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SCIENTIFIC REVIEW

Bioavailability File: Amlodipine

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Amlodipine (AML), a third-generation dihydropiridin, is a long-acting L-calcium channel blocker used in the treatment of hypertension and angina pectoris. It exerts its effects by blocking the voltage-dependent L-calcium channels and binding to both dihydropiridin and nondihydropiridin binding sites. AML is well absorbed (96%) after oral administration and its bioavailability is between 64-90%. Its volume of distribution is about 16 to 21 L/kg and protein binding is 98% after oral administration. AML is extensively metabolized in the liver and its elimination from the plasma is biphasic with a terminal half-life of 30 to 50 h. It is excreted by renal route about 60%. According to Biopharmaceutics Classification System, AML is classified as class I drug by WHO. In this review physicochemical properties, pharmacology, analytical methods, pharmacokinetics and bioavailability of amlodipine are discussed.

Key Words: *Amlodipine, Bioavailability, Pharmacokinetics, Biopharmaceutics Classification System (BCS)*

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Biyoyararlanım Dosyası: Amlodipin

Amlodipin (AML) yüksek tansiyon ve anjina pektoris tedavisinde kullanılan, üçüncü jenerasyon dihidropiridin türevi, uzun etkili L-kalsiyum kanal blokörü bir ilaçtır. Etkisini dihydropiridin ve nondihydropiridin bağlanma bölgelerine bağlanarak ve voltaja bağlı L-kalsiyum kanallarını bloke ederek göstermektedir. AML, oral uygulamadan sonra iyi absorplanmakta (%96) ve biyoyararlanımı %64 ile %90 arasında gerçekleşmektedir. Oral uygulamadan sonraki dağılma hacmi yaklaşık olarak 16-21 L/kg ve proteinlere bağlanması %98 olmaktadır. Amlodipin karaciğerde yüksek oranda metabolize olmakta ve plazmadan eliminasyonu iki fazlı olup eliminasyon yarılanma ömrü 30-50 saattir. Renal yolla %60 oranında itrah edilmektedir. AML Dünya Sağlık Örgütü tarafından biyofarmasötik sınıflandırma sistemine göre sınıf 1 ilaç olarak sınıflandırmaktadır. Bu derlemede amlodipinin fizkokimyasal özellikleri, farmakolojisi, analitik metodları, farmakokinetiği ve biyoyararlanımı tartışılmıştır.

Anahtar Kelimeler: *Amlodipin, Biyoyararlanım, Farmakokinetik, Biyofarmasötik Sınıflandırma Sistemi (BSS)*

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INTRODUCTION

Amlodipine (AML), a third-generation dihydropiridin, is a long-acting L-calcium channel blocker used in the treatment of hypertension and angina pectoris (1-6). Like other calcium channel blockers AML causes relaxation of vascular smooth muscle and cardiac muscle (4-7). Pharmacokinetics of AML is very different from other drugs from its class (8-11). A pKa value of 8.7 means that AML is present in the ionized form at the physiologic pH. Therefore, it possesses a strong affinity for cell membranes. These characteristics are believed to be a reason for AML's unique pharmacokinetics (10, 11). AML has higher bioavailability, longer half-life ($t_{1/2}$), longer time to C_{max}, higher volume of distribution and slower gradual elimination than other calcium channel blockers (3, 4, 6, 8-12). Unique pharmacokinetic profile of AML is directly connected with clinical benefits (13). AML is the most frequently used antihypertensive drug among all dihydropiridines (14) .

PHYSICOCHEMICAL PROPERTIES

Amlodipine besylate (AB) is a salt of AML (CAS 111470-99-6). Although it is used as a racemic mixture, only the S (-)-enantiomer is pharmacologically active, whereas R (+)-enantiomer is 1000-fold less active (2, 15). The chemical name is 3-ethyl-5-methyl(±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl) -1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzene sulphonate. The empirical formula of AB is $C_{20}H_{25}ClN_2O_5 \bullet C_6H_6O_3S$ (1). The chemical structure of AB is shown in Figure 1.

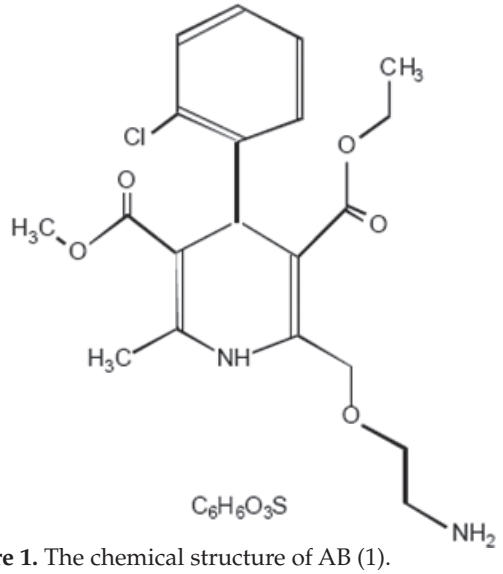

Figure 1. The chemical structure of AB (1).

AB is white to off-white, crystalline powder with a molecular weight of 567.06. It is slightly soluble in water and propanol, freely soluble in methanol, sparingly soluble in ethanol (1). Melting range of AB is 195-204°C. Theoretical and practical octanol/water partition coefficient (log K_{ow}) are 3.00 and 2.66 respectively (16). AB is stable under ordinary conditions. AML has a pKa value of 8.7 (10, 11) .

ANALYTICAL METHODS

Chromatography

Several HPLC methods have been described in the literature for determination of AML in human plasma (2, 3, 17-34), rat plasma (35), tablet and capsule (36) samples (Table 1). Also various HPLC methods have been reported for determination of AML in combination with other drugs (37-46) (Table 2). All these methods differ with respect to the mobile phase, columns and detection methods used for the analysis of compounds. Other methods like thin layer chromatography (45), gas chromatography (47), high-performance thin-layer chromatography (48) were also developed to determine the amount of AML in human plasma or pharmaceutical formulations.

Spectroscopy

Several spectrophotometric methods have been described for determination of AML in pure form (49-52), pharmaceutical formulations (e.g. tablets, capsules) (36, 49, 51-53) and also for AML in combined pharmaceutical dosage forms (37, 54-56). Very few spectrofluorometric methods have appeared in literature for determination of AML in tablets (57, 58) .

Voltammetry

Two voltammetric methods have been reported for determination of AML in human urine (59) and pharmaceutical formulations (59, 60) .

PHARMACOLOGY

AML is a third-generation calcium channel antagonist, and inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. AML blocks voltage-dependent

Table 1. Chromatographic conditions of the reported methods used for the separation and determination of AML.

Samples	Column	Detection	Mobile Phase	Flow Rate (mL/ min)	Extraction (+/-)	LOD/ LOQ (ng/mL)	Retation time (min)	Refe-rence
Human plasma	HyPurity C18 (150x3.9 mm, 3µm)		ACN: 0.05M KH2PO4 buffer: AA (62:38:01) (pH 3.5)	1.8		1.0/10		(17)
Human plasma	Bond Elut C2	Amperometric						(18)
Human plasma	C18 Column	Ultraviolet	10mM/L Ammonium Acetate: MeOH (30:70)	0.2	+	-/0.2		(19)
Human plasma	Supelcosil ABZ+Plus (25cmx4.6mm, 5µm)	Ultraviolet 360 nm	ACN: Water (70:30) with 10mM acetate buffer (pH 5)		+			(20)
Human plasma	Luna RP-C18 (15x2mm, 3µ)	Mass Spectrometry			+	>1.0/-		(21)
Human plasma	Monolithic WCX column				+	0.2/-		(22)
Human plasma	C18 Column (100x2.1mm, 3µm)	Mass Spectrometry	0.1%FA in ACN/0.1%FA in Water (42:58)	0.25	-	0.2/0.2	2.10	(3)
Human plasma	C18 Column	Mass Spectrometry			+		1.9	(23)
Human plasma	ACQUITY UPLC BEH C18 (50x2.1mm, 1.7µm)	Mass Spectrometry	Water: ACN (Both containing 0.3%FA)Gradient conditions	0.35		-/0.15		(24)
Human plasma	Nucleosil C8 (125x4.6mm)	Ultraviolet 239 nm	0.01 M NaH2PO4 buffer :ACN (63:37) (pH 3.5)	1.5	+	0.2/-		(25)
Human plasma	Bondapak C18 (300x3.9mm, 10µm)	Fluorescence	MeOH: Water (80:20)	0.8	+		>20	(26)
Human plasma	X-Terra C18	Mass Spectrometry	0.02M Ammonium formate: ACN (20:80) (pH 4.5)	0.5			1.12	(27)
Human plasma	Chiral-AGP Column (150x10mm) Supelcosil LC8 (20x4.6mm, 5µm)		10mM Acetate buffer with 1% 1-propanolol (pH 4.5)	0.9	+	0.1/0.2	-	(2)
	Symmetry C8 (150x4.6mm, 4µm)	Ultraviolet 240 nm	10 mM Acetate buffer: ACN (55:45) (pH 4.5)					
Human plasma	Genesis C18 (150x4.6mm, 4µm)	Mass Spectrometry	60%CH3CN: 40%Water + 10 mM FA	0.4	+	0.1/-	3.4	(28)
Human plasma		Mass Spectrometry						(29)
Human Plasma	C18 Column	Mass Spectrometry	MeOH:1% HAc (65:35)		+			(30)
Human Plasma	C18 Column	Fluorescence Exc: 470 nm Em: 537 nm	Sodium phosphate buffer with 1mL/L TEA: MeOH (pH 2.5)	2.8	+	-/0.25	>20	(31)
Human Plasma	Waters symmetry C18 (150x4.6, 5µm)	Mass Spectrometry	Water: ACN: FA (30:70:0.03)	1.0	+		1.5	(32)
Human Plasma	Hypersil BDS C18	Mass Spectrometry			+		3.2	(33)
Human plasma	C8 Column (150x4.6 mm,5 µm)	Ultraviolet 238 nm	20 mM/L MeOH: KH2PO4 (42:58) (pH 3.5)	1.0	+	0.1/-		(34)
Rat plasma		Electrochemical						(35)
Tablet Capsules	C18 Column		0.1% H3PO4: ACN (60:40) (pH 3.0)	1.0				(36)

Abbreviations: ACN: Acetonitrile, AA: Acetic Acid, MeOH : Methanol, FA: Formic Acid

Table 2. Chromatographic conditions of the reported methods used for the separation and determination of AML in combination with other drugs.

Samples	Column	Detection	Mobile Phase	Flow Rate (mL/min)	Extraction (+/-)	LOD/ LOQ (µg/mL)	Retation Time (min)	Reference
Binary mixtures (AML+VAL)	RP ACE C18 (150x4.6mm; 5µm)	Ultraviolet 254 nm	MeOH:ACN:NaH_2PO_4 + 5mL/L TEA (42:18:40) (pH 3.0)	2.0				(37)
Commercial formulation (AML+RAM; AML+ ENA)		Ultraviolet 210 nm	MeOH:Water (50:50)					(38)
Rat liver perfusate containing AML and VAL	HICHROM Nucleosil 100-5 C18 (250x4.6mm)	Ultraviolet 240 nm	Phosphate buffer: ACN:MeOH (50:40:10) (0.01M, pH 3.6)	1.0	-	0.02/0.05	8.23	(39)
Tablet (AB+OLMED)	Kromasil C18 (250x4.6 mm)	Ultraviolet 238 nm	KH_2PO_4/K_2HPO_4: ACN (50:50) (0.05 M)	1.0	-		3.69	(40)
Tablet (AB + BIS)	Luna C18-2 (50×4.6mm; 3µm)	Ultraviolet 230 nm	Ammonium buffer: ACN (65:35) (pH 5.0)	0.8	-	-	3.91	(41)
Tablet (AB+LOS+ HYDR)	Kromasil C18 (4.6 mm i.dx250 mm)	Ultraviolet 232 nm	KH_2PO_4/K_2HPO_4:ACN (57:43) (0.025 M, pH=3.7)	1.0 (6.3min) 1.3 (6.3min)	-	-/-	5.12	(42)
Capsule (AB+BH)	Aquity UPLC, BEH C8 (100x2.1mm, 1.7µm)	Ultraviolet	Phosphate buffer: equal mix. ACN+MeOH (45:55) (pH 3.0)	0.3		-/0.01		(44)
Tablet (AB+VAL)	Zarbax ODS (4.6cmx250mm, 5µm)	Ultraviolet 254 nm	ACN: Phosphate buffer (50:50)	1.0	-	0.08/0.22	1.5	(93)
Human plasma Bulk powder Tablet (AB+VAL)	xTerra C18 (250x4.6mm, 5µm)	Ultraviolet 237 nm	MeOH: ACN: Water: 0.05%TEA (40:20:30:10) (pH 3.0 ±0.1)	1.2	-	-	-	(45)
Tablet dissolution samples (AML+VAL)	C18 ODS2 (200x4.6mm, 10µm)	Ultraviolet 240 nm	Phosphate Buffer:ACN:MeOH (44:46:10) (0.01 M, pH 3.6)	1.0	-	0.05/0.1	7.1	(46)

Abbreviations: AML: Amlodpine, AB: Amlodpine Besylate, VAL: Valsartan, RAM: Ramipril, ENA: Enalapril, OLMED: Olmesartan medoxomil, BIS: Bisoprolol fumarate, NH: Nebivolol Hydrochloride, LOS: Losartan Potassium, HYDR: Hydrochlorothiazide, BH: Benazepril Hydrochloride, ACN: Acetonitrile, MeOH : Methanol, TEA: Triethylamine, mix.: mixture.

L-calcium channels (1, 4, 6, 8, 10, 11, 61, 62) and it binds to both dihydropiridin and nondihydropiridin binding sites (1, 10). Because the contractile processes of muscles are dependent on amount of calcium ions in cells, the inhibition of calcium influx leads to vascular smooth muscle relaxation and negative inotropic and chronotropic effects in heart. AML acts selectively and it has greater effect on vascular smooth muscle cells than on cardiac muscle cells (1, 4, 61, 62). Therefore, the negative inotropic effect is not significant *in vivo*, when AML is dosed in therapeutic amounts (1, 4, 10, 61, 62). Also the effects of AML on smooth muscle are more pronounced in arteries than in venous beds. AML causes reduction in peripheral resistance due to arterial dilatation and this is the main mechanism that leads to reduction in blood pressure and antianginal effects (1, 4, 61, 62) .

AML reduces supine and standing blood pressures. The heart rate and plasma catecholamine levels are not significantly changed when AML is chronically administered. With once daily administration the antihypertensive effect is maintained for at least 24 hours. In healthy, normotensive subjects, AML does not change blood pressure significantly (1) .

Wang et al. (63) investigated the effect of AB and dexamethasone combination in a gel formulation (0.5% and 0.3% respectively) on the ischemic skin flap. The results of the study showed that AB and dexamethasone in gel formulation might penetrate into skin tissue and could significantly increase the survival area of ischemic skin flap.

Side effects

Treatment with AB is usually well tolerated at doses up to 10 mg daily. The most common side effects are headache and edema. Other most common adverse reactions are: flushing, palpitation, fatigue, nausea, abdominal pain, and somnolence. The frequency and severity of adverse effects are connected with dose and several side effects (e.g. edema, flushing, palpitation, and somnolence) also with sex (more incidents in women than men). Other side effects such as cardiovascular problems, psychiatric problems, allergy, and musculoskeletal illnesses are very rare (the events occurred in less than 1% in placebo-controlled trials) (1, 64) .

Drug Interactions

In contrast to most of other calcium channel blockers, AML has few significant drug interactions. There is no clinically significant effect on the human plasma protein binding of digoxin, phenytoin, warfarin and indomethacin. Patients receiving drugs that induce or inhibit cytochrome P450 3A4 should be monitored for a potential change in AML response (1) .

FORMULATION TYPES

Although 2.5, 5 and 10 mg conventional tablets of AB (Norvasc®, Pfizer) are commercially available in the market, many researchers are trying to develop its rapidly dissolving tablets or dispersions with different preparation techniques and different excipients in order to increase patient compliance for those who have trouble in swallowing tablets such as elderly or pediatric patients (1, 65-69) .

To investigate the stability of AML, Nahata et al. (70) prepared two suspension formulations containing 1 mg/mL AML using commercially available AML tablets (Norvasc-Pfizer). One of the formulation is in extemporaneously prepared 1% methylcellulose in syrup (1:1), and the other is in commercially available OraSweet®/OraPlus®. The results of the study showed that AML was stable in both suspension formulations stored in plastic prescription bottles for 91 days at 4ºC or 56 days at 25ºC. It was concluded that these formulations may be useful for elderly or pediatric patients who are unable to take tablets. Lyszkiewicz et al. (71) studied the bioavailability of Nahata et al.'s suspension formulations, and found that the bioavailability of the suspension formulations was not different from 5 mg tablet formulations. These findings support the use of the suspensions in children. Although, Nahata et al. claimed that the suspension formulations were stable for 56 days at room temperature, Lyszkiewicz et al. recommended freezing the suspensions during the using period or using it within a shorter period of time (70, 71) .

Various dosage forms of AB were investigated in the literature. The nanoemulsion drug delivery system of AB was designed by Chhabra et al. (72) to improve solubility and oral bioavailability of the drug and to localized delivery of drug at target size. Swamy et al. (73) were prepared intranasal hydroxyproyl guar (HPG) microspheres containing AB by using water in oil emulsification solvent evaporation technique in order to avoid first pass metabolism, to achieve controlled blood level profile and to improve therapeutic efficacy. Based on the results, it is suggested that, HPG is a suitable biodegradable polymer for nasal drug delivery of drugs with first pass metabolism such as AB. Asymetric membrane capsules (AMCs) containing both AB and atenolol were prepared by Garg et al. (74). It was reported that the best AMCs formulation which consist of highest amount of osmotic agents and optimum amount of buffering agents followed zero order release kinetics for AB.

According to the FDA, AB has 184 drug products either alone or in combination with other drug (s). These drug products are registered as oral tablet or capsule dosage forms in combination of AB: Aliskiren Hemifumarate, AB:Atorvastatin Calcium, AB:Benazeprile Hydrochloride, AB:Olmesartan Medoxomil, AB:Telmisartan, AB:Valsartan, AB:Aliskiren Hemifumarate:Hydrochlorothiazide , AB:Olmesartan Medoxomil:Hydrochlorothiazide, AB:Valsartan:Hydrochlorothiazide (75) .

DOSAGE AND ADMINISTRATION

AB is available commercially in 2.5, 5 and 10 mg tablets. In adults the initial dose is 2.5 or 5 mg daily, the maximum dose is 10 mg daily. In pediatric patients, for 6-17 years of age, the effective antihypertensive dose is 2.5-5 mg once daily. The use of more than 5 mg once daily has not been studied in pediatric patients (1) .

PHARMACOKINETICS AND BIOAVAILABILITY

Absorption

AML is a dihydropiridin that has a slow absorption and prolonged effect. The extent of AML absorption is about 96% following oral administration (76). Following oral and i.v. doses of ^{14}C-AML to rat and dog, 40-50% of the dose was excreted in the urine indicating that the oral dose was well absorbed (34). According to the "Martindale The Extra Pharmacopoeia" the bioavailability varies but is usually about 60 to 65% (77). When ^{14}C-AML was administered to two human volunteers by means of single oral and intravenous doses, the drug was well absorbed by the oral route and the mean oral bioavailability for unchanged drug was 62.5% (76). Maximum plasma level is reached 6-12 hours after single oral administration, and absolute bioavailability of the AML tablet is estimated to be 64 to 90% (1, 9, 76, 78, 79) .

Faulkner et al. (79) investigated the pharmacokinetics of AML following single (10 mg, n=12, oral and IV) and repeated dose (15 mg/daily, n=28, 14 days, oral) administrations. The pharmacokinetic parameters determined after single and repeated dose administrations were given in Table 3. Comparative pharmacokinetics after single iv and oral dose showed that bioavailability of oral AML is 64%. In repeated oral administration (once daily for 14 days, 15 mg), the steady state plasma drug concentration was reached by the seventh dose. Relatively long elimination half-life of AML (45 h) after repeated doses resulted in an approximately threefold accumulation.

Table 3. Pharmacokinetic parameters of AML obtained after single and repeated dose administrations (75).

Pharmacokinetic Parameters	Single dose (10 mg)	
	Oral	IV
$AUC_{0-\infty}$ (ng.h./mL)	238±53	371±69
C_{max} (ng/mL)	5.9±1.2	–
t_{max} (h)	7.6±1.8	–
k_{el} (h^{-1})	0.020±0.0036	0.021±0.0032
CL (mL/min per kg)	–	7.0±1.3
$t_{1/2}$ (h)	35.7±6.1	33.8±5.3
V (L/kg)	–	21.4 + 4.4
Bioavailability (%)	64 Range 52-88	–
	Repeated oral dose	
	Day 1	Day 14
C_{max} (ng/mL)	6.9 ± 2.6	18.1 ± 7.1
t_{max} (h)	8.9 ± 3.7	8.7± 1.9
C_{min} (ng/mL)	3.3 ± 1.2	11.8 ± 5.3
C_{av} (ng/mL)	4.5 ± 1.6	14.5 ± 5.8
$t_{1/2}$ (h)	–	44.7 8.6
k_{el} (1/h)	–	0.016 0.0034

Pharmacokinetic parameters of different AML salts (5, 80, 81) or generic AML tablets (78, 82) were examined in several studies. All studies showed no significant differences between pharmacokinetic and pharmacodynamic characteristics among different AML formulations. Carvalho et al. (28) investigated the bioequivalence of 5 mg AML (test formulation) or AB (reference formulation, Norvasc®) tablets in 24 healthy volunteers (Figure 2, Table 4). The study was conducted using an open-label, randomized

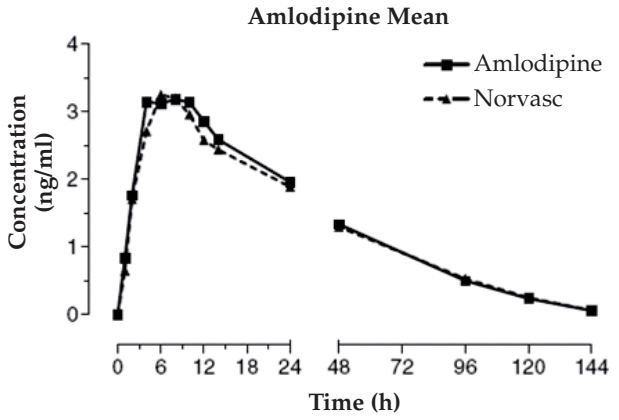

Figure 2. Mean plasma concentrations versus time curve for both AML formulations (28) .

two-period crossover design. It was found that AUC_{last}, AUC_{0-inf} and C_{max} ratios were within the 80–125% interval indicating that 5 mg AML tablet was bioequivalent to 5 mg Norvasc® tablet (28). Similarly, Liu et al. (3) was reported no significant difference between the pharmacokinetic parameters of test (dispersible AML tablet) and reference (Norvasc®) AML tablets obtained after oral administration of single dose (2x5 mg tablet) to healthy Chinese male volunteers.

Pharmacokinetics of AB in an AB/atorvastatin calcium (AC) combination tablet was investigated in a randomized, 2-way crossover design in 126 healthy volunteers. Subjects received a single dose of AB/AC tablet or coadministred matching doses of individual AB and AC tablets at the highest (10/80 mg; n=62) and the lowest (5/10 mg; n=64) dose strengths. The results of the study (Table 5 and Figure 3) demonstrated that there was no significant difference between the pharmacokinetic parameters obtained after oral administration of combination AB/AC tablets and coadministred individual AB and AC tablets (47) .

Chhabra et al. (72) were developed nanoemulsion (NE) of AB by spontaneous emulsification method with the aim to enhance the solubility and oral bioavailability of AB and to achieve localized drug delivery at target site. The drug release from NEs was significantly higher than the marketed tablet formulation ($p < 0.01$). The pharmacokinetics and biodistribution studies of the optimized radiolabeled (99mTc-labeled) formulation (15% Labrafil M, 35% Tween 80: ethanol (2:1), and 50% by weight aqueous phase) in mice (p.o.) demonstrated a relative bioavailability of 475% against AB suspension. In almost all the tested organs, the uptake of AB from NE was significantly higher ($p<0.05$) than AB suspension especially in heart with a drug targeting index of 44.1%, also confirming the efficacy of nanosized formulation at therapeutic site. A three times increase in the overall residence time of NE further signifies the advantage of NEs as drug carriers for enhancing bioavailability of AB (72) .

Table 4. Mean pharmacokinetic parameters obtained from 24 volunteers after oral administration of 5 mg AML tablet (28).

Pharmacokinetic parameter	AML Tablet	Norvasc® Tablet
AUC_{0-last} (ng.h/mL)		
Geometric Mean	151.7	147.4
S.D.	78.1	75.1
AUC_{0-inf} (ng.h/mL)		
Geometric Mean	166.9	166.3
S.D.	78.8	76.7
C_{max} (ng/mL)		
Geometric Mean	3.9	3.8
S.D.	2.5	2.1
k_e (1/h)		
Median	0.02	0.02
Range	0.01-0.03	0.01-0.04
$t_{1/2}$ (h)		
Median	33.9	37.0
Range	24.3-45.7	18.7-63.4
t_{max} (h)		
Median	6.0	6.0
Range	2.0-14.0	4.0-14.0

Distribution

AML has volume of distribution of 16 to 21 L/kg following oral administration (4, 83, 84). Tissue distribution is extensive in particular into the liver (84). AML is approximately 98% bound to plasma proteins in hypertensive patients (1, 83). The effect

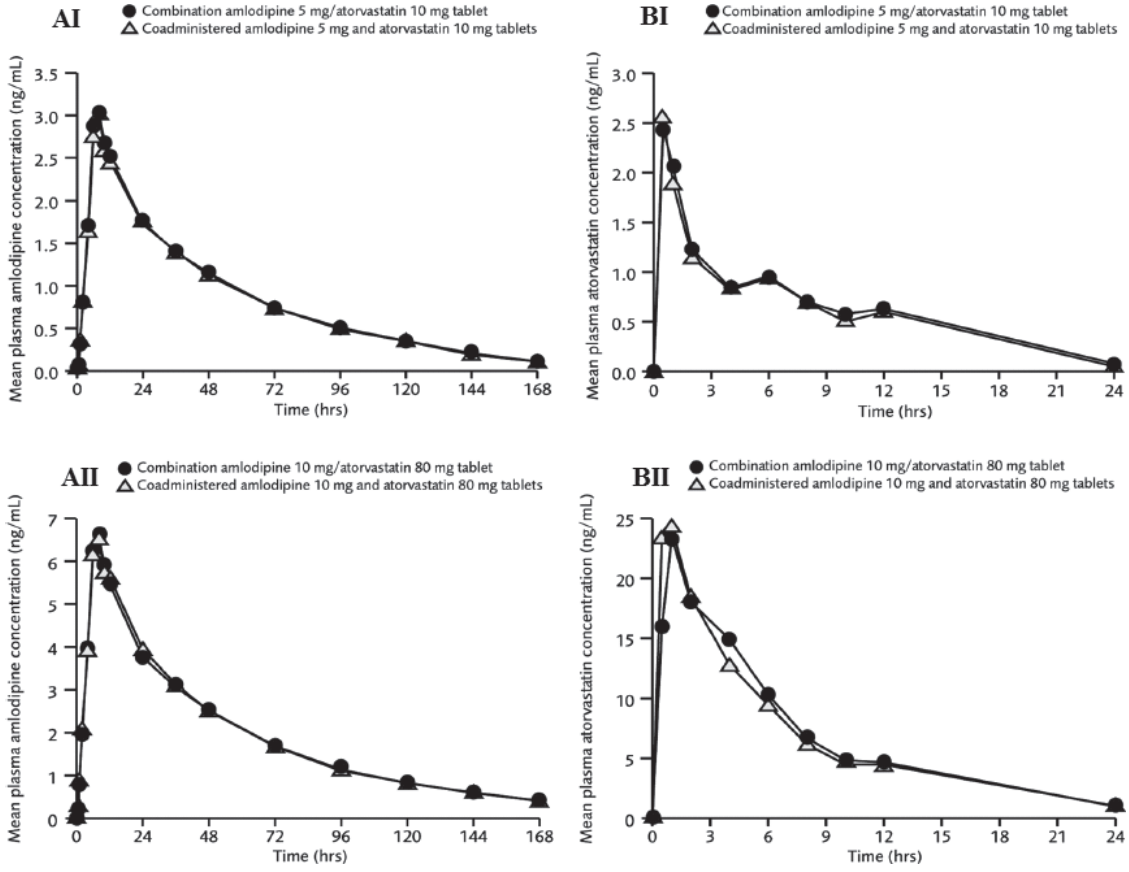

Figure 3. Mean plasma concentrations of AML (AI) and atorvastatin (BI) after the administration of 5/10-mg combination tablets versus the coadministration of AML 5-mg and atorvastatin 10-mg tablets. Mean plasma concentrations of AML (AII) and atorvastatin (BII) after the administration of 10/80-mg combination tablets versus the coadministration of AML 10-mg and atorvastatin 80-mg tablets (47).

Table 5. Mean (n = 63) Pharmacokinetic parameters obtained after oral administration of AML/Atorvastatine Calcium (AML/AC) combination tablets (5/10 mg or 10/80 mg) versus coadministration of individual AML (5 or 10 mg) and AC (10 or 80 mg) tablets (47).

	AML					AC			
	$AUC_{0\text{-inf}}$ (ng.h/mL)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)		$AUC_{0\text{-inf}}$ (ng.h/mL)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)
5/10 mg combination tablet	151a	3.04a	7.80	44.9		16.2a	2.40a	0.79	7.30
AML 5 mg + AC 10 mg	147a	2.94a	7.67	45.1		15.6a	2.43a	0.81	7.60
Ratio of geometric mean, %	102.7	103.4	-	-		103.8	98.8	-	-
90% confidence interval	98.9-105.4	99.6-107.7	-	-		96.4-111.8	88.3-110.6	-	-
10/80 mg combination tablet	336a	6.63	7.61	45.8		163a	25.5a	0.89	9.10
AML 10 mg + AC 80 mg	336a	6.58a	8.07	46.9		156a	27.1a	1.54	9.34
Ratio of geometric mean, %	100.0	100.8	-	-		104.5	94.1	-	-
90% confidence interval	97.2-102.9	97.6-103.9	-	-		98.8-110.8	84.6-104.4	-	-

[a] Geometric mean

of grapefruit juice on the pharmacokinetics of AML was investigated by Vincent et al. Water or grapefruit juice (240 mL) was administered to each subject prior to AML infusion. Amlodipine Maleate (10 mg) was administered via intravenous route and samples were collected for 216 hours from 20 male subjects. The calculated Vd_{ss} values of water (control) and grapefruit juice (test) were 21.0 (±3.8) and 22.7 (±5.1) L/kg, respectively, with no significant difference between the groups (85) .

Metabolism and Elimination

AML is extensively metabolized in the liver and its elimination from the plasma is biphasic with a terminal half-life of 30 to 50 h (1, 4, 6, 8-10, 83, 84). The rate of oxidative metabolism is relatively slow, therefore, AML does not exhibit extensive first-pass or presystemic metabolism after oral administration (83). AML is extensively (about 90%) converted to inactive metabolites via hepatic metabolism (Cytochrome P450 3A4 isozyme) (1) .

Following oral and IV doses of ^{14}C-AML to rat and dog, urinary and faecal excretion in rat was essentially complete within 48 h but was prolonged upto 168 h in dog. The majority (about 95%) of the urinary metabolites were identified for both species; unchanged drug accounted for 10% and 20% of the urinary radioactivity in rat and dog respectively. In rat, the principal route of metabolism involved cleavage of the S-methoxycarbonyl group of both the parent dihydropyridine and its pyridine analogue. In contrast, metabolism in dog involved oxidative deamination of the 2-aminoethoxy-methyl side chain. Secondary metabolism in both rat and dog was similar to that of other calcium channel blockers of the dihydropyridine class, with oxidation to the pyridine form being followed by aliphatic hydroxylation in the 6-position or O-dealkylation in the 2-position and lactonization (34) .

The disposition of AML, has been studied by Beresford et al. (76) in two human volunteers using single oral and IV doses of ^{14}C-AML. It was found that renal elimination was the major route of excretion with about 60% of the dosed radioactivity recovered in urine. Mean total recovered radioactivity in urine and faeces amounted to 84% for both the oral and intravenous routes. Apart from a small amount of unchanged AML (10% of urine ^{14}C**),** only pyridine metabolites of AML were excreted in urine. Nine different metabolites of AML were identified (Figure 4). The major metabolite was 2- ([4- (2-chloropheny1) -3-ethoxycarbonyl-S-methoxycarbonyl-6-methyl-2-pyridyl] methoxy) acetic acid (Figure 4, Met VII) and this represented 33% of urinary radioactivity. The majority (>95%) of these metabolites were excreted in the 0-72 h post-dose period. The data indicate that oxidation of AML to its pyridine analogue is the principal route of metabolism with subsequent metabolism by oxidative deamination, de-esterification and aliphatic hydroxylation. For the two volunteers, AML concentrations in plasma declined with a mean half-life of 33 h, while slower elimination of total drug-related material from plasma was observed, consistent with prolonged excretion (up to 12 days) of metabolites in urine and faeces. Only AML and pyridine metabolites were found in the circulation. As these pyridine derivatives have minimal calcium antagonist activity, the efficacy of AML in man can be attributed to the parent drug (76) .

Food Effect

Absorption of AML is not affected by food (1, 15). Josefsson et al. investigated the effect of grapefruit juice on the pharmacokinetics of AML (5 mg single oral dose) in twelve healthy male volunteers. A single oral dose of AML (5 mg) was administered with a glass of grapefruit juice (250 mL) or water. When AML was coadministered with grapefruit juice, C_{max} (115%) and AUC (0–72 h; 116%) values were comparable with water, and no significant difference between t_{max} values (86). Similar observations were made by Vincent et al. (82). Single dose of oral and intravenous AML (10 mg) was administered to 20 healthy male volunteers. For 9 days beginning with the day of administration of AML, grapefruit juice (or water control) was given once daily, and blood samples, blood pressure and heart rate measures were obtained. Results of the study showed that oral AML has high systemic availability (grapefruit juice: 88%; water: 81%), and pharmacokinetic parameters (AUC, C_{max}, t_{max}, and k_{el}) were not markedly changed with grapefruit juice coadministration. Total plasma

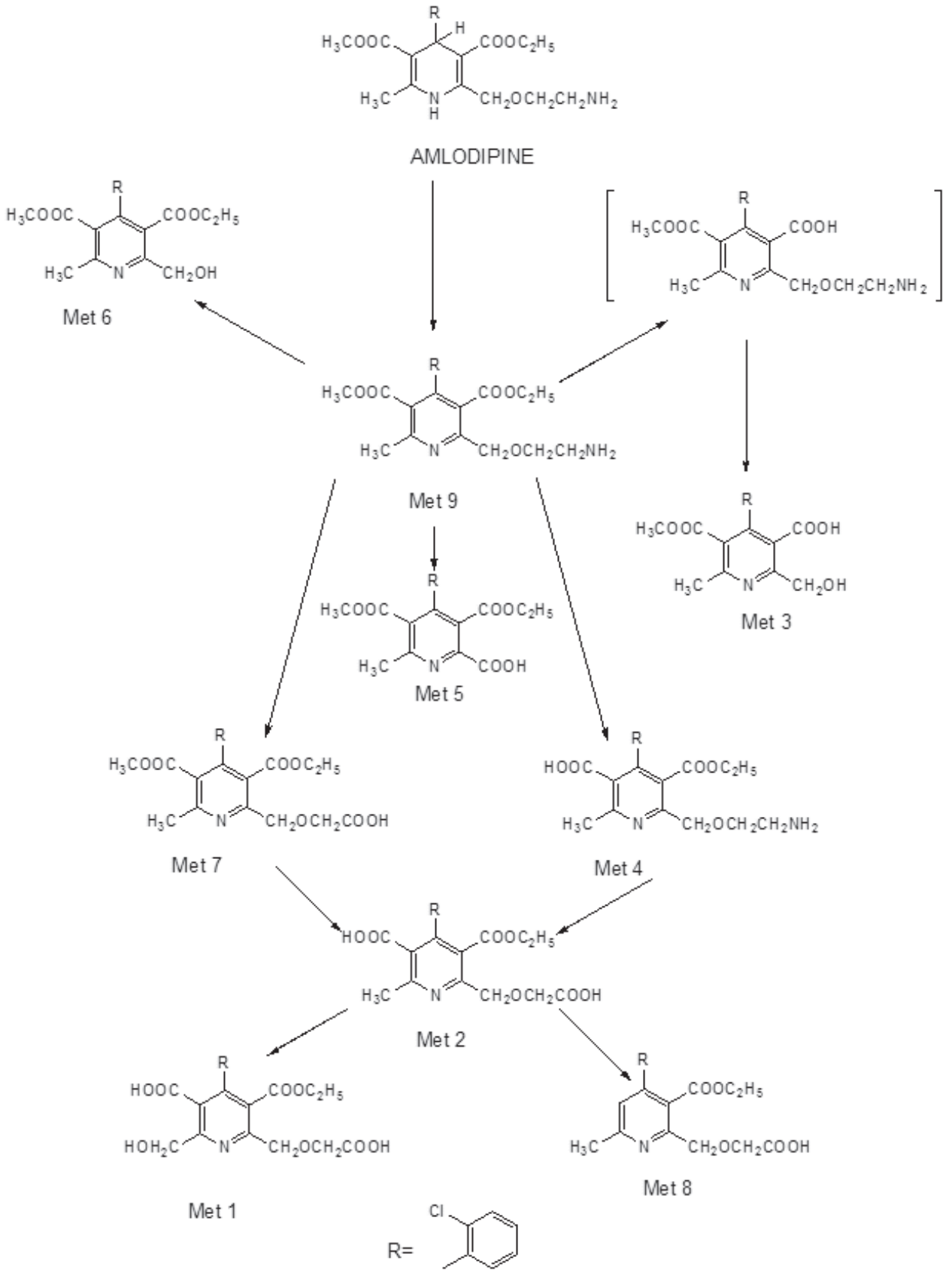

Figure 4. Urinary metabolites of AML in man (Met: Metabolite) (73) .

clearance and volume of distribution, calculated after intravenous AML, were essentially unchanged by grapefruit juice (CL 6.65 mL/ min per kg, juice *vs* 6.93 ml/min per kg, water; V_{ss} 22.7 L/ kg, juice *vs* 21.0 L/ kg, water). Also, grapefruit juice coadministration did not greatly alter the stereoselectivity in AML oral or intravenous kinetics. It was concluded that once daily grapefruit juice administration with usual oral doses of AML is unlikely to alter the profile of response in clinical practice (85) .

Renal impairment

The pharmacokinetics of AML is not significantly influenced by renal impairment and the dosage adjustment is not necessary (1, 87-89). When AML (as a single 5 mg capsule) was administered once daily for 14 days to 27 male subjects with renal functions ranging from normal to dialysis dependent (87), half-life and accumulation of AML were similar to previously reported values and did not vary with renal function. Similar observations were made when AML was administered (2.5-5.0 mg, once daily for 8 weeks) to 35 hypertensive patients with renal dysfunction (88) .

Hepatic impairment

Patients with hepatic insufficiency may require a lower initial dose of AML than healthy patients (1). When a single oral dose of AML (5 mg) was administered to 12 patients with hepatic impairment and 8 healthy convalescing subjects, some of the pharmacokinetic parameters were different in both groups. T_{max} was shorter and $t_{1/2}$ was longer in patients with hepatic insufficiency. Although AUCs were higher in hepatic patients, these differences were not significant. On the other hand, Cmax values were similar in both groups (90) .

Age

The pharmacokinetic parameters of AML in children are not significantly different than those in adults and are not influenced by frequency of dosing (1). On the other hand, elderly patients have decreased clearance and longer $t_{1/2}$ suggesting increased drug accumulation during chronic dosing (1, 91) indicating that those patients may require a lower initial dose of AML (1) .

BIOPHARMACEUTICS CLASSIFICATION SYSTEM EVALUATION

The Biopharmaceutics Classification System (BCS) is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. BCS categorizes drugs into four groups, Class 1 (high solubility and high permeability), Class 2 (low solubility and high permeability), Class 3 (high solubility and low permeability) and Class 4 (low solubility and low permeability). The BCS allows biowaiver for rapid dissolving immediate-release (IR) products of Class 1 drugs (92). On the other hand EMA extends biowaivers to drugs of Class 3 (high solubility and low permeability) (93). In regard to BCS classification of AML, there is conflicting information in "Proposal to Waive in Vivo Bioequivalence Requirements for the Who Model List of Essential Medicines Immediate Release, Solid Oral Dosage Forms". In Table A, AML is classified as BCS Class 1 compound. In Table C, its solubility is given as slightly soluble (5ml) and permeability as BAabs 60-65%, excretion of drug metabolites urine 90-95% (94). AML was classified as BCS Class 1 drug by Olusola et al and Shohin et al (95, 96) .

CONCLUSIONS

AML, which is a dihydropiridin derivative, has a slow absorption and prolonged effect. Following oral administration the extent of AML absorption is about 96%. AML is present in ionized form at physiologic pH because of its pKa value. Therefore, it possesses a strong affinity for cell membranes and has high permeability according to BCS guidance. These characteristics are believed to be a reason for AML's unique pharmacokinetics. AML has higher bioavailability, longer half-life ($t_{1/2}$), longer time to C_{max}, higher volume of distribution and slower gradual elimination than other calcium channel blockers. Because of its unique pharmacokinetics characteristics AML is the most frequently used antihypertensive drug among all dihydropiridines. AML pharmacokinetics is not significantly affected by co-administration with orange juice, age, renal impairment, hepatic impairment or age. According to some researchers and *WHO Model List of Essential Medicines* it was categorized as BCS Class 1 drug. Although it is used as a racemic mixture, only the S

(-)-enantiomer is pharmacologically active, whereas R (+)-enantiomer is 1000-fold less active. In some clinical studies, S-Amlodipine 2.5 mg is found to be equivalent in its efficacy and tolerability when compared to Amlodipine 5 mg in the treatment of mild to moderate hypertension.

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