averages of the total body clearance and volume of distribution in both formulations were found to be different from the other reported ranges, which were 1.0–2.0 mL/min/kg and 1.0–1.9 L/kg, respectively (Elliott et al., 1987).

The multivariate analysis, accomplished through analysis of variance (ANOVA) for assessment of period and formulation effects, revealed the absence of any of these effects in C_{max}, AUC_t, and AUC_∞. The substantial subject variations in pharmacokinetics of doxazosin from the two formulations were significantly found. According to FDA regulations (Thailand FDA, 2001), the interval between doses of each treatment was adequate for avoiding the carryover effect. The design of washout period in this study was calculated by using a half life of 22 h (Penenberg et al., 2000) and was over more than 5 half lives recommended by the guideline (US FDA, 2000). The point estimates for mean of test product/reference product of C_{max}, AUC_t, and AUC_∞ were 0.98, 1.05, and 1.02, respectively; and the 90% confidence intervals for C_{max}, AUC_t, and AUC_∞ were 0.91–1.05, 0.95–1.15, and 0.86–1.20, respectively, which were within the commonly accepted bioequivalence range of 0.80–1.25 (Thailand FDA, 2001). It was concluded that the two doxazosin formulations, Dozozin-2® and Cardura®, were bioequivalent in their rate and extent of absorption.

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