A Randomized, Open, Crossover bioequivalence study and Food Effect Assessment of Two fixed-dose combination of Lisinopril / Amlodipinebesylatein Healthy Chinese Subjects

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Abstract

Background:This study was conducted to compare the PK characteristics, food effect and evaluate the bioequivalence between two fixed-dose combinations of lisinopril /amlodipine besylate in healthy Chinese subjects. Methods: A single center, randomized, open-label, single-dose, crossover bioequivalence study was designed in healthy Chinese subjects under both fasting and fed conditions. Cmax and AUC were used to evaluate bioequivalence. Adverse events were recorded. Results: 75 healthy subjects completed the study. The 90% confidence intervals of the ratio of geometric means of Cmax and AUC0-[?] of lisinopril and amlodipine fell within 0.80-1.25. A fat-high breakfast produced significant alteration in the Cmax and AUC of lisinopril after a dose of either reference or test drug. No severe adverse events were observed. Conclusion: The trial demonstrated that the test and the reference drug of fixed-dose combinations of lisinopril /amlodipine besylate were bioequivalent and well tolerated under fasting and fed condition

Introduction

Hypertension is an independent and major risk factor for cardiovascular diseases, and a lowering blood pressure (BP) substantially reduces prematuremorbidity and mortality[1].The 2019annual report on cardiovascular health and diseases in China indicated that, the number of Chinese residents withhypertensionhas reached 245 million[2]. However, only 45.8% of the patients are treated, and the control of hypertension was 16.8%[3]. According to Chinese Guidelines for Prevention and Treatment of Hypertension,five classes of anti-hypertensive drugs, including calcium channel blockers (CCB), angiotensin-converting enzyme inhibitors (ACEI), angiotensinreceptor blockers (ARB), diuretics, β-blockers, and fixed-ratio preparations composed of the above drugs, are recommended.Highriskgroup of patients with BP [?] 160/100 mmHg and 20/10mmHg higher than that of the target BP, or patientswhoreceive mono-therapy and do not achieve the goal BPshould be treated with combinationtherapy[4]. It is well known that, compared to free-dose combinations, fixed-dose combinations (FDCs) of two or more antihypertensive agents in a single pill can improve medication compliance, an importantconsideration when requiring patients to self-administermultiple medications. One of the preferred specific drug regimens is ACEI/CCB,as the most common adverse effects of CCBs, peripheral edema and tachycardia, are partially neutralized by RAAS inhibitors[5].

Lisinopril, an ACEI, can decreaseperipheral vascular resistance and reduce blood pressure, preload, and afterload, without changes in heart rate[6]. Lisinopril is the only ACE inhibitor that exhibits a linear dose-response curve[7]. The antihypertensive effect of lisinopril usually appears within 1 h after oral administration, and peaks at about 6h. Bioavailability of lisinopril is about 20-28 %, and its cumulativeeffective half-lifeafter

multiple administration is about 12 h. Lisinopril does not bind to other plasma proteins other thanACE, and it is excreted from the urine in its original form without undergoingmetabolic transformation[8].

Amlodipine, a dihydropyridine-based CCB, inhibits the transmembrane influx of calciumions into vascular smooth muscle and is indicated for the management of stable angina and hypertension. Amlodipine is almost completely absorbed and is converted to inactive metabolites by CYP3A4 in liver[9].After single oral administration, amlodipine reaches at C_{max} within 6.0–8.0 hours and has a terminal elimination half-life of40–50 hours, with high oral bioavailability of 60%–65%[10].

The combination of lisinopril and amlodipine, two classes of long acting drugs, has a marked additional effect on blood pressure and fewer side effects than individual monotherapy[11, 12]. Though many pharmacokinetics studies forlisinoprilandamlodipine as a single pill have been reported, very few were focused on an FDC.The FDC of Lisinopril 10mg/ Amlodipine besylate 5mg (Lisonorm®) has been developed by Gedeon Richter Ltd and approvedin multiple countriesin the European Union, but not yet in China.The aim of this study was to compare the PK characteristics and evaluate the bioequivalence and food effect betweenLisonorm and the newly developed lisinopril/amlodipine besylate FDC productin Healthy Chinese Subjects.

Materials and Methods

Formulations

The test product of lisinopril 10mg /amlodipinebesylate 5mg (batch no.:180101;expiration date: December 2019)was produced by Sichuan MEIDAKANG Pharmaceutical Co. Ltd(SichuanProvince,China), and developed by Sichuan Sunrise Biopharm Co. Ltd (Sichuan Province, China).

The reference product of Lisonorm®(batch no.:T79030A;expiration date: September 2019) was produced by Gedeon Richter Ltd (Hungary).

Subjects

Healthy volunteers that meet the inclusion criteria and not the exclusion criteria were enrolled in the study after the clinical and laboratory examinations. The inclusion criteria included as follows:1)healthy male and female aged over 18years;2)the Body Mass Index is in the range of19.0 to 26.0 kg/m^2 (both inclusive), and males with minimum of 50 kg weight, females with minimum of 45kg weight; 3)subjects have no clinically significant abnormalities, including vital signs, physical examinations, laboratory tests, and ECG as determined by clinical examination; 4)agree to follow approved birth control methods.

Subjects were excluded if any of the following conditions were present: 1)allergic diathesis or hypersensitivity toinvestigational products; 2)history or presence of significant cardiovascular, urogenital, pulmonary, hepatic, renal, gastrointestinal, endocrine, immunological, dermatological, neurological or psychiatric disease or disorder, or other medical history affecting drug absorption; 3)use of any drugs or herbal medicinewithin 14 days; 4)smoking more than five cigarettes a day, abuse of alcohol or drugs, drinking too much tea, coffee or caffeinated drinks (more than 8 cups a day, 250ml/cup); 5)donation or loss of blood or plasma >400mL in the past 3 months; 6)consumption of any beverages or food containing caffeine or products rich in grapefruit, such as coffee, tea and chocolate, etc,within 48 hours prior toreceiving study drug.

Ethic

The bioequivalence study has been registered on ClinicalTrials.gov (No.:NCT04885660, retrospectively registered in May 2021) and been approved bythe Medical Ethics Committeeof the Affiliated Hospital of Qingdao University (No.: QYFYEC 2018–055-01). The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice (GCP) and applicable lawsand regulationsof China National Medical Products Administration(NMPA).Writteninformed consent wasobtained from all subjects before their participation in the study.

Study design

This was a single center, randomized, open-label, single-dose, two-period crossover bioequivalence study in both fasting and fed conditions. According to pre-test and previous studies, the coefficient of individual variation of lisinopril/amlodipinebesylate FDC product arranged from 20%-30%[13-15]. Under the condition that α=0.05, statistical test efficiency 1-β= 0.9, coefficient of variationwas 0.28, equivalent lower limitwas 0.80, upper limit was1.25 and actual ratiowas1, 35 samples were needed, using the "equivalence tests for the ratio of two means in a 2x2 cross over design" process insoftwarePower Analysis and Sample Size (PASS,version 15.0).Considering the possibility of shedding, 40 subjects were planned to be selectedin each fasting and fed bioequivalence study. All eligible subjects were randomly assignedaccording to the random table generated by the statistical professionals in the Department of Shanghai Second Military Medical University using SAS9.4. The subjects took the test(T) and reference(R)drugsrespectivelywith 240ml water after an overnight fast of at least 10 hours (fasting study) or after the high-fat breakfast within half an hour before dosing(fed study).The high-fat breakfast contained 929 kcal calories, and consisted of three pork buns with cabbage, spinach mixed with Yuba, and millet gruel. Breakfast composition and energy distribution was showed in Table1. Subjects were forbidden to drink water within 1h before and after taking the drug, and the lunch and dinner were provided at 4 h and 8 h respectively post-drug administration. No other food and beverage intake was permitted except the normal diets provided by the clinical trial center during the study. A washout of 14 days was set between the two administrations, according to the half-life recorded inoriginal drug instructions. 4ml venous blood samples were collected before drug administration and at1,2,3,4,5,6,7,8,9,10,11,12,13,24,36,48,72,96,144,168h after administration. The samples were centrifuged at 1700gear per minute for 10min at 4 to separate the plasma, which was divided into two aliquots(drug monitoring at least 800ul and backup) and stored at -80until analysis.The study flow chart is presented in Figure 1.

Bioanalytical Assay

Plasma concentrations of lisinopril and amlodipine were measured by an established and validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method at Suzhou Haike Pharmaceutical Technology Co., Ltd (Suzhou, Jiangsu Province, China). For the analysis of lisinopril, plasma samples were pretreated by liquid liquid extraction with isopropanol: ethyl acetate(1:2,V/V); LSN-d5 was used as internal standard; 5mM ammoniumacetate aqueous solution, 0.01% formic acid and methanol were used as mobile phase; chromatographic separation was performed on Atlantis-dC18 column(Waters, Massachusetts, USA) and the analytes were detected using Triple Quad TM 5500 tandem mass spectrometer(Sciex, Canada) in positive ion mode, with ion sprayin multiple reaction monitoring mode.The lower limit of quantification was 0.500ng/mL and the assay dynamic range was 0.500-100ng/mL. The analytes in matrix were stable when stored at -20 for 26 days, at -80 for 169 days and after four freeze-thaw cycles.

Amlodipine plasma concentrations were determinedusing a liquid chromatography unit(Shimadzu,LC-30AD,Japan) and a mass spectrometer(Sciex, Triple Quad TM 6500 plus, Canada). Under multiple reaction monitoring, LC-MS/MS system adopts positive ionization mode.Forthe analysis of amlodipine, the lower limit of quantification was 0.050ng/mL and the assay dynamic range was 0.050-10.0ng/mL.The analytes in matrix were stable when stored at -20 for 91days, at -80 for 207 days and after four freeze-thaw cycles.

Data collection and analysis was performed with Analyst 1.6.3 software (Sciex, Canada) and Watson LIMS(Thermo, USA). Calibration curves were constructed using linear regression equation obtained by the weighted ($W=1/X^2$) least square method fitting for both analytes.Quantitation of qualitycontrol and clinical samples were also performed by theAnalyst software using the same mathematical algorithm asthat used in the calibration of standard curves.

Pharmacokinetic Analysis

Pharmacokinetic (PK) parameters for lisinopril and amlodipine in plasma were estimated by a non-compartmental model (NCA) using Phoenix WinNonlin version 7.0 software (Pharsight Corporation, St Louis, MO, USA). For the purpose of bioequivalence analysis, the maximum observed concentration (C_{max}), the area under the plasma concentration-time curve from time 0 to the last measured time point ($AUC_{0\text{-}t}$),

and the area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-[?]}$)were considered as primary PK parameters. The secondary PK parameters were the observed time to C_{max}(T_{max}) and the apparent terminal half-life ($T_{1/2}$). C_{max} and T_{max}were the factually measured data andAUC_{0-t} was calculated using the linear and logarithmic trapezoidal methods. $AUC_{0-[?]}$ was calculated according to the following formula: $AUC_{0-[?]}$= AUC_{0-t}+C_{last}/λ_z($C_{last}$is the last measurable concentration and is the first order rate constant of terminal elimination determined from a linear regression line after logarithmic transformation at the end of concentration time curve. $\lambda_z$is the slope calculated by linear regression after logarithmic conversion at the end of the concentration-time curve). $T_{1/2}$ was calculated to be ln2/λ.

Safety assessment

The safety was evaluated by monitoring vital signs, physical examination, laboratory tests, electrocardiogram(ECG) and adverse events (AEs) collected after dosing throughout the study. Vital signs, including body temperature, blood pressure (BP) and heart rate, were measured at screening, before drug administration and at 2, 4, 6, 8, 12, 24, 36, 48, 72, 96, 144, 168h after administration. Routine laboratory tests (hematology, urinalysis, serum chemistry and pregnancy test for females) and 12-lead ECG were conducted at screening and before removal from the study. The AEs, including all subjective symptoms reported by subjects and objective signs observed by clinical investigators, were recorded and assessed for their severity and the correlation with research drugs.

Statistical analysis

Statistical analysis was performed by SAS 9.4. All data were tested by two-side test and the probability value (P) less than 0.05 was considered statistically significant.AUC and C_{max} were logarithmically transformed and analyzed by linear mixed effect model. Sequence, period and formulation were fixed effects, and subject within sequence was included as a random effect. Analysis of variance (ANOVA) of cross-over design was performed on the log-transformed variables. The geometric mean ratios (GMRs) of the primary pharmacokinetic parameters and their 90% confidence intervals (CIs) were calculated, and the test formulation was judged as bioequivalence if it fell within the equivalent range (80-125%). Bioequivalence was assessed separately in both the fasting and fed groups.

Results

Subject characteristics

A total of 181 subjects were screened for inclusion; 92 healthy subjects (40 fasting group and 52 fedgroup) were randomized into each of the study group, and 75 subjects (39 of fasting group and 36 of fed group) completed the study. 1 subject in fasting group withdrew because of pregnancy before admission in second period. 12 subjects in fed group fell off as a result of failing to finish the high-fat breakfast within 30 minutes, and replaced by the other subjects from the waiting list; besides, another 4 subjects dropped out due to poor compliance, voluntary withdrawal and AE of tonsillitis.

Data from the subjects who received a study drug at least once were used for safety assessment and subjects who completed the study were included in the PK analysis. The baseline demographic characteristics of subjects showed no statistical difference between the sequence groups (Table2).

Pharmacokinetics

The mean plasma concentration versus time profiles of lisinopril and amlodipine following a single dose of the test or reference products under fasting and fed conditions are illustrated in Figure2, the PK parameters are summarized in Table3.

The intra individual variation ofAUC_{0-72} and AUC_{0-t} of lisinopril were 21.2%, 19.3% and 14.5%, 13.3% respectively under fasting and fed condition, indicating that lisinopril has low variability. Therefore, the bioequivalence evaluation results ofAUC_{0-72} were added.

Regarding theC_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}72}$ (only lisinopril) and $AUC_{0\text{-}[?]}$ of lisinopril and amlodipine respectively,the 90% CIs for the GMRs fell within the predefined acceptance range of 80-125%, and provided supportive evidence for bioequivalence (Table4). Accordingly, lisinopril had a relatively long terminal elimination half-life of about 90 hours, which may be related to the binding saturation of the drug and ACE. In the fasting study, although the sample collected at 168 h after administration did not reach 3-5 half-lives, the last detectable concentration of all subjects was lower than 1/20 of the corresponding peak concentration and only 2.5% (2/79) of $AUC_{_\%Extrap}$was more than 20%. Therefore, the plasma concentration from 0-168h can completely describe the pharmacokinetic behavior of lisinopril. Compared with the fasting study, the C_{max} and AUC of lisinopril under fed condition were significantly reduced. Although 54.5% (40/74) of $AUC_{\_\%Extrap}$ was higher than 20%, 89.2% (66/74) of the final concentration at 168h were lower than 1/10 of the corresponding peak concentration, which could basically describe the pharmacokinetic behavior of lisinopril. After eliminating the data with $AUC_{\_\%Extrap}$ greater than 20% for sensitivity analysis, the 90% CI for the GMRs of $AUC_{0\text{-}[?]}$ of the test and reference preparation was 96.2% (86.7-106.7%).

A fat-high breakfast produced significant alteration in the C_{max} and AUC of lisinopril after a dose of either reference or test drug in Chinese healthy subjects.Compared with fasting study, after high -fat postprandial administration, lisinopril C_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}72}$and $AUC_{0\text{-}[?]}$ under fed condition were greatly decreased by 74%, 59%, 66%, 53% for test products ($P<0.001$), and 73%, 57%, 64%, 51% for reference products ($P<0.001$). In addition, there was a nearly 1.5-hour delay in median T_{max} under fed conditions for the test products. However, no changes were observed in $T_{max}$for reference products and in $T_{1/2}$ for both the test and reference products between the two fasting and fed studies.

Safety assessment

The test and reference drug of lisinopril/amlodipinebesylate FDC product showed good tolerance in all subjects. During the study, the vital signs of subjects were stable except that some subjects had signs of blood pressure reduction due to the expected effect of the study drug, and there was no clinically significant change in the follow-up laboratory examination after the administration compared with the baseline value.In the study of fasting condition, a total of 33 treatment emergent adverse events (TEAEs) were recorded in 20 subjects (50% of 40 subjects) after T treatment, and 25 TEAEs were recorded in 16 subjects (40% of 40 subjects) after R treatment. In the fed study, 13 TEAEs were recorded in 10 subjects (22.7% of 44 subjects) after T treatment, and 12 TEAEs were recorded in 9 subjects (20.5% of 44 subjects) after R treatment.All AEs were light and spontaneously recovered without specific intervention except for one instance of tonsillitis, which may be irrelevant to the study drugs, and a case ofatopic dermatitis.No subjects withdrew from the study due to AEs except for one case of tonsillitis and no severe adverse events (SAE) occurred. There was no significant difference in the incidence of AEs between the two treatments. All TEAEs were summarized according to system organ classification (SOC) and preferred term (PT), and were presented in Table5.Hypotension was the most common AE, and Figure 3 illustrates the changes in mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) from baseline to 24 hours.The results showed that the blood pressure decreased maximally from pre-dose values by 6 h after one dose of lisinopril/amlodipinebesylate FDC product and the suppression lasted up to 12 h. There was no significant difference in mean SBP between the fasting and fed groups, except at 8 h after administration of test product ($P=0.038$). However, the DBP decreasedmore than SBP in healthy Chinese subjects, and compared to fed study, the DBPdecreased obviously more at 4 h, 6 h, 8 h following the dosing with both regimens in the fasting state ($P<0.05$).

Discussion

The purpose of thisstudy was to compare the PK properties to examine whether the new lisinopril/amlodipinebesylate FDC product was equivalent to the reference for a new drug application to the NMPA.In this study, the GMR and its 90% CI for the C_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}72}$ (only lisinopril) and $AUC_{0\text{-}[?]}$ of lisinopril and amlodipinerespectively, under both fasting and fed conditions, fell within the conventional bioequivalence criteria of 0.80-1.25.In addition, compared with the reference drug, the incidence of AEs of the test drug had no difference, and showedsimilar safety and tolerance. These results indicated that the two

lisinopril/amlodipinebesylate FDC preparations were bioequivalent and exchangeable in clinical practice.

In this study, food appeared to greatly decrease the extent of lisinopril absorption by more than half and affect the antihypertensive effect for both the reference and test products. These results are inconsistent with the instructions of the original lisinopril tablet (Zestril® produced by AstraZeneca UK limited) and the reference Lisonorm(produced by Gedeon Richter Ltd). The instructions say the gastrointestinal absorption of lisinopril is not affected by food.A previous study investing the influence of food consumption on the rate or extent of absorption of orally administered lisinopril in healthy volunteers observed that, a breakfast (524kcal, consisting of one fried egg, two pieces of toast or bread, 20g of orange marmalade or jelly, two stripes of bacon, 150ml of skimmed milk and 100ml of orange juice)did not affect the bioavailability of lisinopril[16]. This inconsistency may probably be due to the fact that (i) high-caloric and high-fat foods have a more obvious impact on the physiology of the gastrointestinal tract and lead to more significant changes in the bioavailability of pharmaceuticals[17]; (ii)spinach in breakfast is rich in oxalic acid, which may change gastrointestinal PH and gastrointestinal peristalsis [18]; and (iii) the participants of this study were all young Chinese adults, and there may be ethnic differences in the pharmacokinetics of lisinopril. Among ACE inhibitors, lisinopril has a unique property that does not require hydrolysis to exert ACE inhibition, and only lisinopril and captopril are not ester prodrugs and less lipophilic[19]. Food has been shown to reduce the bioavailability of captopril by 35% to 50% after a single oral administration, but not the bioavailability of inhibitors administered as ester prodrugs[20].With high solubility, low membrane permeability and poor metabolism, the pharmacokinetic of lisinopril may be dominated by absorptive transporter effects[21].Only about a quarter of the administered dose is absorbed and the low bioavailability is due to poor gastrointestinal absorption rather than first-pass hepatic metabolism, as demonstrated by the factthat mean fecal recovery of lisinopril was 69% of intact drug[22].

In order to better understand the possible reasons for the decrease of lisinopril absorption in the fed study, we searched for various factors that affect lisinopril bioavailability.Little is known about pharmacokinetic interaction of lisinopril so far.Drugs that often used with lisinopril,such as nifedipine, digoxin,hydrochlorothiazide, have no substantial effect on the pharmacokinetics of lisinopril[23-25].No drug-drug interactions (DDIs) were found between the active components amlodipine and lisinopril. One of the factors that has been reported to affect the kinetic properties of lisinopril was age. Drug concentrations of elderly patients (>65 years) have been reported to be approximately double those of younger patients[26]. And a recent study demonstrated that a concomitant ingestion of epigallocatechin gallate(EGCG)-concentrated green tea extract significantly decreased lisinopril C_{max}, AUC0-24 and AUC0-[?]by 71%,69% and 67%, without altering renal clearance of lisinopril[27]. However, in the present study, the enrolled subjects was all between 18 and 50 years old, and those who have drink too much tea were excluded. Moreover, it was forbidden to taketeawithin 48 hours before taking the first administration and during the test.Therefore, larger studies are needed to evaluate the effect of food on the pharmacokinetics of lisinopril in Chinese.

Conclusions

In conclusion, the new lisinopril/amlodipinebesylate FDC product(specification: lisinopril 10mg / amlodipine 5mg)developed by Sichuan Sunrise Biopharm Co. Ltd (Sichuan Province, China)are equivalent to the Lisonorm (specification: lisinopril 10mg / amlodipine 5mg) produced by Gedeon Richter Ltd (Hungary). If the test formulation can be approved by NMPA, it can be used in the treatment of hypertension in Chinese adult patients. However, patients may need to avoid consumption of high-fat, high-calorie diet during treatment.

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Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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