Bioequivalence and Pharmacokinetics of Two Formulations of Amlodipine Tablets in Healthy Subjects

Jaafar J. Ibraheem Al-Tamimi[*,1]

[*]Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Abstract

The bioequivalence of a single dose tablet containing 5 mg amlodipine as a test product in comparison to Norvasc® 5 mg tablet (Pfizer USA) as the reference product was studied. Both products were administered to twenty eight healthy male adult subjects applying a fasting, single-dose, two-treatment, two-period, two-sequence, randomized crossover design with two weeks washout period between dosing. Twenty blood samples were withdrawn from each subject over 144 hours period. Amlodipine concentrations were determined in plasma by a validated HPLC-MS/MS method. From the plasma concentration-time data of each individual, the pharmacokinetic parameters; C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $C_{max}/AUC_{0-\infty}$, λ_Z, $T_{0.5}$, MRT, Cl/F and V_d/F; were calculated applying non-compartmental analysis. The average values of the above parameters for the test formula were 1.99 ng/ml, 8.3 hours, 82.87 ng.hr/ml, 95.23 ng.hr/ml, 0.0219 hr^{-1}, 0.018 hr^{-1}, 38.5 hr, 56.2 hr, 60.9 l/hr and 3483 liters, respectively. The average values of these parameter for the reference formula were 1.92 ng/ml, 7.9 hours, 76.3 ng.hr/ml, 89.31 ng.hr/ml, 0.0225hr^{-1}, 0.019 hr^{-1}, 36.7 hr, 59.9 hr, 69.5 l/hr, and 3983.4 liters, respectively. The pharmacokinetic parameters mentioned above were statistically analyzed by ANOVA test. Ln-transformed values of the pharmacokinetic parameters used for bioequivalence testing; C_{max}, AUC_{0-t} and $AUC_{0-\infty}$; were also statistically analyzed by ANOVA, 90% Confidence Interval (CI) and Schuirmann's two one-sided t-tests. For the T_{max}, parametric and nonparametric tests were applied. Based on FDA criteria on bioequivalence, the results of the above statistical tests demonstrated bioequivalence of the two products.

Keywords: Amlodipine, Pharmacokinetic, Bioequivalence , HPLC/MS/MS.

دراسة التكافؤ الحيوي و حركية الدواء لمستحضرين من اقراص الاملودبين على متطوعين اصحاء

جعفر جابر ابراهيم التميمي[*،1]

[*]فرع الصيدلانيات، كلية الصيدلة ،جامعة بغداد ،بغداد ،العراق.

الخلاصة

تم دراسة التكافؤ الحيوي وحركية الدواء لمستحضر جنيس على شكل حبوب يحتوي على خمسة مليغرامات من دواء الاملودبين بالمقارنة مع المستحضر المرجعي نورفاسك على شكل حبوب يحتوي ايضا على خمسة مليغرامات من دواء الاملودبين و المنتج من شركة فايزر الامريكية. تم اعطاء كل المنتجين لثمانية وعشرون من المتطوعين الاصحاء. وقد تمت الدراسة باتباع التصميم الذي يشمل اعطاء الدواء للمتطوعين و هم صائمين ولفترتين و بشكل عشوائي ومتقاطع وتفصل بين الفترتين اسبوعين.تم سحب عشرون عينة دم من كل متطوع لفترة ١٤٤ ساعة ثم حساب تراكيز الاملودبين لكل متطوع بواسطة طريقة HPLC/MS/MS.

ومن خلال تراكيز الدواء لكل متطوع تم حساب عوامل حركية الدواء في الجسم وهي :

C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $C_{max}/AUC_{0-\infty}$, λ_Z, $T_{0.5}$, MRT, Cl/F ,V_d/F

وكانت النتائج (المعدل) كما يلي بالنسبة للدواء الجنيس وحسب تسلسل عوامل حركية الدواء المذكورة اعلاه كما يلي:

1.99 ng/ml, 8.3 hours, 82.87 ng.hr/ml, 95.23 ng.hr/ml, 0.0219 hr^{-1}, 0.018 hr^{-1}, 38.5 hr, 56.2 hr, 60.9 l/hr and 3483 liters

اما بالنسبة للدواء المرجعي فكانت النتائج كما يلي:

1.92 ng/ml, 7.9 hours, 76.3 ng.hr/ml, 89.31 ng.hr/ml, 0.0225hr^{-1}, 0.019 hr^{-1}, 36.7 hr, 59.9 hr, 69.5 l/hr, 3983.4 liters.

و بعد التحليل الاحصائي باتباع الدستور الامريكي لدراسة التوافر والتكافؤ الحيوي فقد تبين ان الدواء الجنيس متكافؤ حيويا مع الدواء المرجعي.

الكلمات المفتاحية :- املودبين , حركية الدواء ، التكافؤ الحيوي، طريقة HPLC/MS/MS

Corresponding author E. mail: drjaafarjaber@yahoo.com

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Introduction

Amlodipine is a long-acting calcium channel blocker. Amlodipine is chemically described as (R.S.) 3-ethyl-5-methyl-1-(2- aminoethoxy-methyl) - 4- (2 -chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedi-carboxylate benzenesulphonate. Its empirical formula is: $C_{20}H_{25}CIN_2O_5.C_6H_6O_3S$. The molecular weight is 567.1. Amlodipine is indicated for the treatment of hypertension, chronic stable and vasospastic angina. Absolute bioavailability has been estimated to be between 64 and 90%. The bioavailability of amlodipine is not altered by the presence of food. Amlodipine is extensively (about 90%) converted to inactive metabolites via hepatic metabolism with 10% of the parent compound and 60% of the metabolites excreted in the urine. Approximately 93% of the circulating drug is bound to plasma proteins. Pharmacokinetics of amlodipine are not significantly influenced by renal impairment and patients with renal failure may therefore receive the usual initial dose. After oral administration of therapeutic doses of amlodipine, absorption produces peak plasma concentrations between 6 and 12 hours. Elimination of amlodipine from plasma is biphasic with a terminal elimination half-life of about 30-50 hours. The therapeutic dose of amlodipine is 2.5-10 mg [(1)] .

Due to wide use of amlodipine in clinical practice, concomitant use with other drugs, high interindividual variation, in addition to, the pharmacokinetic characteristics which involve slow absorption, lone time to peak and long elimination half-life; many investigations were conducted to study the pharmacokinetics, pharmacody-namics, bioavailability, and bioequivalence of amlodipine after different dosage forms, after food/fluid intake, and in different populations [(2-17)]. Determination of amlodipine concentrations in human plasma were achieved by HPLC-MS method due to the low therapeutic doses of amlodipine and consequently very low plasma levels (nanograms/ml) [(2-17)].

Bioequivalence studies are considered as pivotal part for registration of generic products since these studies are conducted to show that the rate and extent of bioavailability of the generic product is similar to the brand/innovator product. Consequently, the effect(s) and the side effect(s) of the generic product are essentially equivalent to the brand/innovator product, and hence both products are interchangeable in clinical practice.

The purpose of this study was to investigate the pharmacokinetics and relative bioavailability (bioequivalence) of two amlodipine formulations; a test product as tablet containing 5 mg amlodipine, in comparison to Norvasc® 5 mg tablet (Pfizer USA) as the reference product, after administration to 28 healthy male adult subjects applying randomized crossover design.

Materials and Methods

Study products information

A test product as tablet containing 5 mg amlodipine. The reference product was Norvasc® tablet manufactured by Pfizer USA.

Ethical consideration

The study was carried out according to the provisions of the declaration of Helsinki [(18)] and ICH guidelines for good clinical practice [(19)]. The subjects provided informed consent before the commencement of the study.

Study design

A fasting, single-dose, two-treatment, two-period, two-sequence, randomized crossover design was applied as recommended by FDA guidance for bioavailability and bioequivalence. Twenty eight subjects participated in the study. Equal number of subjects (14 subjects) were randomly assigned to each dosing sequence of the treatments (test and reference formulations). The treatments were separated by two weeks washout interval between period I and period II dosing.

Inclusion criteria

Twenty eight subjects were selected according to the following inclusion criteria: age between 18-48 years; normal Body-Mass-Index (BMI) =18-28; non smokers; no drug or alcohol abuse; no history of contraindication and/or allergy to the drug and any related compounds; normal physical and clinical examinations including vital signs, hepatic, renal, respiratory, cardiac, gastrointestinal and psychiatric; normal clinical laboratory tests including biochemistry, hematology, routine urine analysis, negative for HIV, hepatitis B and C; no consumption of drugs for two weeks prior the study; no blood donation, hospitalization or participation in any study (clinical, pharmacokinetic, bioavailability or bioequivalence) within the last 2 months prior to the present study.

Drug product administration and the conditions of the study

The drug was administered with 240 ml of water after an overnight fasting of 12 hours. No water was permitted 2 hours before and

after dosing. Water was allowed 2 hours after dosing. Standard diets (breakfast, lunch and dinner) were administered and were identical in both periods of the study. Xanthine containing products were not allowed twelve hours before dosing and then twelve hours after dosing. Grapefruit juice or beverages containing grapefruit were not allowed within the past week prior the study and until the completion of the whole study (both periods of the study). The subjects were not allowed to sleep or lie during the first four hours of drug administration, they remained seated upright.

Blood samples collection

Seven ml of blood samples were withdrawn via an Indwelling Cannula placed in the forearm anticubital vein at zero time (one hour before dosing), and then at: 1.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 16.0, 24.0, 36.0, 48.0, 72.0, 96.0, 120.0 & 144.0 hours post dosing. The blood samples were directly transferred to heparinized tubes and then immediately centrifuged for 5 minutes at 4000 rpm. The plasma samples were separated by polypropylene disposable tips and transferred to Eppendorf tubes and then immediately stored at -20°C until analysis for determination of amlodipine concentrations in plasma.

Clinical observations

Vital signs (blood pressure and pulse) of each subject were measured one hour before dosing and then at 3, 6, 9 and 12 hours post dosing.

Analytical procedure

Amlodipine's concentrations in plasma were determined by a modified HPLC-MS/MS method obtained from previously published HPLC-MS/MS methods [20-23]. The validation of the method was evaluated following FDA bioanalytical method validation criteria [24] and GLP guidelines [25]. Samples were extracted using liquid-liquid extraction technique. In each run, 1ml of plasma was alkalinized using 2 ml of 0.2 M borate buffer then extracted using 6 ml of Hexane: ethyl acetate (1:1) extraction solvent. Diazepam was used as an internal standard. The samples were then shaken for 20 minutes at 250 rpm and later centrifuged for 5 minutes at 4000 rpm. The upper organic layer was aspirated and placed into another tube and then evaporated to complete dryness under nitrogen stream. The residual samples were reconstituted with 50 µl of 1% acetic acid in methanol: water 1:1 to be ready for direct injection into the HPLC. Drug quantitation was done using a Finnigan LCQ DUO ion trap mass spectrometer (Finnigan Thermoquest, USA) equipped with an (ESI) source (Finnegan) and run by: Xcalibur 1.2 software (USA). Calibration standard responses were linear over the range of 0.1-10 ng/ml of amlodipine concentrations in human plasma with a lower limit of quantitation (LLOQ) of 0.1 ng/mL.

The plasma samples were analyzed after the completion of the clinical part of the study as recommended in bioequivalence studies. Plasma samples of each subject for both periods were analyzed with their own calibration curve and quality control (QC) samples as one batch in a single run. For each run, six QC samples (dispersed evenly in a low-high and high-low sequence throughout the batch) were analyzed. No determination was done by extrapolation below the LLOQ and above the upper limit of quantitation (ULOQ) of the standard calibration curve as recommended by FDA bioanalytical method validation guidance [24].

Pharmacokinetic analysis

Kinetica 2000 (V4.0) software was used for all pharmacokinetic analysis of data. A non-compartmental analysis was applied for all pharmacokinetic calculations as recommended by FDA and EMEA guidance in bioavailability and bioequivalence [26, 27]. The pharmacokinetic parameters clearance (Cl), apparent volume of distribution (Vd), elimination rate contant (K), terminal elimination half-life ($T_{0.5}$), area under plasma concentration-time curve (AUC), area under moment curve (AUMC), and mean residence time (MRT) were calculated applying standard methods [28].

Statistical analysis

Kinetica 2000 software (V4.0) was used for the statistical analysis of data. For the purpose of bioequivalence evaluation [26, 27, 29], analysis of variance (ANOVA), 90% confidence interval and Schuirmann's two one-sided t-test were applied. ANOVA were carried out to account for the effects of the following sources of variation: treatment, period, sequence and subjects nested in sequence; on the pharmacokinetic parameters; C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $C_{max}/AUC_{0-\infty}$, λ_Z and $T_{0.5}$. ANOVA was also executed for the Ln-transformed values of the pharmacokinetic parameters; C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, and $C_{max}/AUC_{0-\infty}$. The difference between the pharmacokinetic parameters of both products were declared statistically insignificant at 5% significance level ($\propto$ = 0.05) when $P \geq 0.05$. The 90% Confidence Interval (CI) for the ratio of the mean test/mean reference (T/R) for the Ln-transformed values of the pharmacokinetic

parameters; C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were concluded bioequivalent if the lower CI ≥ 80% and the upper CI ≤125%, as recommended by FDA guidance in bioavailability and bioequivalence [26, 29]. Schuirmann's two one-sided t-test [30] was also applied for the pharmacokinetic parameters; C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ as a check and further support of bioequivalence between both products. Both products were concluded bioequivalent by the Schuirmann's test if the lower-T (T_L) ≥ ($T_{0.05\ -26\ df}$) and the upper T (T_U) ≥ ($T_{0.05\ -26df}$). For the T_{max} values, the parametric point estimate was measured as the difference between the mean values of the test and the reference products. The acceptance limit for the T_{max} was within ± 20% of the mean value of the reference product. Nonparametric test was also applied for the T_{max}. ANOVA testing was applied for the pharmacokinetic parameters; MRT, Cl/F and V_d/F; of the test product versus the reference product.

Results and Discussion

Clinical observations

Both test and reference products were well tolerated by all subjects. No incidences of serious side effects or adverse reactions were observed during the study. All the subjects who started the study participated to the end of the study.

Plasma concentrations

The developed LC-MS/MS method presented in this study with LLOQ of 0.1 ng/mL was rapid, sensitive, precise, accurate and specific for quantitation of amlodipine in human plasma. Therefore, the present method can successfully applied to analyze large number of plasma samples for pharmacokinetic, bioavailability and bioequivalence studies of amlodipine in human plasma. No significant differences (P > 0.05) in the plasma concentrations were found in all sampling time points between the test and the reference products. One hour before dosing (pre-dose sample), amlodipine was not detected in plasma samples of any subject indicating the absence of carryover effects and insuring a sufficient washout period. The drug was detected in plasma samples of 21 volunteers and 20 volunteers after 1.0 hour post dosing of the test product and Norvasc® tablets, respectively. This indicates rapid appearance of amlodipine in plasma. Figure 1 shows the profiles of the mean amlodipine plasma concentration-time data of the 28 volunteers for each product. This figure indicates that the plasma concentration-time profiles of amlodipine for both products are to a very good extent superimposable.

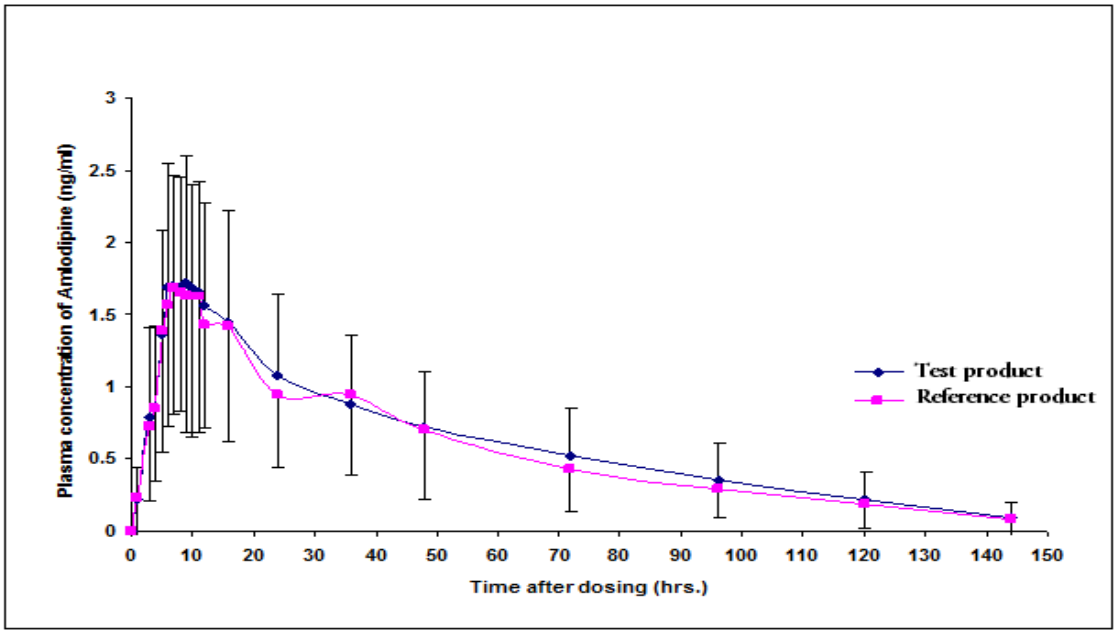

Figure 1: Mean plasma concentrations (± SD) of amlodipine after a single dose administration of a test product (tablet containing 5 mg amlodipine) and the reference product (Norvasc 5 mg tablet) to twenty eight healthy male adult subjects.

Table (1) Mean (± SD) of pharmacokinetic parameters of amlodipine after a single dose administration of a test formulation (tablet containing 5 mg amlodipine) and the reference formulation (Norvasc® 5 mg tablet) to 28 healthy male adult subjects.

Pharmacokinetic Parameters	Test Formula		Reference Formula	
	Mean	± SD	Mean	± SD
C_{max} (ng/ml)	1.99	0.817	1.92	0.94
AUC_{0-t} (ng.hr/ml)	82.87	44.52	76.30	45.68
$AUC_{0-\infty}$ (ng.hr/ml)	95.23	46.12	89.31	48.83
$C_{max}/AUC_{0-\infty}$ (hr^{-1})	0.0219	0.0059	0.0225	0.0057
T_{max} (hr)	8.3	2.19	7.9	2.7
λ_Z (hr^{-1})	0.018	0.0057	0.019	0.0048
$T_{0.5}$ (hr)	38.5	10.07	36.7	11.21
MRT (hr)	56.2	11.78	59.9	15.68
Cl/F (l/hr)	60.9	33.54	69.5	42.09
V_d/F (l)	3483	1954.8	3983.4	2467.5

C_{max} = Maximum concentration of drug in plasma, obtained directly from the concentration versus time curves of individual volunteers.

T_{max} = The time to attain C_{max}, obtained directly from the concentration versus time curves of individual volunteers.

AUC_{0-t} = Area under the plasma concentration-time curve from time zero to t_{last}, calculated by trapezoidal rule.

$AUC_{t-\infty}$ = Extrapolated area under the plasma concentration-time curve from t_{last} to infinity, calculated as C_{last}/λ_Z.

$AUC_{0-\infty}$ = Total area under the plasma concentration-time curve from time zero to infinity, calculated from the sum of $AUC_{0-t} + AUC_{t-\infty}$.

λ_Z = First order terminal elimination rate constant, estimated by linear regression of not less than 3 points of the last points at the terminal phase of the log-concentration versus time curves of individual volunteers.

$T_{0.5}$ = First order terminal elimination half-life, equal to $0.693/\lambda_Z$.

C_{last} = Last measurable concentration which meet or exceed the lower limit of quantitation.

t_{last} = Time at which C_{last} occur.

MRT = Mean residence time, calculated as $AUMC_{0-\infty}/ AUC_{0-\infty}$.

$AUMC_{0-\infty}$ = Area under the moment curve from time zero to infinity.

Cl/F = Oral body clearance, calculated as F x Dose/ $AUC_{0-\infty}$. Dose=5 mg , F=0.7.

Vd/F = Oral volume of distribution, calculated as Cl_{oral} / λ_Z .

F = Oral bioavailability.

Statistical evaluation

ANOVA tests were performed for all the calculated pharmacokinetic parameters presented in Table 1, whereas 90% CI and Schuirmann's two one-sided t-test (Table 2) were applied only for the pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$, since these three parameters are considered as the primary pharmacokinetic parameters for bioequivalence evaluation as recommended by FDA Guidance [26, 29].

ANOVA tests for the C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, $C_{max}/AUC_{0-\infty}$, λ_Z and $T_{0.5}$ values and for the corresponding Ln-transformed values of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, and $C_{max}/AUC_{0-\infty}$, revealed no significant effects ($P > 0.05$) for the sources of variation: treatment, period and sequence. However, for the subjects nested in sequence, a significant effect ($P < 0.05$) was found which may be due to the interindividual variation in the above mentioned parameters as shown in Table 1. Moreover,ANOVA tests for MRT, Cl/F and Vd/F showed no significant difference ($P > 0.05$) between the test and the reference formulas. These findings support the similarity in the pharmacokinetic behaviors of the test and the reference formulas.

The calculated ranges of the 90% CI (Table 2) for the Ln-transformed values of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$, were well within the FDA bioequivalence acceptance criteria [26, 29]. The ranges of Schuirmann's two-one-sided t-test (Table 2) for the above pharmacokinetic parameters were also well within the bioequivalence acceptance criteria [29, 30]. Moreover, power calculations

for C_{max} and AUC demonstrated that sample size of 28 subjects is adequate to obtain power above 80% for bioequivalence evaluation of amlodipine tablets. Therefore, according to FDA Guidance on bioequivalence, it is concluded from the results of the above statistical tests that the test product and the reference brand product (Norvasc® tablet) are bioequivalent.

Table (2) 90% Confidence Interval and Schuiramann's two one-sided T-tests for the pharmacokinetic parameters of the test versus the reference products.

Pharmacokinetic Parameters	T/R Geometric Mean Ratio	90% Confidence Interval*		Schuiramann's Two One-Sided T-Test**	
		Lower limit	Upper limit	Lower limit	Upper limit
C_{max}	1.03	95.29	109.82	3.3996	6.7666
AUC_{0-t}	1.07	100.3	115.23	2.3308	8.0969
$AUC_{0-\infty}$	1.04	97.76	112.48	2.8818	7.4447

* Acceptance criteria = lower limit ≥ 80 and upper limit ≤ 125.0.

** Acceptance criteria = lower limit and upper limit ≥ 1.7081.

Conclusion

The present study introduced pharmacokinetic characteristics of amlodipine after therapeutic oral dose to healthy male adult subjects. The pharmacokinetics of the test product are statistically similar to the reference brand product (Norvasc® tablet) produced by Pfizer USA. Therefore, according to FDA guidance on bioavailability and bioequivalence, it is concluded that the test product is bioequivalent to Norvasc® tablet. Therefore, both products are interchangeable in therapy with amlodipine, and the test formula can be considered presecribable as Norvasc® tablets produced by Pfizer USA.

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