

Short Communication

Amlodipine Metabolism in Human Liver Microsomes and Roles of CYP3A4/5 in the Dihydropyridine Dehydrogenation[§]

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ABSTRACT

Amlodipine is a commonly prescribed calcium channel blocker for the treatment of hypertension and ischemic heart disease. The drug is slowly cleared in humans primarily via dehydrogenation of its dihydropyridine moiety to a pyridine derivative (M9). Results from clinical drug-drug interaction studies suggest that CYP3A4/5 mediate metabolism of amlodipine. However, attempts to identify a role of CYP3A5 in amlodipine metabolism in humans based on its pharmacokinetic differences between CYP3A5 expressers and nonexpressers failed. Objectives of this study were to determine the metabolite profile of amlodipine (a racemic mixture and S-isomer) in human liver microsomes (HLM), and to identify the cytochrome P450 (P450) enzyme(s) involved in the M9 formation. Liquid chromatography/mass spectrometry analysis showed that amlodipine was mainly converted to M9 in HLM

incubation. M9 underwent further O-demethylation, O-dealkylation, and oxidative deamination to various pyridine derivatives. This observation is consistent with amlodipine metabolism in humans. Incubations of amlodipine with HLM in the presence of selective P450 inhibitors showed that both ketoconazole (an inhibitor of CYP3A4/5) and CYP3cde (an inhibitor of CYP3A4) completely blocked the M9 formation, whereas chemical inhibitors of other P450 enzymes had little effect. Furthermore, metabolism of amlodipine in expressed human P450 enzymes showed that only CYP3A4 had significant activity in amlodipine dehydrogenation. Metabolite profiles and P450 reaction phenotyping data of a racemic mixture and S-isomer of amlodipine were very similar. The results from this study suggest that CYP3A4, rather than CYP3A5, plays a key role in metabolic clearance of amlodipine in humans.

Introduction

Amlodipine, a dihydropyridine calcium channel blocker, is one of the most commonly prescribed drugs for the treatment of hypertension and ischemic heart disease. In a clinical study, amlodipine shows a long elimination of half-life (35 hours) after a single 10-mg intravenous dose (Abernethy, 1991), likely due to its high volume of distribution and a low rate of plasma clearance. The drug also exhibits a good oral bioavailability in the range of 52%–88% following a single 10-mg oral dose to human subjects (Faulkner et al., 1986). After an oral dose of radiolabeled amlodipine to humans, the total radioactivity recovery is 59.3% in urine and 23.4% in feces (Beresford et al., 1988a; Stopher et al., 1988). Similarly, 62% and 22.7% of a radiolabeled intravenous dose is recovered in human urine and feces, respectively. S-amlodipine is the active component of racemic amlodipine. There are no significant differences in pharmacodynamic and pharmacokinetic parameters after a single dose of 5 mg of S-amlodipine and 10 mg of amlodipine racemate (Kim et al., 2010). The amlodipine pyridine metabolite (Fig. 1C, M9), raised from dehydrogenation of the dihydropyridine moiety, and its derivatives are major drug-related components in human urine. The mass balance and metabolite profiling data suggest that amlodipine dehydrogenation to M9 followed by multiple oxidative reactions of M9 is the major clearance pathway of amlodipine in humans. Similar metabolic pathways have been observed

in rats and dogs (Beresford et al., 1988b). It has been reported that many dihydropyridine analogs undergo CYP3A4-mediated dehydrogenation to form the corresponding pyridine metabolites in vitro (Guengerich et al., 1991). However, information on in vitro metabolism of amlodipine in humans, including P450 reaction phenotyping data, is not available in the literature.

Telaprevir, a potent inhibitor of both CYP3A4 and CYP3A5, increases the amlodipine mean area under the curve (AUC) by 2.79-fold and the mean half-life from 41.3 to 95.1 hours when the two drugs are coadministered (Lee et al., 2011). Similarly, combined dosing of indinavir and ritonavir, both of which are CYP3A inhibitors, increases the median amlodipine AUC₀₋₂₄ by 90% (Glesby et al., 2005). These clinical drug-drug interaction observations suggest that CYP3A4/5 enzymes play significant roles in metabolic clearance of amlodipine in humans. CYP3A4 and CYP3A5 are two major enzymes of the CYP3A family and have similar catalytic specificities. However, unlike CYP3A4, CYP3A5 is a polymorphic enzyme. To determine if CYP3A5 contributes to the metabolism of amlodipine, the pharmacokinetics of amlodipine in CYP3A5 nonexpressers (CYP3A5*3/*3 carriers) and CYP3A5 expressers (CYP3A5*1/*1 and CYP3A5*1/*3 carriers) have been recently determined and compared with each other. After a single dose of amlodipine, its exposure in CYP3A5 expressers is slightly higher than that in CYP3A5 nonexpressers (Kim et al., 2006). However, the AUC and the maximal concentrations of amlodipine in CYP3A5 nonexpressers are about 2-fold higher than those in CYP3A5 expressers after 5-mg once-daily oral doses for 7 days (Zuo et al., 2013). Although roles of CYP3A5 in the disposition and elimination of

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ABBREVIATIONS: AUC, area under the curve; AUC₀₋₂₄, area under the curve during 24 hours; HLM, human liver microsomes; LC, liquid chromatography; MH⁺, protonated molecular ions; MS, mass spectrometry; MSⁿ, multiple stage MS/MS fragmentation; 1'-OH-midazolam, 1'-hydroxy-midazolam; Thio-TEPA, N,N',N'-triethylenethiophosphoramidate.

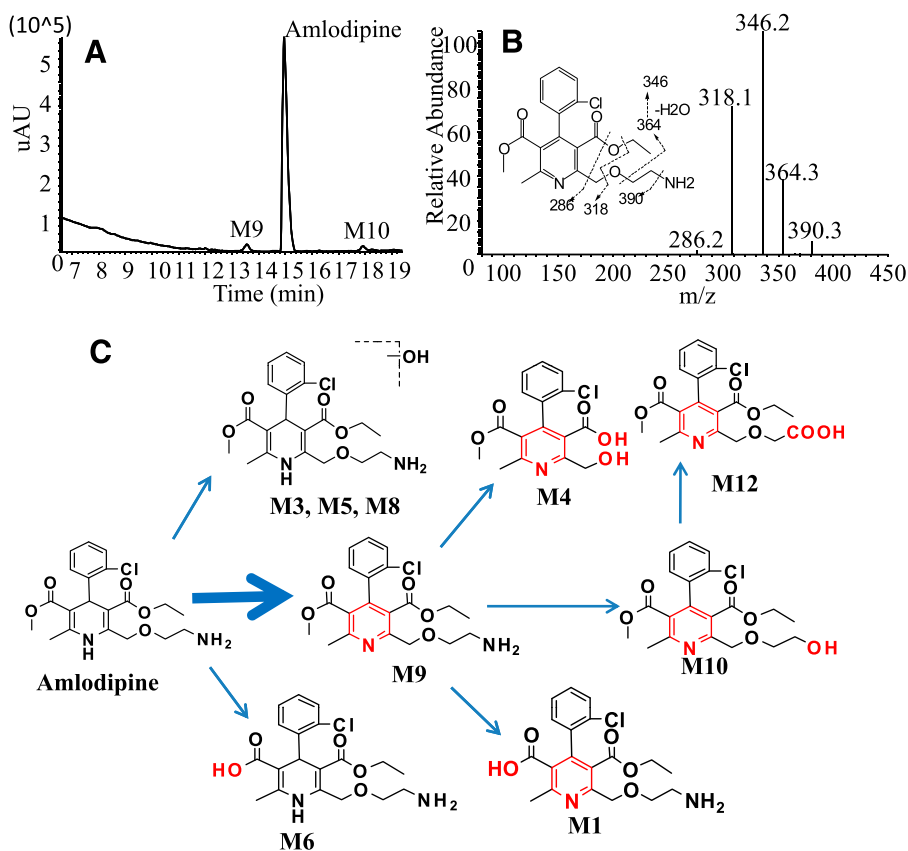


Fig. 1. Amlodipine metabolite profile in HLM. (A) UV profile of metabolites of racemic amlodipine in HLM (30 μ M, 60 minutes, 2 mg protein/ml). (B) MS² spectrum and the structures of the pyridine metabolite (M9). (C) Proposed structures and formation pathways of amlodipine metabolites in HLM.

amlodipine in humans have been suggested by investigators of these clinical studies, it is very challenging to draw meaningful conclusions without *in vitro* P450 phenotyping data of amlodipine. Furthermore, several studies have investigated the role of CYP3A5 polymorphism in blood pressure response to amlodipine, but results are not consistent (Zhang et al., 2013). The major objectives of this study were to determine the amlodipine metabolite profile in human liver microsomes (HLM) and P450 enzyme(s) responsible for amlodipine dehydrogenation to M9. In particular, the roles of CYP3A4 and CYP3A5 in amlodipine metabolism were investigated. In addition, the *in vitro* metabolism of racemic amlodipine was compared with that of *S*-amlodipine. Results from this study provide a biochemical basis for better understanding of the clinically observed amlodipine drug-drug interactions with CYP3A inhibitors and pharmacokinetics of amlodipine in both CYP3A5 non-expressers and expressers.

Materials and Methods

Chemicals and Reagents. A racemic amlodipine (2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester benzene sulfonate norvasc), quinidine, ketoconazole, N, N'-triethylenethiophosphoramidate (Thio-TEPA), montelukast, furafylline, sulfaphenazole, CYP3cide, and benzylnirvanol were purchased from Sigma (St. Louis, MO). *S*-amlodipine was purchased from Energy Chemical (Shanghai, China). All solvents (acetonitrile, methanol, and water) were of high-performance liquid chromatography (LC) grade. Pooled HLM was obtained from BD Biosciences (Woburn, MA). Expressed P450 enzymes, CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, and 3A5 were purchased from BD Biosciences (San Jose, CA).

HLM Incubation for Metabolite Profiling. Racemic amlodipine and *S*-amlodipine (30 μ M) were separately incubated with HLM (2 mg protein/ml)

for 60 minutes. The reaction mixtures (1 ml) also contained potassium phosphate buffer (69 mM, pH 7.4) and NADPH (4 mM). Metabolic reactions were initiated by addition of NADPH after a 3-minute preincubation and stopped by the addition of 2 ml of acetonitrile. Precipitate was removed by centrifugation. Supernatant was dried under nitrogen and then reconstituted in 200 μ l of 5% acetonitrile/water (v/v) for LC/mass spectrometry (MS) analysis.

Time Course of Amlodipine Metabolism in HLM. Racemic amlodipine and *S*-amlodipine (1 μ M) were separately incubated in duplicates in HLM (1 mg protein/ml) for 0, 5, 10, 15, 30, and 60 minutes. The reaction mixtures (0.25 ml) also contained potassium phosphate buffer (69 mM, pH 7.4) and NADPH (2 mM). Metabolic reactions were initiated by the addition of NADPH after a 3-minute preincubation and stopped at designated time points by the addition of 0.5 ml of acetonitrile. Precipitate was removed by centrifugation. Supernatant was dried under nitrogen and then reconstituted in 50 μ l of 5% acetonitrile/water (v/v) for determination of disappearance of the parent drug and the formation of the pyridine metabolite (M9).

Inhibition of Amlodipine Dehydrogenation HLM by P450 Inhibitors.

Racemic amlodipine and *S*-amlodipine (1 μ M) were separately incubated in HLM (0.5 mg/ml) in triplicates for 13 minutes. Reaction mixtures (0.25 ml) also contained potassium phosphate buffer (69 mM, pH 7.4), a chemical inhibitor of P450 enzyme, and NADPH (2 mM). Final concentrations of the P450 inhibitors were 1 μ M for benzylnirvanol, quinidine, ketoconazole, montelukast, and CYP3cide; 10 μ M for furafylline and sulfaphenazole; and 50 μ M for Thio-TEPA. For the incubations with time-dependent inhibitors (Thio-TEPA, furafylline, and CYP3cide), the reaction was initiated by the addition of amlodipine or *S*-amlodipine after 10-minute preincubations of HLM and NADPH in the presence of the individual P450 inhibitors. All other reactions were initiated by the addition of NADPH after 3-minute preincubations of HLM with amlodipine or *S*-amlodipine in the presence of the individual P450 inhibitors. All of the reactions were stopped by the addition of 0.5 ml of acetonitrile, and the precipitate was removed by centrifugation. Supernatants were

dried under nitrogen and then reconstituted in 50 μ l of 5% acetonitrile/water (v/v) for LC/MS analysis.

Amlodipine Dehydrogenation by Expressed P450 Enzymes. Racemic amlodipine and *S*-amlodipine (1 μ M) were separately incubated with individual expressed human P450 enzymes (100 nM) (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5) in triplicates for 13 minutes. Reaction mixtures (0.25 ml) also contained potassium phosphate buffer (69 mM, pH 7.4) and NADPH (2 mM). After 3-minute preincubation, metabolic reactions were initiated by the addition of NADPH and stopped by the addition of 0.5 ml of acetonitrile. Precipitate was removed by centrifugation. The supernatant was dried under nitrogen and then reconstituted in 50 μ l of 5% acetonitrile/water (v/v) for LC/MS analysis. In addition, racemic amlodipine was incubated with expressed CYP3A4 and CYP3A5 with or without CYP3Cide under the conditions described earlier. In the same experiment, midazolam (10 μ M) was incubated with expressed CYP3A4 or CYP3A5 (100 nM) in triplicates for 5 minutes. Reaction mixtures (0.04 ml) also contained potassium phosphate buffer (69 mM, pH 7.4) and NADPH (2 mM). After 3-minute preincubation, the metabolic reaction converting midazolam to 1'-hydroxy-midazolam (1'-OH-midazolam) was started by the addition of NADPH and stopped by the addition of 0.08 ml of acetonitrile. Precipitate was removed by centrifugation. Concentrations of 1'-OH-midazolam in the supernatant were quantitatively determined using LC/MS and 1'-OH-midazolam standard.

Liquid Chromatography/Mass Spectrometry Analysis. An LC/MS system consisted of an LC instrument (Thermo Accela HPLC with a photodiode array detector; Thermo Fisher Scientific, Waltham, MA) equipped with an Xbridge C18 column (5 μ m, 2.1 \times 150 mm; Waters, Milford, MA) and an ion trap mass spectrometer (LTQ XL; Thermo Fisher Scientific) was used for profiling and identification of metabolites formed in HLM. LC separation was carried out using a mobile phase composed of 0.1% formic acid (v/v) in water (solvent A) and 0.1% formic acid in acetonitrile (v/v) (solvent B). A linear gradient of solvent A starting at 95% to 10% from 0 to 23 minutes was applied followed by remaining at 10% for 3 minutes before returning to 95%. The total run time was 30 minutes, and the flow rate was 0.3 ml/min. The mass spectrometer was operating in the positive electrospray ionization mode in the *m/z* range of 100–1000. Capillary temperature was at 275°C. Capillary voltage was at 46 V. The UV profile of racemic amlodipine and its metabolites in HLM incubations was acquired at 245 nm for semiquantitative estimation of the relative abundance of metabolites to the parent drug. Their full-scan MS and MSⁿ data were acquired using a data-dependent method for metabolite structure characterization. The same LC/MS instrument and method, except for a short LC run time (13 minutes), were used for the determination of relative amounts of

the dehydrogenation metabolite (M9) in the time course, HLM inhibition, and metabolism in P450 experiments. In the analysis, full-scan MS data were acquired, and then extracted ion chromatogram at *m/z* 409 was generated. The peak area corresponding to M9 was calculated as the measurement of the relative abundance of M9.

Results and Discussions

Identification of Amlodipine Metabolites Formed in HLM. LC/MS analysis showed that a total of nine metabolites were formed in HLM incubations with 30 μ M racemic amlodipine or *S*-amlodipine (Table 1). M9 and M10 exhibited relatively higher abundances and accounted for 5.9% and 3.2% of racemic amlodipine, respectively, based on a UV profile (Fig. 1A). Other metabolites were detectable only by LC/MS (Supplemental Fig. 1). Protonated molecular ions and multiple stage MS/MS fragmentation (MSⁿ) spectra of the parent drug and its metabolites are summarized in Table 1. Based on mass spectral interpretation and comparisons with those reported in the literature (Suchanova et al., 2006, 2008), metabolite structures and their formation pathways are proposed in Fig. 1C. For example, the structure of M9 was ascertained based on the comparison of its MS/MS spectrum (Fig. 1B) to that of the parent drug (Supplemental Fig. 1) as well as that of the amlodipine pyridine metabolite reported previously. Amlodipine dehydrogenation to M9 was the major metabolic pathway in HLM. M9 underwent further metabolism including oxidative deamination to M10, *O*-demethylation to M1, and *O*-dealkylation to M4. In addition, amlodipine underwent *O*-demethylation to M6 and monohydroxylation to M3, M5, and M8 (Fig. 1C; Table 1), which constituted minor pathways in HLM since the metabolites were not displayed in the UV profile (Fig. 1A). Metabolic profiles of racemic amlodipine and *S*-amlodipine in HLM were similar except that *O*-demethylation leading to M1 and M6 was not observed for *S*-amlodipine (Table 1). The major metabolic pathway observed in HLM is consistent with the major clearance pathway of amlodipine in human subjects: amlodipine dehydrogenation followed by oxidations to multiple metabolites (Beresford et al., 1988a; Stopher et al., 1988).

Time Course of Amlodipine Metabolism in HLM. To optimize incubation conditions for assessing the contribution of P450 enzymes

TABLE 1
Summary of amlodipine metabolites detected in HLM by LC/MS

Metabolite	RT	Identity	MH ⁺	MS ⁿ Spectral Data	Racemic Amlodipine	<i>S</i> -amlodipine
	<i>min</i>					
M1	10.06	P-2H-CH ₂	393	MS ² : 376, 350, 332, 314, 304; MS ³ (332): 304, 286	✓	—
M3	11.79	P + O	425	MS ² : 379, 336, 294, 364; MS ³ (336): 318, 304, 180	✓	✓
M4	12.02	P-2H-C ₂ H ₄ NH-C ₂ H ₄	336	MS ² : 318, 286; MS ³ (318): 286	✓	✓
M5	12.06	P + O	425	MS ² : 417, 390, 379, 336, 294; MS ³ (336): 318, 304, 286, 181	✓	✓
M6	12.06	P-CH ₂	395	MS ² : 352, 334, 306, 180; MS ³ (180): 163, 144	✓	—
M8	12.71	P + O	425	MS ² : 390, 379, 336, 294, 364; MS ³ (336): 318, 304, 248, 181	✓	✓
M9	13.57	P-2H	407	MS ² : 390, 364, 346, 318, 286; MS ³ (346): 318, 286	✓	✓
M10	17.88	P-2H-NH+O	408	MS ² : 346, 318, 286, 390; MS ³ (346): 318, 286	✓	✓
M12	18.38	P-2H-NH+2O-2H	422	MS ² : 376, 346, 318, 286; MS ³ (346): 318, 286	✓	✓
P	15.02	Amlodipine	409	MS ² : 392, 346, 348, 320, 294, 238; MS ³ (392): 360, 346	✓	✓

MH⁺, protonated molecular ions; MSⁿ, multiple stage MS/MS fragmentation; RT, retention time.

to amlodipine dehydrogenation, racemic amlodipine and *S*-amlodipine were incubated at a lower concentration (1 μ M) with HLM, respectively. Metabolite profiles were determined and time courses of the M9 formation were semiquantified by LC/MS. Under the condition investigated, the formation of M9 in HLM increased linearly with increasing incubation time within 15 minutes. No sequential metabolites of M9 were detected, reinforcing the idea that M9 is the intermediate to other pyridine derivatives during amlodipine metabolism. Disappearance of either racemic amlodipine or *S*-amlodipine was less than 76% of the initial concentrations following 15-minute incubations. Based on these data, 1 μ M substrate concentration and 13-minute incubation time were chosen for the subsequent P450 reaction phenotyping experiments. It was expected that the formation of M9 under this condition would be in a linear range.

Identification of P450 Enzyme Responsible for Amlodipine Dehydrogenation. As shown in Fig. 2, the formation of M9 from amlodipine or *S*-amlodipine was NADPH-dependent and nearly completely inhibited by ketoconazole, a potent inhibitor of both CYP3A4 and CYP3A5, and CYP3Cide, a potent and selective inhibitor of CYP3A4 (Walsky et al., 2012). Chemical inhibitors of other major P450 enzymes, including sulfaphenazole (CYP2C9), benzylnirvanol (CYP2C19), quinidine (CYP2D6), montelukast (CYP2C8), furafylline (CYP1A2), and Thio-TEPA (CYP2B6), had no or minimal inhibitory effect on amlodipine dehydrogenation. Similarly, results from metabolism of racemic amlodipine and *S*-amlodipine by a panel of expressed P450 enzymes showed that CYP3A4 was the single P450 enzyme catalyzing the formation of M9 (Fig. 3).

To further confirm that CYP3A5 has no or minimal catalyzing activity toward amlodipine dehydrogenation, the relative formation rate of M9 from racemic amlodipine in CYP3A4 and CYP3A5 was compared with that of 1'-OH-midazolam from midazolam in CYP3A4 and CYP3A5. Midazolam is a substrate of both CYP3A4 and CYP3A5. Results from the current study showed that the formation rates of 1'-OH-midazolam in expressed CYP3A4 and

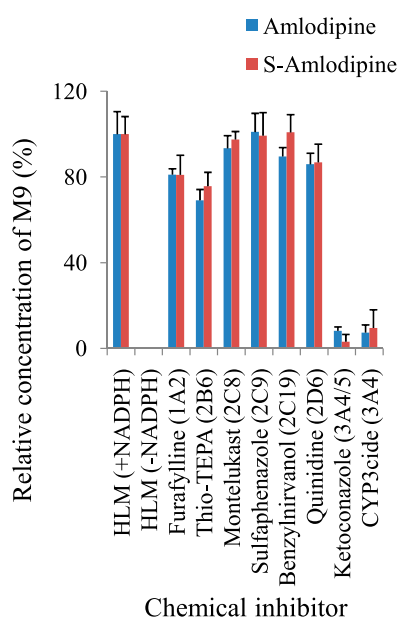


Fig. 2. Inhibition of the formation of M9 from racemic amlodipine and *S*-amlodipine in HLM by P450 inhibitors.

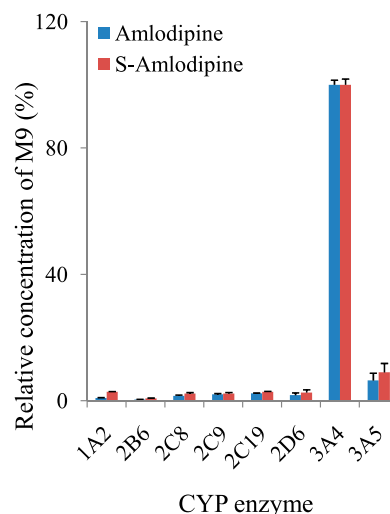


Fig. 3. Relative abundance of M9 formed in incubations of racemic amlodipine and *S*-amlodipine with recombinant human P450 enzymes.

CYP3A5 were 3.96 and 7.21 (nmol/min/nmol P450), respectively, and the relative formation of 1'-OH-midazolam in CYP3A4 and CYP3A5 was 100:182, which is in agreement with that reported in the literature (Christensen et al., 2011; Li et al., 2012). In contrast, the relative formation of M9 in CYP3A4 and CYP3A5 was 100:11. When CYP3Cide, a CYP3A4-specific inhibitor, was coincubated with CYP3A4 and CYP3A5, the M9 formation in CYP3A4 was greatly reduced to the levels in CYP3A5, whereas CYP3Cide had no effect on the M9 formation in CYP3A5. These results indicate that CYP3A4 is responsible primarily for the formation of M9 via amlodipine dehydrogenation. CYP3A5 and other P450 enzymes have no or little role in catalyzing amlodipine dehydrogenation. The differences in amlodipine exposures observed between CYP3A5 nonexpressers and expressers (Kim et al., 2006; Zuo et al., 2013) could be due to differences in CYP3A4 expressions between the two groups since CYP3A5 has minimal, if any, effect on the in vitro metabolism of amlodipine (Figs. 2 and 3). The in vitro data also support an early clinical observation that blood pressure response to amlodipine among high-risk African Americans appears to be determined by CYP3A4, but not CYP3A5, genotypes (Bhatnagar et al., 2010).

In summary, racemic amlodipine and *S*-amlodipine were slowly metabolized in HLM. Amlodipine dehydrogenation to the pyridine metabolite (M9) was the single most important metabolic pathway in HLM; M9 further underwent oxidative deamination, *O*-demethylation, and *O*-dealkylation. These in vitro metabolism data are consistent with amlodipine metabolism and disposition observed in vivo in humans. The data derived from amlodipine metabolism in expressed P450 and HLM with P450 inhibitors indicate that CYP3A4, not CYP3A5, is the primary contributor to amlodipine dehydrogenation. Metabolite profiles and P450 reaction phenotyping data of a racemic mixture and *S*-isomer of amlodipine were very similar, consistent with pharmacokinetics of racemic amlodipine and *S*-amlodipine in humans. These findings suggest that the observed clinical drug-drug interactions between amlodipine and CYP3A4/5 inhibitors are mediated by CYP3A4 rather than CYP3A5. In addition, polymorphic expressions of CYP3A5 would not affect the pharmacokinetic variability of amlodipine and the blood pressure response to amlodipine in humans.

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References

- Abernethy DR (1991) Amlodipine: pharmacokinetic profile of a low-clearance calcium antagonist. *J Cardiovasc Pharmacol* **17** (Suppl 1):S4–S7.
- Beresford AP, Macrae PV, and Stopher DA (1988b) Metabolism of amlodipine in the rat and the dog: a species difference. *Xenobiotica* **18**:169–182.
- Beresford AP, McGibney D, Humphrey MJ, Macrae PV, and Stopher DA (1988a) Metabolism and kinetics of amlodipine in man. *Xenobiotica* **18**:245–254.
- Bhatnagar V, Garcia EP, O'Connor DT, Brophy VH, Alcaraz J, Richard E, Bakris GL, Middleton JP, Norris KC, and Wright J, et al.; AASK Study Investigators (2010) CYP3A4 and CYP3A5 polymorphisms and blood pressure response to amlodipine among African-American men and women with early hypertensive renal disease. *Am J Nephrol* **31**:95–103.
- Christensen H, Hestad AL, Molden E, and Mathiesen L (2011) CYP3A5-mediated metabolism of midazolam in recombinant systems is highly sensitive to NADPH-cytochrome P450 reductase activity. *Xenobiotica* **41**:1–5.
- Faulkner JK, McGibney D, Chasseaud LF, Perry JL, and Taylor IW (1986) The pharmacokinetics of amlodipine in healthy volunteers after single intravenous and oral doses and after 14 repeated oral doses given once daily. *Br J Clin Pharmacol* **22**:21–25.
- Glesby MJ, Aberg JA, Kendall MA, Fichtenbaum CJ, Hafner R, Hall S, Grosskopf N, Zolopa AR, and Gerber JG; Adult AIDS Clinical Trials Group A5159 Protocol Team (2005) Pharmacokinetic interactions between indinavir plus zidovudine and calcium channel blockers. *Clin Pharmacol Ther* **78**:143–153.
- Guengerich FP, Brian WR, Iwasaki M, Sari MA, Bäärnhielm C, and Berntsson P (1991) Oxidation of dihydropyridine calcium channel blockers and analogues by human liver cytochrome P-450 IIIA4. *J Med Chem* **34**:1838–1844.
- Kim BH, Kim JR, Kim MG, Kim KP, Lee BY, Jang JJ, Shin SG, and Yu KS (2010) Pharmacodynamic (hemodynamic) and pharmacokinetic comparisons of S-amlodipine gentisate and racemate amlodipine besylate in healthy Korean male volunteers: two double-blind, randomized, two-period, two-treatment, two-sequence, double-dummy, single-dose crossover studies. *Clin Ther* **32**:193–205.
- Kim KA, Park PW, Lee OJ, Choi SH, Min BH, Shin KH, Chun BG, Shin JG, and Park JY (2006) Effect of CYP3A5*3 genotype on the pharmacokinetics and pharmacodynamics of amlodipine in healthy Korean subjects. *Clin Pharmacol Ther* **80**:646–656.
- Lee JE, van Heeswijk R, Alves K, Smith F, and Garg V (2011) Effect of the hepatitis C virus protease inhibitor telaprevir on the pharmacokinetics of amlodipine and atorvastatin. *Antimicrob Agents Chemother* **55**:4569–4574.
- Li X, Song X, Kamenicka TM, and Cameron MD (2012) Discovery of a highly selective CYP3A4 inhibitor suitable for reaction phenotyping studies and differentiation of CYP3A4 and CYP3A5. *Drug Metab Dispos* **40**:1803–1809.
- Stopher DA, Beresford AP, Macrae PV, and Humphrey MJ (1988) The metabolism and pharmacokinetics of amlodipine in humans and animals. *J Cardiovasc Pharmacol* **12** (Suppl 7): S55–S59.
- Suchanova B, Kostianen R, and Ketola RA (2008) Characterization of the in vitro metabolic profile of amlodipine in rat using liquid chromatography-mass spectrometry. *Eur J Pharm Sci* **33**:91–99.
- Suchanova B, Sispara L, and Wsol V (2006) Liquid chromatography-tandem mass spectrometry in chiral study of amlodipine biotransformation in rat hepatocytes. *Anal Chim Acta* **573**:273–283.
- Walsky RL, Obach RS, Hyland R, Kang P, Zhou S, West M, Geoghegan KF, Helal CJ, Walker GS, and Goosen TC, et al. (2012) Selective mechanism-based inactivation of CYP3A4 by CYP3A4 inhibitor (PF-04981517) and its utility as an in vitro tool for delineating the relative roles of CYP3A4 versus CYP3A5 in the metabolism of drugs. *Drug Metab Dispos* **40**:1686–1697.
- Zhang YP, Zuo XC, Huang ZJ, Cai JJ, Wen J, Duan DD, and Yuan H (2013) CYP3A5 polymorphism, amlodipine and hypertension. *J Hum Hypertens* DOI: 10.1038/jhh.2013.67 [Epub ahead of print].
- Zuo XC, Zhou YN, Zhang BK, Yang GP, Cheng ZN, Yuan H, Ouyang DS, Liu SK, Barrett JS, and Li PJ, et al. (2013) Effect of CYP3A5*3 polymorphism on pharmacokinetic drug interaction between tacrolimus and amlodipine. *Drug Metab Pharmacokinet* **28**:398–405 [Epub ahead of print].

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