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Determination of Felodipine Enantiomers Using Chiral Stationary Phase Liquid Chromatography and Gas Chromatography/Mass Spectrometry, and the Study of their Pharmacokinetic Profiles in Human and Dog

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A stereoselective and sensitive method for the determination of the enantiomers of felodipine, a dihydropyridine calcium antagonist, has been developed and the pharmacokinetic profiles of the enantiomers comparatively studied after oral administration to dogs and humans. D_6-Felodipine, the internal standard, was added to the plasma, extracted with a solvent and then optically resolved into *S*(−) and *R*(+) enantiomers on a high performance liquid chromatographic Chiralcel OJ column. Each enantiomer in the effluent was analysed by capillary column gas chromatography/positive ion electron impact mass spectrometry. After oral administration of the felodipine racemate, the T_{max} and $t_{1/2}$ values hardly differed between the two enantiomers in dogs and humans. The C_{max} and AUC_{0-24h} values of the *S*(−) enantiomer were slightly higher than those of the *R*(+) enantiomer in humans but the difference between the enantiomers was not significant. These results suggested that there is no large difference in the absorption, distribution and elimination of felodipine enantiomers after oral administration of the racemate in either dog or human.

INTRODUCTION

Felodipine (3,5-pyridinecarboxylic acid, 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-5-ethyl-3-methyl ester) is a dihydropyridine calcium antagonist synthesized by Astra Hässle AB (Mölndal, Sweden) (Ljung *et al.*, 1987; Meyer *et al.*, 1983), which is the racemate consisting of two optical isomers, *S*(−) and *R*(+), due to one asymmetric carbon atom in the structure (Lamm and Simonsson, 1989). Many chiral drugs have been reported to show stereoselectivity in their pharmacological activity (Boer *et al.*, 1989; Towart *et al.*, 1982; Gopolakrishnan and Triggle, 1985) and in pharmacokinetic behaviour such as distribution and metabolism. Therefore, it is of much importance to clarify the pharmacokinetics of the optical isomers to ensure the efficacy and safety of chiral drugs. Regarding dihydropyridine calcium antagonists having asymmetric structures, such as nilvadipine (Tokuma *et al.*, 1987a) and benidipine (Takayama *et al.*, 1991), various analytical methods have been developed to investigate the pharmacokinetic profiles of their enantiomers (Tokuma *et al.*, 1987b, 1989). The plasma level of unchanged felodipine is extremely low after oral administration due to the high hepatic clearance (Bäärnhielm *et al.*, 1986). Accordingly, a method for the complete separation and highly sensitive analysis of the optical isomers is necessary for the study on the pharmacokinetic characteristics of felodipine enantiomers. The present study describes the determination of felodipine enantiomers, using chiral stationary phase high performance liquid chromatography (HPLC) and a gas chromatographic/mass spectrometric (GC/MS) selected ion monitoring (SIM) method, and the study of their pharmacokinetic profiles after oral administration of felodipine racemate in dog and human.

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EXPERIMENTAL

Materials. The standard materials of *S*(−)-felodipine (Lot No. H183/96), *R*(+)-felodipine (Lot No. H183/91) and D_6-felodipine (Lot No. 8/1689; internal standard, IS; Fig. 1) were provided by Astra Hässle AB. The optical purities of the enantiomers were each ≥99% by HPLC using the Chiralcel OJ column mentioned below. The isotopic abundance of the IS was ≥99.9% by GC/MS analysis under the conditions described below. *n*-Hexane and toluene were of HPLC grade and the other reagents were of analytical grade.

Instrumentation. HPLC was conducted with a Waters 6000A system connected with a U6 K universal injector (NIHON Waters) and a Chiralcel OJ column (4.6 mm i.d. × 250 mm; column temperature, 40 °C) using *n*-hexane:isopropanol (5:1 v/v) as the mobile phase. The chromatogram was monitored with a JASCO UV-875 detector at a wavelength of 240 nm. The GC/MS system used was a JEOL JMS-Dx-300 where a fused silica cross-linked methylsilicone capillary column (0.20 mm i.d. × 12.5 m, Ultra 1; Hewlett Packard) was directly introduced into the MS ion source. The injector was regulated to a splitless mode (260 °C) and helium gas was

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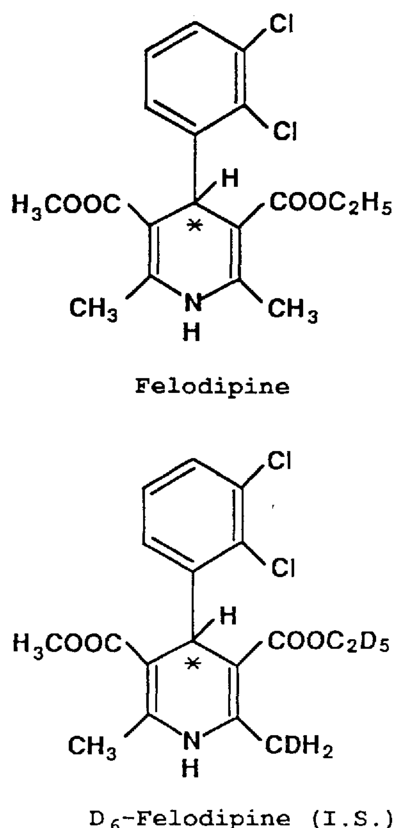

Figure 1. Chemical structure of felodipine and D_6-felodipine (IS). * = Centre of asymmetry.

used as the carrier at an inlet pressure of 0.25 kg/cm^2. The temperature of the column oven was maintained at 220° for the first minute and was then raised to 290 °C at a rate of 24 °C/min. Mass spectra were measured in a positive ion electron impact (PIEI) mode with an electron energy of 70 eV and an ion source temperature of 300 °C. The obtained data were processed with a JEOL JMA-3100 system.

Extraction and optical resolution. To 1 mL of a human plasma sample, 20 ng of IS and 1 mL of 0.1 M borate buffer (pH 9.0) were added. After the addition of 4 mL of *n*-hexane:toluene (4:1 v/v), the mixture was shaken for 5 min and centrifuged at 1,500 × g for 10 min to separate the organic phase. After washing of the organic phase with 1 mL of 0.1 M borate buffer (pH 9.0), the organic phase was evaporated under a stream of nitrogen at 60 °C. The dog plasma sample was extracted, after the addition of IS, with toluene instead of the *n*-hexane:toluene (4:1 v/v) used to avoid co-extraction of an interfering peak in the HPLC for the human sample. Then, the organic phase was evaporated in the same manner. The residue after evaporation was redissolved in 0.2 mL of the mobile phase for HPLC and 0.1 mL was injected into the HPLC column. The column effluents containing the *S*(−) and *R*(+) enantiomers were collected on the basis of the retention time of the standard material of the felodipine racemate which had been injected prior to the sample (Fig. 2). Each collected fraction (ca. 1.5 mL) was evaporated under a stream of nitrogen at 60 °C. The residue was dissolved by the addition of 30 µL of toluene and analysed by capillary column GC/PIEI-MS.

Quantification by GC/MS. Plasma samples spiked with known amounts of each enantiomer and the IS were pretreated as described above and subjected to GC/MS(SIM) to obtain the calibration curve from the peak-height ratio of felodipine (*m*/*z* 238) to that of the IS (*m*/*z* 243). The precision and accuracy of this procedure were validated by intra- and inter-day assays of the plasma spiked with the enantiomers. The stability of the enantiomers in plasma was investigated as follows: each of the enantiomers was added to plasma to a concentration of 5.00 ng/mL which was stored at −20 °C for 1 month and after the addition of the IS, the samples were extracted, fractionated by HPLC and analysed by GC/MS to estimate the concentration of each enantiomer.

***In vivo* dog study.** Four male dogs (Hazleton Research Animals Inc., Cumberland, VA, USA, 6 months old, 9–10 kg body weight) which had been allowed free access to diet and water were orally given felodipine racemate (ground with a jet mill; Lot No. 10422-1) suspended in 0.25% carboxymethylcellulose at a dose of 1 mg/kg with 10 mL of distilled water. Before the dosing, and 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 24 h postdosing, 3 mL of blood sample was collected from the cephalic vein into a heparinized syringe, immediately cooled with ice and centrifuged to separate plasma, which was frozen at −20 °C until analysed.

***In vivo* human study.** One felodipine tablet 10 mg (Lot No. 121/13/10) was orally administered with 100 mL of water to each of six healthy male volunteers aged 20–27 years. They

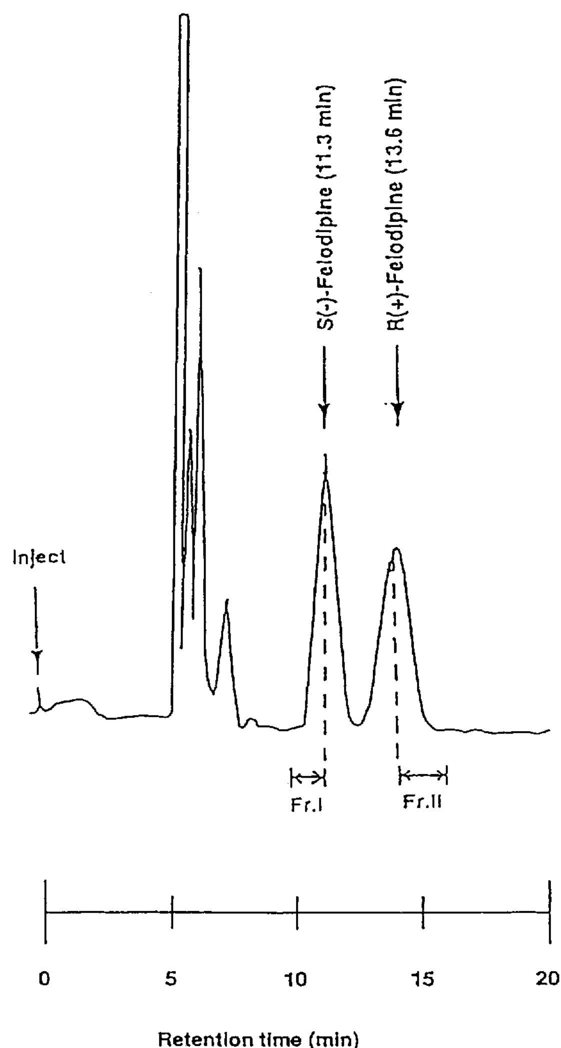

Figure 2. Chiral stationary phase liquid chromatogram of the extract of human plasma containing felodipine. Fractions I and II were collected for capillary column GC/PIEI-MS analysis. Fraction I: fraction range for *S*(−)-felodipine; fraction II: fraction range for *R*(+)-felodipine.

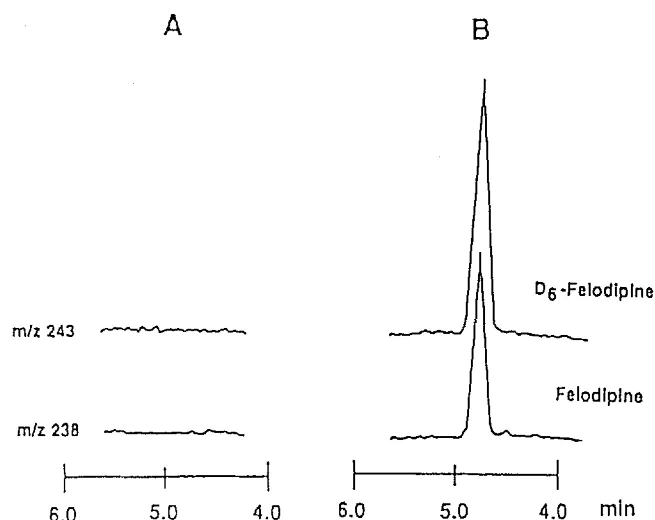

Figure 3. Selected ion current profiles from capillary column GC/PIEI-MS analysis of HPLC effluents. (A) Blank human plasma and (B) human plasma extract containing felodipine (10 ng/mL plasma) and D_6-felodipine (10 ng/mL plasma).

went without food for more than 11 h prior to the dosing. Before dosing, and 0.5, 1, 1.5, 2, 4, 6, 9, 12 and 24 h postdosing, 4 mL of blood sample was collected by venipuncture into a tube and centrifuged to separate plasma, which was frozen at −20 °C until analysed.

Pharmacokinetic parameters. The elimination half-life was calculated by fitting the plasma concentrations of the compounds in the elimination phase to the monoexponential equation by the linear least squares method. The maximum plasma concentration (C_{max}) and the time to reach the maximum concentration (T_{max}) were obtained from the found values. The area under the curve ($AUC_{0-24\,h}$) for up to 24 h postdosing was calculated using the trapezoidal rule.

RESULTS

Extraction and optical resolution of enantiomers

Figure 2 shows the analytical results obtained by using a Chiralcel OJ column after extraction with *n*-hexane:toluene (1:1 v/v) in the presence of 0.1 M borate buffer (pH 9.0). The retention times of the *S*(−) and *R*(+) enantiomers from felodipine and the IS were 11.3 min and 13.6 min, respectively, and no interfering peak was seen, which indicated favourable HPLC separation of felodipine enantiomers.

Analysis by GC/MS

In the mass spectra of felodipine and the IS in a round mass mode, strong base peaks were observed at *m/z* 238 for felodipine (theoretical value: 283.3) and at *m/z* 243 for the IS (243.3), which corresponded to the fragment ions after elimination of the dichlorophenyl group and H(D) moieties of the ethyl ester group. When these fragment ions were monitored by GC/MS analysis, felodipine and the IS showed the same retention time, of about 4.8 min, in sufficient intensity. Thus, those fragment ions were adopted as the monitoring ions in the SIM method. Figure 3 shows the ion current profiles of felodipine (10 ng/mL) and the IS (10 ng/mL) by the SIM method.

Validation of quantification

The calibration curve of each enantiomer obtained by GC/MS analysis of the spiked human plasma showed a favourable linearity by a lack-of-fit test over the range of 0.05–10.00 ng/mL with a detection limit of 0.05 ng/mL ($S/N=3$). Table 1 shows the results of the intra- and inter-day assays. The coefficient of variation (CV) was ≤11.3% for each enantiomer and the experimental values accorded well with the theoretical ones (100–106% of the added amount), indicative of satisfactory reproducibility and accuracy. Similar assays using dog plasma also gave favourable results (CV≤6.3%).

Stability in plasma

The concentration of each enantiomer added to the human and dog plasma and then stored at −20 °C for one month was nearly equal to the initial one (100–102%). Furthermore, the concentration of the opposite enantiomer which had not been added was below the detection limit. These results suggested that the enantiomers in human plasma are extremely stable and undergo very little chiral conversion.

Time-coursed enantiomer level in dog plasma

The time course of plasma enantiomer levels after the oral dosing of felodipine racemate (1 mg/kg) to male dogs is shown in Fig. 4 and the pharmacokinetic parameters on each enantiomer are summarized in Table 2. Plasma *S*(−) and *R*(+) enantiomer concentrations reached maximum values 2 h postdosing (*S*(−), 19.8 ng/mL; *R*(+), 18.1 ng/mL) and then decreased with a half-life of 6.1 h. The $AUC_{0-24\,h}$ value of the *S*(−) enantiomer (54.0 ng.h/mL) was almost comparable to that of the *R*(+) enantiomer (50.2 ng.h/mL). The *S*(−)/*R*(+) ratio of the plasma level was almost constant (1.0–1.1) after the administration of the felodipine.

Table 1. Precision and accuracy of felodipine enantiomers in human plasma

	Enantiomer	Actual conc. (ng/mL)	Found conc.[a] (ng/mL)	Percentage of actual conc. (%)	Coefficient of validation (%)
Intraday assay (*n*=5)	*S*(−)	5.00	5.05±0.20	101	3.9
		0.50	0.50±0.02	100	4.4
		0.05	0.05±0.01	100	11.3
	R(+)	5.00	5.04±0.09	101	1.8
		0.50	0.52±0.01	106	2.3
		0.05	0.05±0.00	100	6.8
Interday assay (*n*=4)	*S*(−)	5.00	5.11±0.04	102	0.9
		0.50	0.50±0.02	100	3.1
		0.05	0.05±0.00	100	5.1
	R(+)	5.00	5.07±0.06	101	1.2
		0.50	0.53±0.01	106	2.1
		0.05	0.05±0.00	100	3.3

[a] Each value represents the mean±SD.

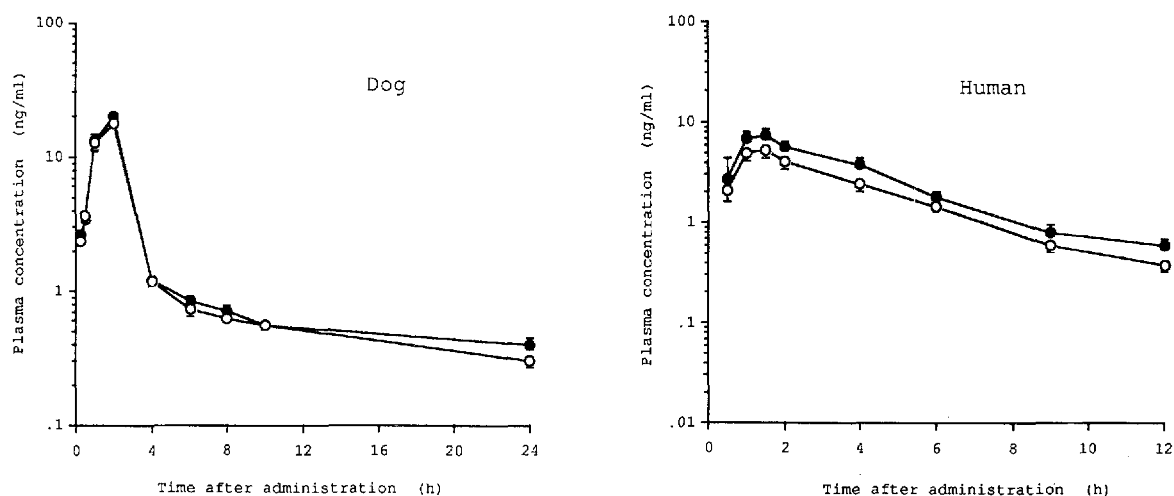

Figure 4. Plasma concentration–time profiles after oral administration of felodipine racemate to dogs ($n = 4$) and humans ($n = 6$): ●–●: $S(-)$-felodipine, ○–○: $R(+)$-felodipine. Data represents the mean±SE.

Time-coursed enantiomer level in human plasma

Figure 4 shows the time course of the plasma enantiomer concentration after oral dosing of felodipine 10 mg tablets to healthy male subjects (20–27 years old) with 100 mL of water. Table 2 shows the pharmacokinetic parameters calculated from the time-coursed plasma enantiomer concentration. The plasma $S(-)$ and $R(+)$ enantiomer concentrations reached maximum values 1.6 h postdosing ($S(-)$, 8.3 ng/mL; $R(+)$, 6.2 ng/mL) and then decreased with half-lives of 2.7 h and 2.5 h, respectively. The AUC_{0-24h} value of the $S(-)$ enantiomer was slightly higher than the $R(+)$ enantiomer ($S(-)$, 30.1; $R(+)$, 21.3 ng.h/mL). The $S(-)/R(+)$ ratio of the plasma level was almost constant (1.0–1.4) after the administration of the felodipine.

DISCUSSION

Recently it has been clarified that the stereoselective hepatic clearance and protein binding, etc. cause the stereoselectivity in the pharmacokinetics and the pharmacological profiles of dihydropyridine calcium channel blockers (Tokuma *et al.*, 1987b; Takayama *et al.*, 1991). *In vivo* pharmacokinetic studies of optical isomers are carried out in two ways: administration of each isomer to different animals or administration of the racemate to the same individuals. The former administration method, however, has the disadvantages of the influence of between-day variation and individual variation or the possibility that the *in vivo* kinetics itself may differ from that of the racemate (interaction between the isomers). These facts lead to the necessity of the administration of the racemate in the pharmacokinetic study of racemate drugs. In this study, we have established the stereoselective assay for felodipine enantiomers in plasma by an off-line coupling of stereoselective HPLC and capillary GC/MS and have examined their pharmacokinetic profiles after oral administration of felodipine racemate in dog and human. Each enantiomer could be separated favourably on a Chiralcel OJ column by HPLC and analysed by the SIM method of capillary column GC/PIEI-MS. In both species, the T_{max} and $t_{1/2}$ values hardly differed between the enantiomers. The C_{max} (human alone) and AUC_{0-24h} values of the $S(-)$ enantiomer were higher than those of the $R(+)$ enantiomer, but the differences were very slight (enantiomeric ratio $S(-)/R(+)$: 1.1–1.4). These results suggested that there is no large difference between the absorption, distribution and elimination of felodipine enantiomers in the dog and human species. Similar results have recently been reported in dogs after oral administration of a pseudoracemic mixture (Eriksson *et al.*, 1991a) and in humans by GC-electron capture detector (Soons *et al.*, 1990). Dihydropyridine calcium channel blockers such as nilvadipine and benidipine have been reported to show about three-fold differences in C_{max} and AUC_{0-24h} values from their optical antipodes (Tokuma *et al.*, 1987b; Niwa *et al.*, 1989), possibly due to the asymmetric effect of the side-chains of the dihydropyridine ring on the stereoselective protein binding and metabolism by cytochrome P-450 in liver microsomes (Niwa *et al.*, 1988). The elimination of felodipine from the body depends on the metabolic clearance by cytochrome P-450 (Bäärnhielm *et al.*, 1984). The metabolism rate of $R(+)$-felodipine was more rapid than that of $S(-)$-felodipine in human liver microsomes (Eriksson *et al.*, 1991b). However, because of the relative symmetry of the dihydropyridine ring of felodipine compared with nilvadipine, benidipine, etc., it can be

Table 2. Pharmacokinetic parameters of felodipine enantiomers in dog and human after oral administration[a]

Species	Dose (mg/kg)		C_{max} (ng/mL)	T_{max} (h)	AUC_{0-24h} (ng.h/mL)	$t_{1/2}$ (h)
		$S(-)$	19.8±0.8	2.0±0.0	54.0±2.0	6.1±0.7
Dog		$R(+)$	18.1±0.6	2.0±0.0	50.2±1.3	6.1±0.6
($n = 4$)	1					
		$S(-)/R(+)$	1.1±0.0	1.0±0.0	1.1±0.0	1.0±0.0
		$S(-)$	8.3±1.1	1.6±0.5	30.1±3.5	2.7±0.1
		$R(+)$	6.2±1.0	1.6±0.5	21.3±2.6	2.5±0.1
Human						
($n = 6$)	0.17					
		$S(-)/R(+)$	1.4±0.1	1.0±0.0	1.4±0.0	1.1±0.1

[a] Each value represents the mean±SE.

speculated that the small difference between the felodipine enantiomers in the steric effect on the intermolecular action with the hepatic cytochrome P-450 and serum protein is reflected as a smaller difference in their pharmacokinetic profiles.

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