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Research Article

Effect of food on Bioavailability of Amlodipine in Indian population

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ABSTRACT

Effect of food on bioavailability of amlodipine was assessed by comparing data of reference product (NORVASC tablet 10mg of Pfizer Pharmaceuticals, USA) from two bioequivalence studies. Fasting and non fasting study were conducted on 30 male healthy subjects. Drug products administration were done after 12 hours overnight fast and after 30.0 minutes of high fat breakfast in fasting and non-fasting study respectively. Serially blood samples were collected from time of dosing to 168.00 h of the post dosing at predefined time. Advance analytical technology (LC/MS/MS) was used for quantification of biological samples. Food intake increases C_{max} of Amlodipine by about 5.5%, increases AUC_t and AUC_i by about 2%, expedited T_{max} by about 9.6% and no change for kel. 90% confidence interval (C I90) of C_{max}, AUC_t and AUC_i within limit (80% to 125%). Bioavailability of Amlodipine is not affected by the presence of food.

Keywords: Amlodipine; food effect; Pharmacokinetics; bioavailability.

INTRODUCTION

Amlodipine is a vaso-selective, dihydropyridine derivative, calcium-channel blocking agent[1,2]. Amlodipine is used for management of hypertension alone or in combination with other antihypertensive agents[3-6]. with an angiotensin II receptor blocker would enhance antihypertensive activity with greater efficacy and better tolerability, which maximize the blood pressure-lowering effects and minimize the severity of their side effects of each component[7-9]. Chemically Amlodipine is (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate.[10] It has high bioavailability, large volume of distribution and long elimination half-life[11,12]. The metabolism and excretion of Amlodipine have been studied in healthy volunteers following oral administration of 14C-labelled drug[10]. Amlodipine is well absorbed by the oral route with a mean oral bioavailability of approximately 60%. Renal elimination is the major route of excretion with about 60% of an administered dose recovered in urine, largely as inactive pyridine metabolites. The major metabolite identified was 2- ([4- (2-chlorophenyl) -3- ethoxycarbonyl -5- methoxycarbonyl -6- methyl- 2- pyridyl]methoxy) acetic acid, and this represented 33% of urinary radioactivity. Amlodipine concentrations in plasma

declined with a mean half-life of 33 h, while elimination of total drug-related material from plasma was slower[11-13]. Amlodipine lowers blood pressure by relaxing arterial smooth muscles, which decreases total peripheral resistance and therefore reduces blood pressure. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extracellular calcium ions into these cells through specific ion channels. Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells[14]. Effectiveness of drug is depends on its bioavailability. One of the major factor affecting bioavailability is food effect. To study evaluate food effect it is necessary to perform bioavailability study in fasting and non fasting condition. Such study was not performed on Indian population. Hence aim of research work is to perform food effect study on Indian population.

MATERIAL AND METHODS

Study design

Both the bio-equivalence studies were Single dose, open label, two sequence, two period, randomized, balanced, crossover studies conducted on 30 healthy, adult, male human subjects for fasting and non-fasting phase. Amlodipine 10 mg tablet (NORVASC tablet 10mg of Pfizer Pharmaceuticals, USA) was administered at '0 hour' after 12h fasting to the subjects in sitting posture with about 240 ml of water at ambient temperature, and at about 30 minutes after serving of standardized high-fat & high calorie breakfast to the subject in sitting posture with about 240 ml of water at ambient temperature for fasting and non fasting study respectively under the supervision of trained personnel. This activity was followed by mouth check to assess compliance to dosing. Subjects were served food at different time which contained the nutrients as per Table-1.

Participants

All the subjects were informed of the aim and risk involved in the study and written consent were obtained. Written consent is the process by which subjects voluntarily confirm there willingness in a particular trial after having been informed of all aspects of the trial which are relevant to decision to participate in trial like purpose, procedures how to conduct, benefits, risks/discomforts of trial in written. The inclusion criteria for subject selection was based on the age (18years or above), body mass index (between 18.5 and 30.0kg/height^2), general physical examination, electrocardiogram and laboratory tests like hematology, blood chemistry, urine examination and immunological tests, demographics data given in Table-2. The exclusion criteria included subject with history of alcoholism, smokers and having a disease which may compromise the haemopoietic, gastrointestinal, renal, hepatic, cardiovascular, respiratory, central nervous system, diabetes, psychosis or any other body system. All the subjects who meet inclusion and exclusion criteria mentioned in protocol and were judged eligible for study, based on medication history, physical examination, vital signs and clinical laboratory tests. Approval for the study was obtained from Independent Ethics Committee. The study was conducted in accordance with internationally accepted standards of good clinical practices (ICH), good laboratory practices (21 CFR 51 and local regulatory requirements)[15].

Blood sampling

Blood samples of all the subjects under fasting and non fasting conditions were collected in vacutainers containing K_3 EDTA at predefine sampling time points as follows, prior to dosing (0.00 h), 0.50, 1.00, 1.50, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 10.00, 12.00, 16.00, 24.00, 48.00, 72.00, 96.00, 120.00, and 168.00 h Post dosing. Blood samples were centrifuged at 4ºC for 15 minutes, plasma was separated, transferred to labelled polypropylene tubes and stored in the freezer set at -20ºC ±10ºC until analysis.

Bioanalytical Method

It has been reported that low plasma concentration of Amlodipine achieved after oral administration[16], therefore, a sensitive and specific analytical method is essential for determination of Amlodipine in human plasma. Plasma samples of different time point were analyzed for the concentration of Amlodipine using validated LC-MS/MS method. Liquid-liquid extraction procedure was used to extract Amlodipine from plasma samples. Amlodipine-D4 was used as an internal standard. Chromatographic separation was achieved by Kinetex 5µ biphenyl (100*4.6mm, 5µm) column. The linear dynamic calibration curves range was kept from 0.1000ng/mL to 20.00ng/mL. API 3000 LC/MS/MS (equipped with turbo ion spray) mass spectrometer was used for analysis.

Pharmacokinetics Analysis

For generation of concentration data from LC/MS/MS "Analyst" software of Applied
Bio-system was used. All concentration data were used for application of bio-statistic using WinNonlin® professional software and SAS® Statistical analysis software. The concentration data was used to calculate the pharmacokinetic parameters C_{max}, T_{max},

AUC_t (the area under the plasma concentration time curve from time zero to the time of the last non zero concentration) and AUC_i (the area under the plasma concentration time curve from time zero to the infinite time) using a non-compartmental analysis of WinNonlin® professional software. The pharmacokinetic parameters and drug plasma concentration were evaluated statistically using SAS® for effect of food.

RESULTS AND DISCSSION

All oral doses were well tolerated by the subjects, and no adverse events were reported. Pharmacokinetic parameters for Amlodipine and percentage different are presented in Table-3. AUC comparison in fasting and non fasting conditions of Amlodipine given in Figure:-1.

Average C_{max} of all the subjects for Amlodipine found 5.928ng/mL and 6.255ng/mL in fasting and non fasting condition respectively. Food intake increases C_{max} of Amlodipine by about 5.5%.
Average AUC_t and AUC_i of all the subjects for Amlodipine was found 298.131 hr*ng/mL and 321.172 hr*ng/mL respectively in fasting condition. AUC_t and AUC_i for Amlodipine was found 304.800 hr*ng/mL and 329.630 hr*ng/mL respectively in non fasting condition. Food intake increases AUC_t as well as AUC_i of Amlodipine by about 2%.

Table 1
Nutrition given in fasting and non-fasting condition

Timing	Nutrition							
	Carbohydrates (gm)		Proteins (gm)		Fat (gm)		Energy (K.Cal.)	
	Fasting	Non Fasting	Fasting	Non Fasting	Fasting	Non Fasting	Fasting	Non Fasting
DINNER*	138.200	138.200	24.800	24.800	22.500	22.500	851.000	851.000
BREAKFAST*	NA	61.600	NA	42.600	NA	66.350	NA	1012.50
LUNCH	165.950	141.900	37.800	30.300	23.750	23.100	1026.00	890.000
SNACKS	63.100	63.100	5.300	5.300	11.200	11.200	357.000	357.000
DINNER	150.000	150.000	32.600	32.600	23.500	23.500	923.000	923.000
BREAKFAST	57.750	57.750	14.250	14.250	6.500	6.500	345.000	345.000

*= served Prior to dosing
**= served for non fasting study only

Table 2
Demographic data

		Non fasting study	Fasting study
Subjects enrolled		30	30
Study completed		30	30
Gender		Males	Males
Age (Years)	Mean(±SD)	28(±6)	28(±7)
	Median	28	27
	Min	19	19
	Max	43	43
Weight (kg)	Mean(±SD)	61.0(±7.3)	60.4(±7.0)
	Median	60.4	60.0
	Min	48.3	48.3
	Max	75.0	74.5
Height (cm)	Mean(±SD)	167.5(±5.6)	167.2(±5.5)
	Median	167.3	167.3
	Min	156.0	156.0
	Max	180.0	180.0
Race		Asian	Asian

Table 3
Statistical data of pharmacokinetics parameters of Amlodipine

Parameters		Amlodipine (n=30)		% Different
		Fasting	Non-fasting	
AUC_t (hr*ng/mL)	Mean	298.131	304.800	2.2
	Std	91.567	78.636	
AUC_i (hr*ng/mL)	Mean	321.172	329.630	2.6
	Std	99.958	87.214	
C_{max} (ng/mL)	Mean	5.928	6.255	5.5
	Std	1.575	1.337	
T_{max} (hr)	Mean	8.333	7.533	9.6
	Std	1.583	2.047	
Kel (1/hr)	Mean	0.017	0.017	0.0
	Std	0.004	0.003	

n: Number of subjects enrolled, C_{max}: Maximum plasma concentration, T_{max}: Time to achieve maximum plasma concentration AUC_t: (the area under the plasma concentration time curve from time, zero to the time of the last non zero concentration), AUC_i: (the area under the plasma concentration time curve from time, zero to the infinity time), kel: Elimination constant

Table 4
Statistical result on log transferred data of Amlodipine

Dependent	Unit	NFGeoLSM	FGeoLSM	Ratio_%	CI90 Lower	CI90 Upper
$Ln(C_{max})$	ng/mL	6.121	5.746	106.53	96.41	117.73
$Ln(AUC_t)$	hr*ng/mL	296.367	285.235	103.90	92.59	116.60
$Ln(AUC_i)$	hr*ng/mL	319.633	306.685	104.22	92.45	117.50

NFGeoLSM= Non fasting geometric least square mean , FGeoLSM= Fasting geometric least square mean
CI 90= Confidence interval at 90, Ln= Natural logarithm

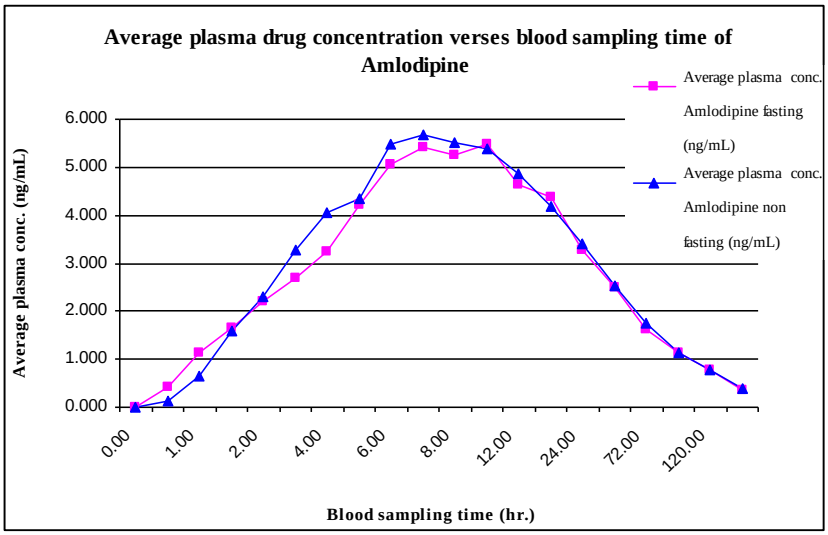

Figure 1
AUC comparison of Amlodipine in fasting and non fasting condition

Average T_{max} of all the subjects for Amlodipine in fasting condition were found 8.333 hr and in non fasting condition 7.533 hr. Food intake expedited T_{max} of Amlodipine by about 9.6%.
Average Elimination constant (kel) of all the subjects for Amlodipine in fasting and in non fasting condition found 0.017 hr. Elimination constant (kel) of Amlodipine remain unchanged in fasting and non fasting condition.
Results of 90% confidence interval of Amlodipine for C_{max}, AUC_t and AUC_i are within limit (80% to 125%). Ratio% of Geometric Least Square Means of non fasting to fasting for C_{max}, AUC_t and AUC_i found 106.53, 103.90 and 104.22 respectively. Table:-4 Statistical result on log transferred data of Amlodipine.

CONCLUSION

Most advance analytic technique high-performance, liquid chromatography (HPLC)-electrospray ionization tandem mass spectrometry (MS-MS) was used for quantification of Amlodipine in biological matrix. Use of advance technique gives more accurate results which help to study pharmacokinetics profile of Amlodipine in fasting and non fasting condition. Effect of food on pharmacokinetics parameters may or not clinically significant. [17-18] Constituents of food can change pharmacokinetics of drugs by many ways like delayed gastric emptying time, complexation of food constituents with drug, change in solubilisation of drug, alteration of hepatic blood flow and change of metabolism of drug by enzymes due to food effect.[19-22] Present research indicates Food intake increases C_{max} of Amlodipine by about 5.5%, increases AUC_t and AUC_i by about 2%, expedited T_{max} by about 9.6% and no change for kel.

For Amlodipine, it was found that 90% confidence interval (C I90) of C_{max}, AUC_t and
AUC_i within limit (80% to 125%). Hence, it can be said that no food effect was found for pharmacokinetics parameters C_{max}, AUC_t and AUC_i of Amlodipine, which indicates that the efficacy of Amlodipine is not affected by presence of food.
Thus, it can be concluded that the bioavailability of Amlodipine is not affected by the presence of food.

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