CHAPTER II

REVIEW OF RELATED LITERATURE AND RESEARCH

Amlodipine is chemically described as 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester. Its empirical formula is $C_{20}H_{25}ClN_2O_5$ [3]. It has molecular weight of 408.9. It is sparingly soluble in water and has a pKa of 8.6 [4].

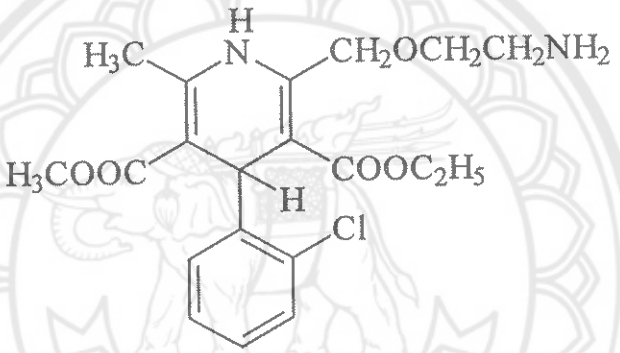


Figure 1 Structure of amlodipine

Mechanism of action:

Amlodipine is a dihydropyridine calcium channel blocker that inhibits the transmembrane influx of calcium ions through slow channel in vascular smooth muscle and cardiac muscle. Amlodipine binds at both dihydropyridine and nondihydropyridine binding sites. The contractile processes of cardiac and vascular muscle are dependent on the movement of extracellular calcium ions into these cells. Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells. Serum calcium concentration is not affected by amlodipine. Within the physiologic pH range, more than 90% of amlodipine is an ionized compound, and its kinetic interaction with the calcium channel receptor is characterized by a gradual rate of association and dissociation with the receptor binding site, resulting in a gradual onset of effect. As the main site of action of amlodipine is a

peripheral vasculature, its antihypertensive effect is related to peripheral vasodilation and a subsequent reduction in systemic vascular resistance [2, 4, 5].

Pharmacokinetics:

Absorption

After oral administration to human, amlodipine is absorbed slowly and almost completely absorbed from human gastrointestinal tract. In healthy volunteers, oral bioavailability of amlodipine was relatively high (60-65%) [2, 6]. In a trial in which healthy male volunteers received amlodipine 2.5, 5 and 10 mg amlodipine orally, the AUC and the C_{max} increased in the ratios of 1:2.3:5.1 and 1:2.2:4.6, respectively [2]. As such, its pharmacokinetic profile showed a linear relationship in studies range with positive relations between oral amlodipine dosage and C_{max} and AUC_{0-72}. C_{max} of amlodipine usually attained 6 to 9 hours after oral administration [2,6]. Faulkner and associates (1989) investigated the influence of food on systemic amlodipine availability in 12 healthy volunteers and the result showed no significant difference in the extent of drug absorption [7].

Distribution

Amlodipine has a large volume of distribution (Vd) of 21 L/kg after intravenous administration and is highly bound to plasma proteins (98%). At physiological pH, over 90% of amlodipine are ionized. Thus, high Vd may associate with the strong electrostatic interaction between the positive charge of basic side chain on the dihydropyridine ring of the drug and the phosphate head of phospholipid molecule which is a component of biological membrane [2,6].

Metabolism

Amlodipine is metabolized slowly and extensively by hepatic, though the first-pass metabolism of amlodipine is minimal. Initial biotransformation of amlodipine in human is oxidation of the dihydropyridine moiety to the pyridine analogue then followed by side chain oxidation and hydrolysis. There are no reports that metabolites of amlodipine show any significant activity [2, 4, 6].

Excretion

The recovery of radioactive amlodipine from urine and faeces of volunteers receiving [^{14}C] amlodipine were similar after oral and intravenous administration, with 60% of the dose recovered in urine and 20-25% in the faeces. The excretion of radioactive drug in faeces after intravenous administration indicated that amlodipine and metabolites are excreted across the gut wall and/or in the bile [2, 4, 6].

Plasma concentrations and half-life

After the oral administration of a single dose of amlodipine 10 mg, C_{max} of 5.9 $\pm$ 1.2 ng/ml was obtained at 6-9 hours. A prolonged terminal elimination half-life ($t_{1/2}$) of 36 $\pm$ 6 hours and total body clearance (CL) of 0.42 $\pm$ 0.078 L/h/kg were studied in healthy volunteers [2, 6].

Indications

Amlodipine is indicated for the treatment of hypertension, chronic stable angina and vasospastic angina (prinzmetal's or variant angina) [5].

Dosage and administration

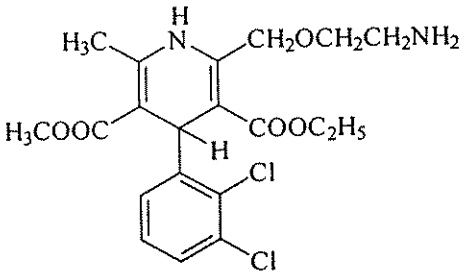
The oral dose of amlodipine is 5-10 mg once daily. Young, elderly and hepatic insufficiency patients may be started on 2.5 mg once daily [5].

Adverse reactions

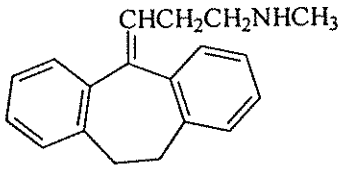
Most adverse reactions of amlodipine are at mild to moderate severity. The discontinuation of amlodipine due to adverse reactions was reported to be about 1.5% of patients and was not significantly different from placebo (1%). The most common side effects were headache and edema. Other side effects were dizziness, flushing, palpitation. However, these effects were disappeared after treatment is withdrawn [5, 6].

Methods for amlodipine analysis

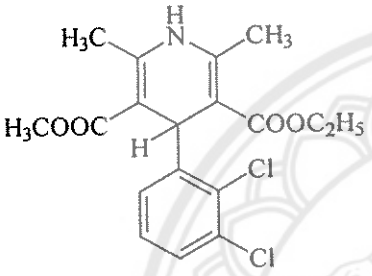
There were several reports about the analysis of amlodipine. Various chromatographic methods such as high-performance liquid chromatography (HPLC), gas chromatography (GC), liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) or high-performance thin-layer chromatography (HPTLC) were studies. All of these analytical methods were comprised of a chromatographic technique for separating amlodipine from the other substances and a detection technique for amlodipine. The reported analytical methods are summarized in Table 1. The most popular technique for measurement of amlodipine concentration in biological samples was HPLC coupling with various detectors such as MS/MS, UV, amperometric and fluorescence detectors. The analytical columns used were the column packed with reversed phase material (C8, C18) and the chiral column for separating the enantiomer of amlodipine. The sensitivity of HPLC analysis depends on the detector types. MS/MS detector gave the highest sensitivity with the limit of quantitation (LOQ) of 0.09 ng/ml. Amperometric and fluorescence detectors provided the sensitivity of about 0.2 ng/ml. UV detector gave low sensitivity with LOQ in nanogram per milliliter. The other reported technique was GC. The sample was separated in the gaseous condition and detected with the electron-capture (ECD) detector. Sensitivity of GC-ECD is similar to the detection with amperometric and fluorescence detector which LOQ was about 0.2 ng/ml. HPTLC gave the lowest sensitivity with 6 mcg/ml of LOQ. In order to measure amlodipine in human plasma, a very selective and sensitive method with a limit of quantitation in a range of nanogram per milliliter or lower is required. Several substances were used as an internal standard i.e. chloroamlodipine or UK 52.829 (2', 3'-dichlorophenyl analogue of amlodipine), felodipine, nitrendipine, nortriptyline, desipramine, propanolol and 4'-hydroxy-propafenone. UK 52.829 was the most popular internal standard used in the analysis because its structure and chemical properties were close to those of amlodipine. Felodipine and nitrendipine were used as they had dihydropyridine skeleton like amlodipine. Nortriptyline was used as an internal standard in the analysis using HPLC-fluorescence because it had the secondary amino on the side chain which was needed for reacting with 4-chloro-7-nitrobenzofurazan (NBD-Cl) (Figure 2).



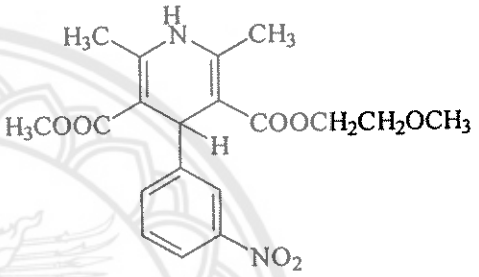
UK 52.829



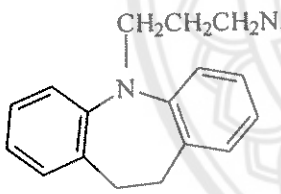
nortriptyline



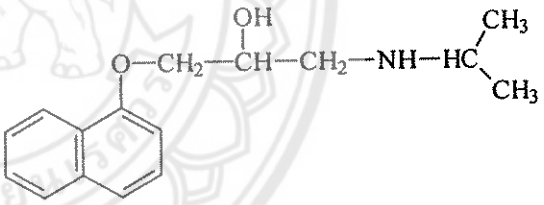
felodipine



nitrendipine



desipramine



propanolol

Figure 2 Structures of internal standards used in amlodipine analysis

Not only the analytical method, but also the extraction method for sample preparation had to be optimized. The plasma samples could not directly be analyzed because they contained many substances such as plasma proteins. Such substances could interfere the analyses. The extraction methods reported can be classified to two main methods i.e. solid phase extraction (SPE) and liquid-liquid partition. The extraction methods are summarized in Table 2. After extraction, the samples were analyzed by using several analytical methods. For GC and HPLC with fluorescence detector methods,

the sample has to be derivatized before analysis. For the GC analysis, the sample has to be derivatized with trimethylacetyl chloride or trifluoroacetic anhydride to make amlodipine evaporated [8, 9]. Using HPLC with fluorescence detector method, the substance should have fluorescence property. Amlodipine does not have this property, therefore, the derivatization with fluorescing agent such as NBD-Cl is necessary [10].

Table 1 The list of analytical methods for amlodipine determination.

No.	method	column	mobile phase	flow rate (ml/min)	detector	type of sample	internal standard	LOQ (ng/ml)	researcher
1	HPTLC	HPTLC plate (Merck)	methylene chloride:methanol:25%ammonia 8.8:1.3:0.1		UV 230 nm	solution		6,000	Argekar, Powar [11]
2	HPLC	Chiral AGP 150X4 mm (ChromTech)	1-propanol:10mM pH 4.5 acetate buffer 1:99	0.9	UV 240 nm	plasma	chloramlodipine		Luska et al. [12]
3	HPLC	Chiral AGP 150X10 mm (ChromTech)	n-propanol:10mM pH 4.5 acetate buffer 1:99	4	UV 240 nm	solution			Luska et al. [12]
4	HPLC	Chiral AGP (ChromTech),Supelcosil LC-8 (phenomenex), Symmetry C8 (water)*	(A) n-propanol:10mM pH 4.5 acetate buffer 1:99 for sample clean up, (B) MeCN:10mM pH 4.5 acetate buffer 45:55 for separate S-,R- amlodipine	0.9	UV 240 nm	plasma	chloramlodipine	<5	Luska et al. [12]

Table 1 (CONT.)

No.	method	column	mobile phase	flow rate (ml/min)	detector	type of sample	internal standard	LOQ	researcher
5	HPLC	Symmetry C8 (Waters)	(A)50mM pH 3.8 sodium phosphate buffer, (B) MeCN A:B 85:15 6.5 min A:B 65:35 18.5 min A:B 20:80 3 min	1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min	UV 200.5 nm	blood, urine			Gaillard et al. [12]
6	HPLC	Metaphase C18 (Jasco)	0.01M phosphate buffer:MeCN 1:1 pH 4.5	1.5	UV 250 nm	solution	felodipine	25	Patel et al. [13]
7	HPLC	Zorbax SB-Phenyl (Rockland Technology)	Methanol:0.1M pH 4.0 acetate buffer 65:35 + 2mM sodium dodecyl sulfate + 1mg EDTA / L	0.3	amperometric	plasma	UK 52.829	0.2	Josefsson et al. [14]
8	HPLC	Cosmosil 5 C18 (Nakarai Chemicals)	0.05M pH 3.1 phosphate buffer:MeCN 65:35 v/v + 0.005M sodium octane sulfonate + 5 mg EDTA / L	1	amperometric	serum	UK 52.829	0.2	Shimooka et al. [15]

Table 1 (CONT.)

No.	method	column	mobile phase	flow rate (ml/min)	detector	type of sample	internal standard	LOQ	researcher
9	HPLC	Bondapak C18 (Water)	methanol:water 80:20	0.8	fluorescence Ex 459 nm, Em 528 nm	plasma	nortriptyline	0.25	Tatar, Atmaca [10]
10	GC	DB1 (JW Scientific)	Carrier and make up gas: hydrogen gas with nitrogen column temperature: 250°C		electron-capture	plasma, gingival crevicular fluid	nitrendipine	500	Monkman et al. [8]
11	GC	Fused silica coated with crosslinked 5% phenylmethyl silicone gum (Hewlett-Packard)	carrier and make up gas; nitrogen column temperature: 280-320°C with the rate of 20°C/min 320°C for 9 min		electron-capture	plasma	UK 52.829	0.2	Faulkner et al. [9]

Table 1 (CONT.)

No.	method	column	mobile phase	flow rate (ml/min)	detector	type of sample	internal standard	LOQ	researcher
12	HPLC	Genesis C18 (Jones Chromatography)	60%MeCN + 10mM formic acid	0.4	MS/MS	plasma	desipramine	0.1	Carvalho et al. [16]
13	HPLC	Zorbax C8 (Phenomenex)	MeCN:water:formic acid 75:35:1	0.4	MS/MS	plasma	4'-hydroxy-propafenone	0.4	Zhong et al. [17]
14	HPLC	Chiral AGP (ChromTech)	10mM ammonium acetate buffer pH 4.5 : 1-propanol 99:1	0.9	MS/MS	plasma	propanolol	0.09	Streel et al. [18]

* Three columns were used for three steps of separation. Chiral AGP was used for sample clean up. Supelcosil LC-8 and Symmetry C8 were used to separate S- and R- amlodine respectively.

Table 2 The list of extraction methods of amlodipine in biological fluid.

No.	method	type of sample	internal standard	Sample volume (ml)	Recovery (%)		researcher
					amlodipine	Internal standard	
1	liq-liq	serum	UK 52.829	1	50.2 ± 3.4	58.3 ± 6.3	Shimooka et al. [15]
2	liq-liq	plasma, gingival crevicular fluid	nitrendipine	1			Monkman et al. [8]
3	liq-liq	plasma	UK 52.829	1			Faulkner et al. [9]
4	liq-liq	plasma	nortriptyline	0.5	72.00- 93.56	92.52	Tatar, Atmaca [10]
5	liq-liq	plasma	chloamlodipine	5			Luska et al. [12]
6	liq-liq	blood, urine		1			Gaillard et al. [12]
7	C2 SPE	plasma	UK 52.829	1	98	95	Josefsson et al. [14]
8	liq-liq	plasma	desipramine	0.2	63.7 -77.9	52.7	Carvalho et al. [16]
9	C2 SPE	plasma	propanolol	1.8	86-88		Streel et al. [18]