Determination of *S*- and *R*-Amlodipine in Rat Plasma using LC-MS/MS After Oral Administration of *S*-Amlodipine and Racemic Amlodipine

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Abstract: The pharmacokinetic properties of *S*-amlodipine were studied using racemic amlodipine and single *S*-enantiomer (SK310) administration to rats. Plasma levels of the drug were determined using chiral liquid chromatography coupled with tandem mass spectrometry following solid phase extraction. The stereospecific analysis of amlodipine was performed on an α-acid glycoprotein (AGP) column using a mobile phase comprising 10 mM ammonium acetate (pH 4.0) and propanol at a flow rate of 0.2 mL/min. This method was used to perform a comparative study of the pharmacokinetics of amlodipine and SK310. The results revealed that the pharmacokinetic profile of *S*-amlodipine after the administration of SK310 was comparable to that following the administration of the racemic mixture.

Key words: Amlodipine, LC-MS/MS, Pharmacokinetics, Rat plasma

Introduction

Amlodipine, chemically dihydropyridine (Fig. 1), is a potent calcium channel blocker, used in the treatment of hypertension and angina pectoris.[1-3] Similar to most other calcium blocking agents of the dihydropyridine class, a racemic mixture of amlodipine is used for therapeutical purposes. However, it has been reported that the vasodilatation effect of amlodipine can be ascribed to its *S*-enantiomer. As reported previously, the *S*-enantiomer is largely associated with amlodipine's antihypertensive effects, while the *R*-enantiomer is thought to be responsible for nitric oxide-mediated vasodilatation, which is associated with adverse effects, including peripheral edema and facial flushing.[4,5] Therefore, it would be worthwhile to develop a pharmaceutical formulation of amlodipine consisting entirely of the *S*-enantiomer. In this context, we investigated the pharmacokinetics of racemic amlodipine and the *S*-isomer in rats to evaluate the pharmaceutical equivalence between these 2 formulations.

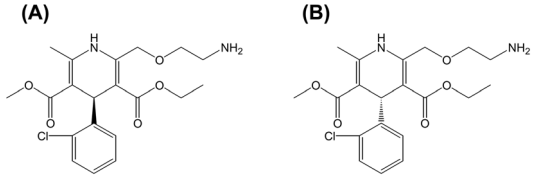

Figure 1. Chemical structures of amlodipine (A) *R*- and (B) *S*-isomers.

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Several analytical methods have been reported previously for the determination of amlodipine, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) with ultra-violet detection, fluorometric detection, amperometric detection, mass spectrometric detection, and gas chromatography (GC) with electron capture detection in various biological matrices.[6-11] A pharmacokinetic study by Streel et al. has previously described the enantioselective determination of amlodipine in human plasma by LC-MS/MS.[13]

In the present study, the plasma concentration profiles of amlodipine were investigated and compared using LC-MS/MS analysis after administering a single *S*-enantiomer(SK310) and a racemic mixture of amlodipine to rats.

Experimental

Chemical and Reagents

S-amlodipine and (±)-amlodipine were provided by SK Chemical Ltd. (Seongnam, Korea) with a purity of 99%. Polyethylene glycol (PEG, average molecular weight 300), ammonium formate, sodium carbonate, and desipramine (internal standard) were obtained from Sigma Chemical (St. Louis, MO, USA). Methanol and propanol were of HPLC grade and purchased from Avantor Performance Materials (Phillipsburg, NJ, USA). All the other chemicals used were of analytical grade unless otherwise specified.

Animals

Male Sprague-Dawley rats weighing 250-310 g were

purchased from DaeHan Laboratory Animal Research Center (Taejeon, Korea). The animals were housed in a temperature- and moisture-controlled room at 23 ± 2°C with 55 ± 10% humidity; a 12 h light/dark cycle was maintained and they were allowed free access to food and water.

Pharmacokinetic experiment

Two days before drug administration, the femoral artery was cannulated using polyethylene-50 tubing (Becton Dickinson, Lincoln Park, NJ, USA). The cannulae were fixed to the back of the neck. SK310 and amlodipine besylate as a free base were administered orally at a dose of 5 and 10 mg/kg body weight, respectively, after dissolving the same in a 10% PEG solution. The rats were fasted overnight before drug administration and until 6 h after drug dosing. Blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h post drug administration. After each sample collection, an identical amount of saline was injected in order to compensate for blood loss. Plasma was harvested after centrifugation and stored frozen at −70 °C until analysis.

Sample pretreatment

The extraction of the compounds from plasma was carried out using a solid-phase extraction method. A C_{18} cartridge sorbent was first conditioned with 1 mL methanol and equilibrated with 1 mL distilled water. After applying 200 μL of plasma spiked with the internal standard, the cartridge was washed with 3 mL of 0.1 M sodium carbonate (pH 9). The elution of the compound of interest was performed by applying 1 mL of methanol. The resultant extract was then evaporated to dryness under nitrogen and reconstituted with 30 μL of mobile phase. Then, 15 μL of the reconstituted sample was subjected to LC-MS/MS analysis.

Calibration and quality control standards

The calibration curves for *S*-amlodipine and *R*-amlodipine were prepared by adding varying amounts of the drugs' solutions to 200 μL of blank rat plasma spiked with the internal standard (desipramine). Further sample processing was carried out similar to that performed for the plasma samples from the dosed rats. The calibration curves were prepared by plotting the peak area ratios of *S*-amlodipine/desipramine and *R*-amlodipine/desipramine against the concentration of *S*-amlodipine and *R*-amlodipine, respectively; these were analyzed by the linear least-square regression analysis. The calibration curves for *S*-amlodipine and *R*-amlodipine remained linear over the concentration range of 0.5-50 ng/mL, with correlation coefficients (r^2) > 0.99. For the validation of the LC-MS/MS method, precision and accuracy were determined by repeated analysis of 3 concentration levels of the quality control samples (0.5, 5, and 50 ng/mL; i.e., low, medium, and high) on 4 separate days.

LC-MS/MS analysis

Quantitation of *S*- and *R*-amlodipine was performed in the LC-MS/MS system. The HPLC apparatus comprised an LC-10ADvp binary pump, SIL-10ADvp autosampler, and CTO-10ASvp oven (Shimadzu, Kyoto, Japan). The separation of isomers was achieved on a REGIS Chiral AGP column (150 × 2.0 mm, 5 μm) with a flow rate of 0.2 mL/min for the mobile phase. The mobile phase consisted of 10 mM ammonium acetate (pH 4.0) and propanol (99:1, V/V). The HPLC apparatus was coupled to an API 2000 triple-quadrupole mass spectrometer (Applied Biosystem SCIEX, Concord, Canada) equipped with a Turbo Ion Spray source. Electrospray ionization (ESI) was performed in positive acquisition mode, with nitrogen used as a nebulizing agent, turbo spray, and curtain gas with optimum values set at 40, 75, and 40 (arbitrary units), respec-tively. The turbo-gas temperature was set at 400 °C and the ESI needle voltage was adjusted to 5500 V. Multiple-reaction monitoring detection was applied, using nitrogen as the collision gas, with a dwell time of 150 ms for each transition; the transitions of the protonated molecular ions at m/z 409 and 267 to product ions occurred at m/z 238 and 208 for amlodipine and desipramine, respectively. The representative MRM chromatograms for *S*-amlodipine and *R*-amlodipine and desipramine in rat plasma are shown in Fig. 2.

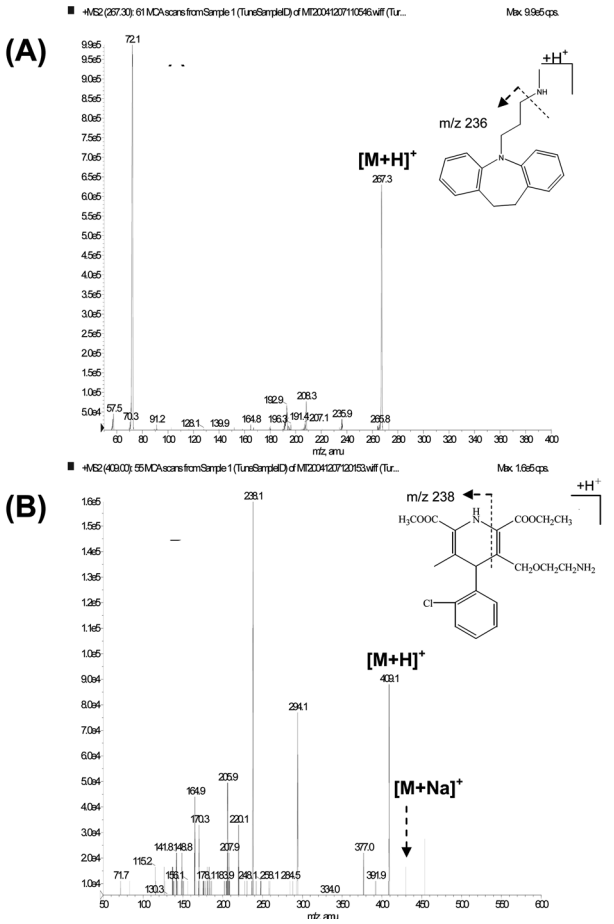

Figure 2. Product ion mass spectra of (A) desipramine (IS) and (B) amlodipine.

Data analysis

Plasma-concentration data were analyzed by a non-compartmental method using WinNonlin (Scientific Consulting, Inc., Lexington, KY, USA). Comparison of pharmacokinetic parameters such as Tmax, Cmax, AUC, Cl/F, $T\lambda_{1/2}$, Vd/F, and MRT were performed using a one-way analysis of variance followed by Duncan's test. Statistical significance was set at $P < 0.05$.

Results and Discussion

The molecular weight of (±)-amlodipine is 408, and that of the protonated molecular ion in the positive ion mode is 409. In the MS/MS product ion spectrum, a major fragment ion was observed at m/z 238. SK310, one of (±)-amlodipine's optical isomers, i.e., the *S*-isomer, showed an identical MS/MS product ion spectrum. The structures of the *R*-isomer and *S*-isomers of amlodipine are shown in Fig. 1. The molecular weight of the internal standard desipramine was 266 and that of the protonated molecular ion in the positive ion mode was 267, with a main fragment ion in the MS/MS product ion spectrum being detected at m/z 208 (Fig. 2). The internal standard and the (±)-amlodipine *R*- and *S*-isomers were separated using a HPLC Chiral AGP column, and these were detected at retention times of 7.0, 9.2, and 11.1 min, respectively. Under the HPLC conditions, no significant interference or ion suppression from endogenous substances was observed at the retention time of the analyte or IS (Fig. 3).

Linear calibration curves with correlation coefficients greater than 0.999 were obtained in the concentration ranges 0.5-50 ng/mL for both *R*- and *S*-isomers. The method thus demonstrated good precision and accuracy. Table 1 summarizes the intra-and inter-day precision and accuracy for amlodipine *R*- and *S*-isomers from the QC samples (0.5, 5, and 50 ng/mL), respectively. In this assay, the intra-run precision for amlodipine *R*- and *S*-isomers was 1.3-4.2% and 1.9-7.9%, respectively. The inter-run precision for amlodipine *R*- and *S*-isomer was 2.7-5.5% and 6.0-11.7%, respectively. The intra-run accuracy for amlodipine *R*- and *S*-isomers was 98.4-108.2% and 102.4-109.6%, respectively. The inter-run accuracy for amlodipine *R*- and *S*-isomers was 96.7-106.2% and 96.4-99.4%, respectively.

The plasma levels of *S*- and *R*-amlodipine after single enantiomer and racemic mixture administration are shown in Fig. 4, and the relevant pharmacokinetic parameters are summarized in Table 2. Generally, the pharmacokinetic parameters of *S*-amlodipine are comparable between the racemate- and SK-310-treated groups. When the racemic mixture of amlodipine was administered to rats, the blood concentration of the *R*-isomer was lower than that of *S*-isomer. It was considered that the metabolic selectivity of the 2 isomers caused the difference in the blood concentration profiles. The P values for the parameters of *S*-isomer after single oral administration of SK310 and (±)-amlodipine were 0.21-0.50, i.e., no statistically significant differences were observed. Thus, comparable plasma levels of *S*-amlodipine were obtained after the administration of 5 mg/kg of pure enantiomer and 10 mg/kg of racemic mixture of amlodipine. The data obtained in this study therefore indicate that the pharmacokinetic behaviors of the enantiomers were comparable and independent of the administration of the single enantiomer or the racemic mixture.

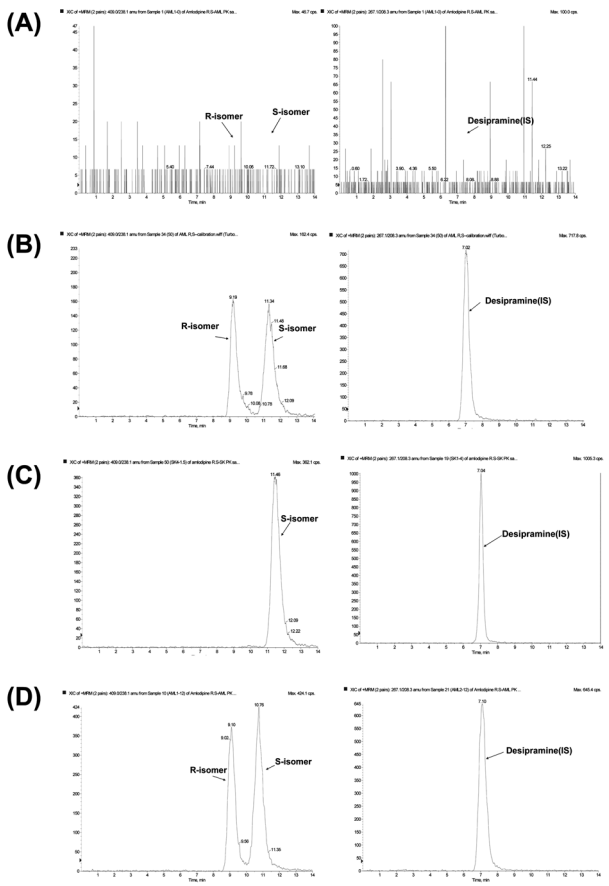

Figure 3. Multiple reaction monitoring chromatograms of (A) blank plasma, (B) blank plasma spiked with *R*- and *S*-isomer amlodipine (2 ng/mL), (C) plasma obtained 2 hr after oral administration of SK310 (5 mg/kg), and (D) plasma obtained 2 hr after oral administration of (±) amlodipine (10 mg/kg). Retention times of IS and *R*- and *S*- isomers were 7.0, 9.2, and 11.1 min, respectively.

Conclusion

In the present study, the plasma concentration profile of amlodipine was determined using LC-MS/MS analysis after oral administration of the *S*-isomer or a racemic mixture of amlodipine to rats. The comparative study of the pharmacokinetic profiles of *S*-and racemic amlodipine at a dose of 5 mg/kg and 10 mg/kg each demonstrated no significant differences. Furthermore, the *R*-enantiomer was not detected in the plasma samples after the administration of *S*-amlodipine. Therefore, based on the low dose and low toxicity associated with *S*-

Table 1. Intra-day and inter-day coefficient of variation (CV) and accuracy for determination of amlodipine *R*- and *S*-isomer in rat plasma (n = 4)

	Theoretical concentration (ng/mL)	Concentration found (ng/mL) (Mean±SD)	CV (%)	Accuracy (%)
R-isomer	Intra-day			
	0.5	0.5±0.01	1.3	108.2
	5	4.9±0.21	4.2	98.4
	50	51.4±1.68	3.3	102.9
	Inter-day			
	0.5	0.5±0.01	2.7	106.2
	5	4.9±0.27	5.5	97.3
	50	48.3±1.48	3.1	96.7
S-isomer	Intra-day			
	0.5	0.5±0.03	6.7	102.4
	5	5.2±0.41	7.9	104.8
	50	54.8±1.02	1.9	109.6
	Inter-day			
	0.5	0.5±0.06	11.7	99.4
	5	4.9±0.1	3.5	98.7
	50	48.2±2.90	6.0	96.4

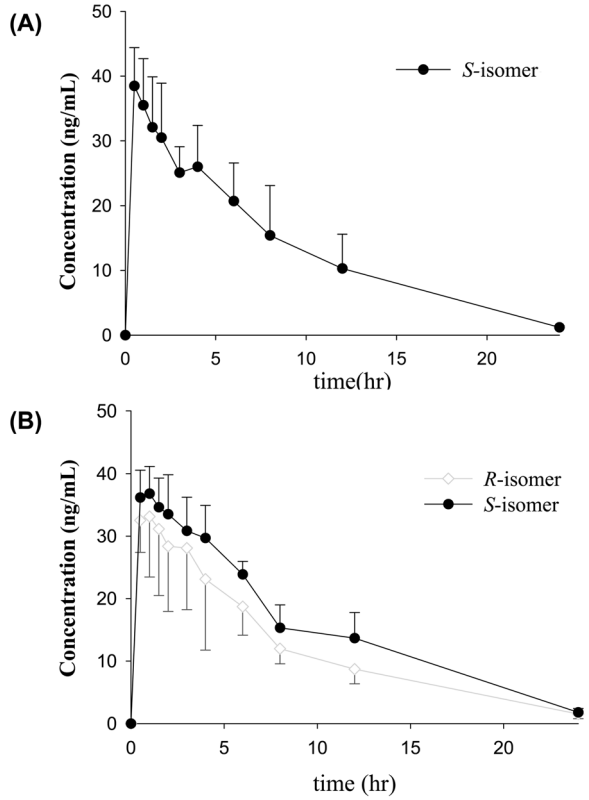

Figure 4. Plasma concentration of *S*- or/and *R*-amlodipine after single oral administration of (A) SK310 (5 mg/kg) and (B) (±)-amlodipine (10 mg/kg) to rats (n = 6).

Table 2. Pharmacokinetic parameters of *S*- or/and *R*-amlodipine after single oral administration of (A) SK310 (5 mg/kg) and (B) (±)-amlodipine (10 mg/kg) to rats (n = 6).

Parameter	SK310 (5 mg/kg)	(±)-Amlodipine (10 mg/kg)	
	S-isomer	*R*-isomer	*S*-isomer
AUC(ng·hr/mL)	329±85.9	285±71.6	368±44.2
Tmax(hr)	0.8±0.41	0.8±0.42	0.9±0.58
Cmax(ng/mL)	42.0±5.68	35.0±8.75	38.8±2.47
Cl/F(mL/hr/kg)	263±76.2	295±67.9	222±29.6
$T\lambda_{1/2}$(hr)	4.4±0.52	5.0±1.14	4.9±0.70
Vd/F(L/kg)	102±39.5	126±39.9	93.4±16.5
MRT(hr)	6.6±0.54	6.6±0.73	7.0±0.67

*No statistically significant difference was observed between *S*-isomer levels of SK310 and (±)-amlodipine-treated groups.

amlodipine, we consider that developing a single-enantiomer formulation would be worthwhile.

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References

1. Meredith, P. A.; Elliot, H. L. *Clin Pharmacokinet*. **1992**, 22, 22.
2. Murdoch, D.; Heel, R. R. *Drugs* **1991**, 41, 478.
3. Arrowsmith, J. E.; Campbell, F.; Cross, E.; Stubbs, J. K.; Burges, R. A.; Gardiner, D. G.; Blackburn, K. J. *J. Med. Chem*. **1986**, 29, 1696.
4. Goldmann, S.; Stoltefuss, J.; Born, L. *J. Med. Chem*. 1992, 35, 3341.
5. Zhang, X. P.; Loke, K. E.; Mital, S.; Chahwala, S.; Hintze, T. H. *J. Cardiovasc. Pharmacol*. **2002**, 39, 208.
6. Pandya, K. K.; Satia, M.; Gandhi, T. T.; Modi, I. A.; Modi, R. I.; Chakravarthy, B. K. *J. Chromatogr. B* **1995**, 667, 315.
7. Lukša, J.; Josić, D. J.; Podobnic, B.; Furlan, B.; Kremser, M. *J. Chromatogr. B* **1997**, 693, 367.
8. Tatar, S.; Atmaca, S. *J. Chromatogr. B* **2001**, 758, 305.
9. Shimooka, K.; Sawada, Y.; Tatematsu, H. *J. Pharm. Biomed. Anal*. **1989**, 7, 1267.
10. Massaroti, P.; Moraes, L. A.; Marchioretto, M. A.; Cassiano, N. M.; Bernasconi, G.; Calafatti, S. A.; Barros, F. A.; Meurer, E. C.; Pedrazzoli, *J. Anal. Bioanal. Chem*. **2005**, 382, 1049.
11. Scharpf, F.; Riedel, K. D.; Laufen, H.; Leitold, M. *J. Chromatogr. B* **1994**, 655, 225.
12. Streel, B.; Lainé, C.; Zimmer, C.; Sibenaler, R.; Ceccato, A. *J. Biochem. Biophys. Methods* **2002**, 54, 357.
13. Kim, B. H.; Kim, J. R.; Kim, M. G.; Kim, K. P.; Lee, B. Y.; Jang, I. J.; Shin, S. G.; Yu, K. S. *Clin. Ther*. **2010**, 32, 193.