l Comparative bioavailability (bioequivalence) of a newly developed formula of Clopidogrel against Actavis tablets in fasting healthy male adult Iraqi subjects

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Abstract

Objective: The study was performed to compare the bioavailability (bioequivalence) of a newly developed generic formula of clopidogrel 75 mg tablet as a test product against Actavis tablet containing 75 mg clopidogrel as the reference formula.
Methods: The newly developed generic formula of clopidogrel tablet was prepared using co-processed excipients composed mainly of microcrystalline cellulose, superdisintegrant, glidant, and magnesium stearate. *In vitro* dissolution test was done first to compare the test to the reference formulas, followed by the *in vivo* study. Both drug products were administered to 40 Iraqi male healthy adult subjects under fasting state applying randomized, two periods, two sequences, two-way crossover design with one week washout period between dosing. Blood samples were obtained over 72 hours interval from each subject, and the concentrations of the inactive metabolite of clopidogrel (clopidogrel carboxylic acid) were determined in serum by LC-MS/MS method. From serum concentration versus time data of each subject, the pharmacokinetics parameters AUC0-t, AUC0-infinity, Cmax, Tmax, and T0.5 were calculated applying non-compartmental data analysis.
Results: The dissolution profile of the test product was found to be similar to the reference product with similarity factor (*f2*) equal to 79.4%. The geometric mean ratios of the primary pharmacokinetic parameters used for bioequivalence testing of the test/reference products were; 102.47% for AUC0-t, 102.68% for AUC0-infinity and 101.85% for Cmax. The 90% confidence intervals for AUC0-t, AUC0-infinity and Cmax were 93.46-109.18 %, 98.78-108.32% and 91.65-104.33%, respectively. Since the 90% confidence intervals for these parameters were within the 80–125% interval proposed by FDA, it was concluded that the newly developed clopidogrel 75 mg tablet was bioequivalent to the reference product produced by Actavis in term of both the rate and extent of absorption and bioavailability. Consequently, the newly developed clopidogrel 75 mg tablet is interchangeable with clopidogrel 75 mg tablet manufactured by Actavis and can be prescribed as an alternative in the Iraqi market.

مقارنة توافر حيوي (تكافؤ) لصيغة مطورة حديثا للكلوبيدوغريل مقابل أقراص الاكتافيز بمشاركة أشخاص عراقيين بالغيين من الذكور وأصحاء وفي حالة الصيام

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الخلاصة:

أجريت الدراسة لمقارنة التوافر البيولوجي (التكافؤ الحيوي) لصيغة جنيسة حضرت حديثا من كلوبيدوغريل 75 ملغ قرص كمنتج اختبار ضد قرص أكتافيس تحتوي على 75 ملغ كلوبيدوغريل كصيغة مرجعية. تم تحضير الصيغة الجنيسة التي تم تطويرها حديثا من قرص كلوبيدوغريل باستخدام سواغ معالج يتألف بشكل اساسي من المايكروكريستلاين سليلوز ، مفتت عالي السرعة، مزلق، وستيرات المغنيسيوم. في اختبار مختبري للانحلال تم أولا لمقارنة الصيغة الجنيسة مع الصيغة المرجعية، تلتها دراسة في الجسم الحي. تم إعطاء كلا المنتجات الدوائية ل 40 شخصا عراقيا بالغا من الذكورذو حة جيدة وفي حالة الصيام مع اتباع تصميم دراسة ذو عشوائية، وفترتين، تسلسلين، في اتجاه ثنائي متبادل مع فترة غسل أسبوع واحد بين الجرعتين. تم الحصول على عينات الدم على مدى 72 ساعة على فترات من كل شخص، وتم تحديد تركيزات المستقلب غير النشط من كلوبيدوغريل (كلوبيدوجريل حمض الكربوكسيلك) في مصل الدم بواسطة تقنية أل سي- ماس / ماس. من تركيز المصل مقابل الوقت لكل شخص ، تم حساب معايير حركية الدواء التي تشمل المساحة تحت المنحني من الصفر لغاية اخر قراءة، المساحة تحت المنحني من الصفر لغاية اللانهاية، أعلى تركيز للدواء بالمصل، الوقت للو ول الى أعلى تركيز للدواء بالمصل ، وعمر النصف T0.5 بتطبيق تحليل البيانات غير المقسم. وقد تبين أن مظهر الانحلال لمنتج الاختبار مشابه للمنتج المرجعي مع معامل التشابه (f2) يساوي 79.4 في المائة. وكانت نسب المتوسط الهندسي لمعايير حركية الدواء المستخدمة لاختبار التكافؤ الحيوي للمنتجات الاختبار / المرجعية؛ 102.47٪ للمساحة تحت المنحني من الصفر لغاية اخر قراءة ، 102.68٪ للمساحة تحت المنحني من الصفر لغاية اللانهاية و 101.85٪ لأعلى تركيز للدواء بالمصل. كانت نسبة الثقة 90٪ للمساحة تحت المنحني من الصفر لغاية اخر قراءة ، ٪ للمساحة تحت المنحني من الصفر لغاية اللانهاية و لأعلى تركيز للدواء بالمصل هي 93.46-109.18٪، 98.78-108.32٪ و 91.65-104.33٪، على التوالي. وبما أن 90٪ من نسبة الثقة لهذه ا معايير كانت ضمن الفا ل الزمني 80-125٪ الذي اقترحته ادارة الاغذية والعقاقير، نستنتج أن قرص كلوبيدوغريل 75 ملغ الذي تم تطويره حديثا كان مكافئ بيولوجيا للمنتج المرجعي الذي ينتجه أكتافيس على حد سواء من حيث معدل ومدى الامتصاص والتوافر البيولوجي. ونتيجة لذلك، قرص كلوبيدوغريل 75 ملغ الذيحضر حديثا قابلة للتبديل مع كلوبيدوغريل 75 ملغ قرص المصنعة من قبل أكتافيس ويمكن و فها كبديل في السوق العراقية.

1. INTRODUCTION

Clopidogrel bisulfate is an inhibitor of ADP-induced platelet aggregation and mechanism of action is by inhibition of adenosine diphosphate (ADP) binding to its receptor. Chemically it is methyl (+)-(*S*)-α- (2-chlorophenyl)-6, 7-dihydrothieno [3, 2-c] pyridine-5(4*H*)-acetate sulfate (1:1). The empirical formula of clopidogrel bisulfate is $C_{16}H_{16}ClNO_2S•H_2SO_4$, and its molecular weight is 419.9. Each 98 mg of clopidogrel bisulfate is equivalent to 75 mg clopidogrel. Clopidogrel is chemically related to ticlopidine but lower side effects and dose. Since Clopidogrel inhibits platelet aggregation thus it can be used for patients undergoing placement of a coronary stent [(1)].

Clopidogrel is a prodrug, it is rapidly, but incompletely absorbed after oral administration and extensively metabolized to active and inactive metabolites. The blood levels of the parent drug and its active metabolite are low in plasma; however the major circulating compound is an inactive carboxylic derivative, which its blood concentration is used to analyze the pharmacokinetic profile of clopidogrel [(2)].

Clinical studies have shown that clopidogrel combined with aspirin is useful in the patient's treatment have myocardial infarction with ST-elevation. Patients have myocardial infarction without ST-elevation or unstable angina must be treated with clopidogrel combined with aspirin for at least nine months to lower the risk vascular death, nonfatal myocardial infarction, and nonfatal stroke [(3)].

Co-processed excipients are combinations of more than one excipient which have new physical properties better than individual excipient regarding compressibility and flowability, in addition to the low cost of production.

The newly developed generic drug product is a drug which is formulated and prepared by the company or researcher other than the innovator after finishing patent protection time. The generic drug formulation knowhow is belonging to the producer except for the active ingredient. A generic must contain the same active ingredients as the original formulation. According to the US Food and Drug Administration (FDA), generic drugs are identical or within an acceptable bioequivalent range to the brand name counterpart concerning to pharmacokinetic and pharmacodynamic properties.

The objective of this study was to compare by *in vitro* dissolution test and *in vivo* comparative bioavailability (bioequivalence) study a newly developed generic formula (clopidogrel 75 mg tablet) as a test product with the reference clopidogrel 75 mg tablet produced by Actavis and marketed in Iraq.

2. SUBJECTS, MATERIALS, AND METHODS

Study protocol

The study was performed following the Helsinki Declaration and Good Clinical Practice Guideline described in International Conference of Harmonization (ICH). Informed consent was signed and received from each volunteer before starting the study. The study was conducted at bioequivalence laboratory, College of Pharmacy, University of Baghdad, Baghdad, Iraq. The study protocol was approved by the ethical committee of the College of Pharmacy before study conduct.

Subjects

Forty healthy male adults volunteers, ages range of 20-45 (mean ±SD: 31±5.3 years), heights between 165.0 -195.0 (182.0± 0.96 cm), and weight between 62 -95 kg (73±5.7 kg), and deviation not more than 15% of their ideal body weight were accepted to enroll in the study. Subjects were got acceptance for enrolment in this study if they have complied with all the inclusion and exclusion criteria stated in the protocol.

All the participants were considered healthy depending on physical examination, ECG, and the following laboratory tests which include blood glucose, urea, creatinine, AST, ALT, alkaline phosphatase, Gamma GT, total bilirubin, albumin, total protein, triglycerides, total cholesterol, hemoglobin, hematocrit, total and differential white cell counts, routine urine tests, and negative for HIV and HBV.

Materials

Clopidogrel bisulfate was a gift from Pioneer Company for pharmaceutical industries, Iraq. Microcrystalline cellulose, superdisintegrant, glidant, and magnesium stearate were a gift from State Company for Drugs Industry and Medical Appliances (SDI), Iraq. All other reagents used in the study were of HPLC grade.

Drug products

The test product (Clopidogrel tablet) was a newly developed generic formulation containing 75 mg clopidogrel. The reference formulation was clopidogrel 75 mg manufactured by Actavis. The generic tablet was prepared using co-processed excipients composed mainly of microcrystalline cellulose, superdisintegrant, glidant, and magnesium stearate granulated using ethanol as binding solvent. Simple mixing of prepared granules with drug and directly compressed into 4 kg hardness tablets each contains 75 mg of the active ingredient in single punch machine (type F3, Manesty, Liverpool, UK).

Dissolution test

The *in vitro* dissolution studies were performed based on USP 32 to compare the dissolution profiles of the test versus the reference tablets using USP apparatus 2 (Copley dissolution 8000, Copley scientific, U.K.). The dissolution medium consisted of 1000 mL of pH 2.0 hydrochloric acid buffer maintained at 37 ± 0.5 °C. The buffer was prepared according to USP monograph. The rotation speed of the paddles was set at 50 rpm. Samples (5 mL) were withdrawn at 5, 10, 15, 20, 25, 30, and 35 min with replacement by fresh medium. The samples were then analyzed by validated in-house HPLC method.

Study Design

The study was conducted as fasting, randomized, two periods, two sequences, crossover balanced design with one week washout period between dosing. At each period, the volunteers were hospitalized at about 6 pm the day before dosing, and then evening standard meals were served at 8:00 pm. After an overnight fast the participants received at 7:00 am a single 75 mg tablet clopidogrel dose of either the test or the reference formulations. Water (200 mL) was given immediately after drug administration. All volunteers have then fasted 4 hours following the drug administration after which standard lunches were served. Evening meals were taken ten hours after dosing. No other food was permitted during the "in-house" period. Liquid consumption was permitted ad libitum after lunch, but xanthine-containing drinks including tea, coffee and cola were avoided. Systolic and Diastolic arterial pressure (measured on invasively with a sphygmomanometer), heart rate and temperature were recorded just before and hourly after drug administration until 12 hours after dosing, and eventually before the discharge of the subjects from the clinical site (72 hours post dosing of each period).

Drug analysis

Blood samples (5 mL) from a suitable antecubital vein of each subject were collected into EDTA containing tubes before (zero time), and then at 0.25, 0.5, 1.5, 2, 2.5, 3.5, 4.5, 5, 5.75, 7.25, 8.5, 9.75, 11.75, 12.25, 14.75, 19.25, 28.5, 38.25, 43, 45, 48, 54.5, 60.25, and eventually at 72 hours after administration of each of the test and the reference products. A total of 25 blood samples were obtained from each subject at each period. The total volume of blood withdrawn during the entire study from each participant was about 250 ml.

Blood samples were cooled in a bath and centrifuged at 3000 rpm for at least 10 min at approximately 4°C. The separated serum samples were transferred into polypropylene tubes and maintained frozen at –20°C until analysis. All samples from a single volunteer were analyzed on the same day to avoid interassay variation. Serum concentrations of the carboxylic acid of clopidogrel, the major inactive metabolite of clopidogrel, were determined by HPLC coupled with tandem mass spectrometry (LC-MS/MS) according to the in-house procedure.

Pharmacokinetic and statistical analysis

The elimination half-life (T½), the first-order terminal elimination rate constant (Ke), the maximum observed serum concentration (Cmax) and the time taken to achieve this concentration (Tmax), the areas under the clopidogrel metabolite serum concentration versus time curves from 0 to 72 hours (AUC0-72h), and areas to infinity (AUC0-infinity) were calculated by using the software (Kinetica®, Version 5) applying non-compartment data analysis approach as recommended by international bioequivalence guidance (FDA and EMEA).

The bioequivalence between both formulations was determined by calculating individual Cmax, AUC0-72h, AUC0-infinty, arithmetic and geometric means of these parameters, ratio of the mean (test/reference), together with their corresponding 90% confidence intervals (CI), after log transformation of the data.

3. RESULTS

The new developed generic formula (test product) of clopidogrel complies with USP general requirement of tablets. Studying the release profiles as shown in Figure 1 of the test product in comparison to the reference tablet produced by Actavis using the similarity factor (*f2*) indicates that there is no difference between them since *f2*=79.378 which is more than 50% which is the recommended FDA value that insures similarity in the dissolution profiles of two formulas (i.e., test versus reference drug products).

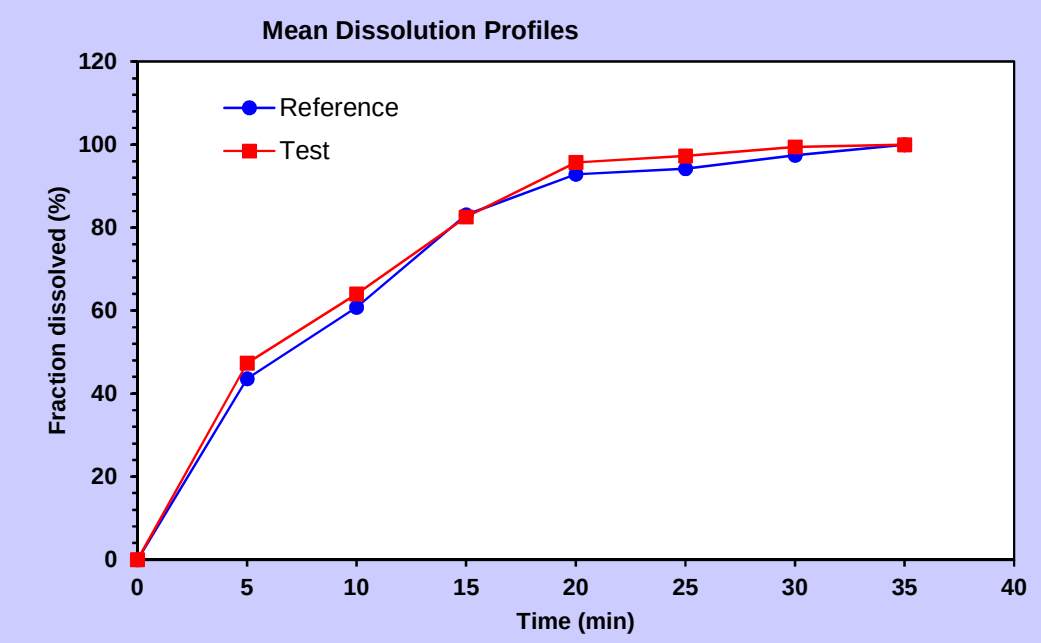

Figure 1: Dissolution profiles a newly developed generic clopidogrel (the test) versus the reference tablets.

Medical examination

Clopidogrel of both test and reference products was well tolerated at the administered dose. All the biochemical parameters did not show any clinical relevant alterations. No adverse effects were either reported or observed.

Pharmacokinetic and statistical analysis

The mean (± SD) serum concentration time profiles of both test and reference formulations shown in Figure 2 were similar and superimposable. The arithmetic mean pharmacokinetic parameters for both formulations are shown in Table 1, the geometric means are presented in Table 2, the geometric mean ratios and the 90% confidence intervals are summarized in Table 3.

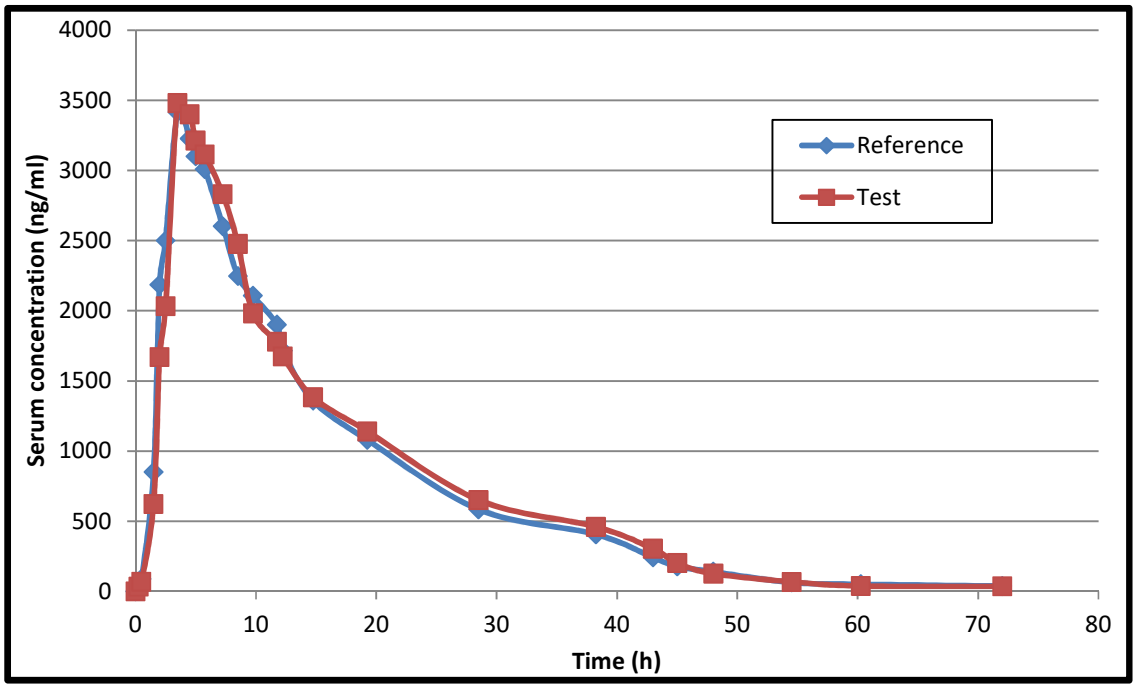

Figure 2: Mean concentrations of clopidogrel carboxlic acid versus time profiles obtained after single oral administration of 75 mg clopidogrel of the reference and the test formulations.

Table 1: Arithmetic mean of pharmacokinetic parameters of clopidogrel carboxlic acid obtained after single oral administration of 75 mg clopidogrel of the reference and the test formulations.

	REFERENCE		TEST	
Parameter (unit)	Means	Standard Deviation	Means	Standard Deviation
AUC0-72h (ng.h/mL)	64564.62	3865.82	65677. 53	4725.47
AUC0-infinity (ng.h/mL)	58463.76	4636.65	59246.76	3684.51
Cmax (ng/mL)	3648.52	994.23	3572.83	877.43
Tmax (median) (h)	3.5	0.65	3.5	0.63
Kel (1/h)	0.07	0.017	0.071	0.018
T½ (median) (h)	9.90	16. 34	9.75	14.53

Table 2: Geometric mean of pharmacokinetic parameters of clopidogrel carboxylic acid of the reference and the test formulations

	REFERENCE	TEST
Parameter (unit)	Geometric Mean	Geometric Mean
Cmax (ng/mL)	3415.87	3479.37
AUC0-72hr (ng.h/mL)	52810.39	54257.68
AUC0-infinity (ng.h/mL)	53208.32	54637.12

Table 3: Ratios of geometric means and 90% confidence interval of the test versus the reference formulations

Parameter	Ratio T/R (%)	Lower Limit (%)	Upper Limit (%)
Cmax	101.85	91.65	104.33
AUC0-72h	102.74	93.46	109.18
AUC0-infinity	102.68	98.78	108.32

Tables 1 and 2 demonstrated that all the calculated pharmacokinetic parameters for the test product were found to be similar to the corresponding values of the reference product. Beside, the geometric mean ratios of the pharmacokinetic parameters Cmax, AUC0-72h and AUC0-infinity were; 101.85, 102.74 and 102.68, and their corresponding 90% confidence intervals were 91.65-104.33, 93.46-109.18 and 98.78-108.32, respectively; which insure close similarity in the pharmacokinetic behaviors of the test and the reference products, as shown in Table 3.

Discussion

Clopidogrel is an analogous molecule to ticlopidine and quickly binds to the platelet inhibition platelet aggregation [5]. Clopidogrel is metabolized by the liver into its major inactive metabolite, the carboxylic acid. Due to its extensive metabolism, methods were developed for the quantifications of clopidogrel carboxylic acid in serum.

The quantification of various drugs by chromatography with tandem mass spectrometry has become the more common analytical method due to improvement in the sensitivity and the selectivity of this method. With the advance of the chromatography, the quality in the determination of the concentrations is more precise, getting lower LOQ and better analysis of results for the determination of clopidogrel in human plasma and serum [6-11].

The bioavailability of a pharmaceutical dosage form refers to the extent and rate of absorption of the active ingredient incorporated in it. Two pharmaceutical forms are said bioequivalent when; to be administered to the same individual, in the same experimental conditions and at the same dose; showed no significant differences about the rate and extent of absorption and bioavailability.

Table 3 indicated that the test formula is bioequivalent to the reference formula in term of the rate and extent of absorption and bioavailability since the 90% confidence intervals of all the pharmacokinetic parameters used for bioequivalence testing, namely Cmax, AUC0-t (AUC0-72h) and AUC0-infinity were well within the accepted ranges of 80-125% as per FDA and EMEA guidance for bioequivalence. Therefore, it can conclude from the current investigation that clopidogrel 75 mg tablet (newly developed generic product) is bioequivalent to the reference clopidogrel 75 mg tablet produced by Actavis, and consequently both products can be considered interchangeable in therapeutic application.

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