Studies on the Metabolic Fate of Felodipine (V) : Absorption, Distribution, Metabolism and Excretion after a Single Oral Administration to Dogs

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Summary

The absorption, distribution, metabolism and excretion of radioactivity were investigated after a single oral administration of ^{14}C -felodipine 1 mg/kg to dogs of both sexes.

1. Radioactivity concentration in the plasma of male dogs reached a $C_{\max}$ of 2,228 ng eq./mL at 2 hr and then declined with half-lives of 4.8 hr up to 8 hr, 16 hr from 12 hr to 48 hr and 123 hr from 72 hr to 168 hr after administration. Radioactivity concentrations in the blood were 51~62% of those in the plasma. The $\text{AUC}_{0-\infty}$ was 41.3 $\mu\text{g}\cdot\text{eq}\cdot\text{hr}\cdot\text{mL}^{-1}$. Profiles of radioactivity concentrations in the blood and plasma of female dogs were similar to those in male dogs.

2. In male dogs, 54.9 and 41.4% of the dose was excreted in the urine and feces within 168 hr, respectively. In female dogs, 55.8 and 38.9% of the dose was excreted in the urine and feces within 168 hr, respectively. The excretion of radioactivity was not sex dependent.

3. In the plasma, the parent compound was a minor substance and M-III and M-IV were mainly found. In the urine, M-III, M-IV and M-V were the major metabolites, while in the feces, M-III, M-IV, M-VI (lactone) and M-VII (lactone) were mainly found. Hardly any sex difference was present in the relative amounts of metabolites in the plasma, urine and feces. The major metabolites UM1 and UM2 found in the plasma of rats were not present in the plasma of dogs.

INTRODUCTION

Felodipine, ethylmethyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridine-dicarboxylate is a dihydropyridine type calcium antagonist.

The absorption, distribution, metabolism and excretion in rats have been studied after a single or repeated administration of ^{14}C -felodipine^{1~4)}, and sex differences were present in the metabolic fate of felodipine.

In this study, the absorption, distribution, metabolism and excretion in dogs of both sexes was investigated after a single oral administration of ^{14}C -felodipine to elucidate sex differences. The species difference in the metabolic fate of felodipine are also discussed.

MATERIALS AND METHODS

1. Labelled Compound

^{14}C -felodipine was supplied by AB Hässle(Sweden). The structure with labelled position is shown in Fig.1 The specific radioactivity of ^{14}C -felodipine was 5.00MBq/mg, and the radiochemical purity was more than 99% through the experimental period. The radiochemical purity was determined by thin-layer chromatography (TLC) as described previously¹⁾.

Fig.1 Chemical structure with labelled position of ^{14}C -felodipine

2. Experimental Animals

Male and female Beagle dogs (MARSHALL FARMS) aged 7~9 months and weighing about 10kg were used in this study.

Dogs were given about 300 g of pellet food (DS-5, Oriental Yeast Co., Ltd.) once daily at about 1 p.m. (water allowed *ad libitum*), and were acclimatized to room temperature conditions of $23 \pm 2^\circ\text{C}$ and a relative humidity of $55 \pm 15\%$ on a 12hr light/dark cycle for more than 1 month. Three animals were assigned to each test group.

3. Dose Level and Administration

^{14}C -felodipine mixed with unlabelled felodipine was suspended in 0.5% CMC sodium solution to prepare the 0.5mg/ml dosing solution. The dose suspension was administered orally to dogs via catheters (Nelaton Catheter No.7, Izumo Rubber Industries) at a dose

of 2 ml/kg. Animals were not fasted. The radioactivity dose administered was about 1.85 MBq/kg.

4. Measurement of Radioactivity

Radioactivity in each test sample was counted for 2min with a liquid scintillation counter (LSC-903, ALOKA). Counting efficiency was corrected by a Channel Ratio method using external standard sources.

5. Determination of Radioactivity Concentrations in blood and plasma and Excretion of Radioactivity in Urine and Feces

After oral administration of ^{14}C -felodipine 1mg/kg, male and female dogs were housed in metabolic cages (Tokiwa Scientific Instrument, designed by Daiichi Pure Chemicals Co., Ltd.). Blood was withdrawn from the cephalic vein using a vacuum tube (Terumo) containing heparin sodium or a heparinized syringe at 15 and 30min, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144 and 168hr after administration. Radioactivity in each 100 μl of blood and plasma obtained after centrifugation ($1,800 \times g$, 4°C , 15min) was counted as described previously¹⁾. The transfer ratio of radioactivity into blood cells was calculated from hematocrit value, radioactivity concentrations in blood and plasma as described previously³⁾ using blood samples obtained 30min, 2 and 8hr after drug administration. The radioactivity concentrations in the blood and plasma were expressed as ng equivalent to felodipine/ml. The concentration described is total radioactivity concentration unless otherwise stated.

Urine and feces were collected 0~4, 4~8, 8~24, 24~48, 48~72, 72~96, 96~120, 120~144, and 144~168hr, and 0~8, 8~24, 24~48, 48~72, 72~96, 96~120, 120~144, and 144~168hr after administration, respectively. The urinary sample obtained within 24 hr after administration was combined with bladder urine collected by urethral catheters (Fr.8 for X-ray, Takei Ika Koki Seisakusho). Radioactivity in the urine and feces was counted as described previously¹⁾. After blood, plasma, urine and feces were collected at 168hr after administration, the metabolic cages were washed out with water and radioactivity in each 1 ml of washings was counted after addition of 13ml of scintillator ATO-MLIGHT (Du Pont NEN Research Products).

6. Analysis of Metabolites

1) Samples for Analysis

Urinary sample (0~24hr), fecal sample (0~48hr) and plasma samples (30min, 2, 4, 8 and 24hr) of male and female dogs after oral administration of ^{14}C -felodipine 1mg/kg were used for the analysis of metabolites.

2) Analytical Methods

The pretreatment methods and TLC of plasma and untreated urinary and fecal samples were performed as described previously¹⁾. In a preliminary study, no conjugates were found in the urine. Therefore, in this study the presence of conjugates was not investigated.

The authentic samples of anticipated metabolites were supplied by AB Hässle(Sweden).

7. Calculation of Pharmacokinetic Parameters and Statistical Analysis

Half-lives were calculated from blood and plasma concentration-time curve of radioactivity in each dog by least-squares regression analysis. The area under the radioactivity concentration-time curve ($AUC_{0-\infty}$) was calculated by the trapezoidal method. Data were expressed as the mean values $\pm$ standard errors (S.E.).

Student t-test with two-tailed probabilities was used for analysis of the significance of difference.

Fig. 2 Blood and plasma concentrations of radioactivity after single oral administration of ^{14}C -felodipine 1 mg/kg to non-fasting dogs. Data are expressed as the mean values $\pm$ S.E. for three animals.

RESULTS

1. Radioactivity Concentration in Blood and Plasma

The radioactivity concentrations in the blood and plasma (equivalent to felodipine), after oral administration of ^{14}C -felodipine 1 mg/kg to male and female dogs, are shown in Fig. 2. In male dogs, the radioactivity concentration in blood (A) reached a $C_{\max}$ of 1,157 ng eq./ml which occurred 2 hr after administration, radioactivity concentration then declined with half-lives of 5.1 hr up to 8 hr, 16 hr from 12 hr to 48 hr and 115 hr from 72 hr to 168 hr after administration. A $C_{\max}$ of 2,228 ng eq./ml in plasma (B) was found 2 hr after administration, radioactivity concentration then declined with half-lives of 4.8 hr up to 8 hr, 16 hr from 12 hr to 48 hr and 123 hr from 72 hr to 168 hr after administration. The radioactivity concentration in blood at each time was 51~62% of that in the plasma.

The radioactivity concentrations in blood (C) and plasma (D) of female dogs were similar to those found in male dogs.

Pharmacokinetic parameters are calculated from blood and plasma concentration-time curve of radioactivity in each dog and summarized in terms of each group (Table I).

2. Transfer of Radioactivity into Blood Cells

After 1 mg/kg ^{14}C -felodipine was administered orally to male and female dogs, the transfer ratios of radioactivity into blood cells were 0.0 ± 0.0 and $1.7 \pm 1.4\%$ at 30 min, 0.7 ± 0.4 and $1.5 \pm 1.0\%$ at 2 hr and 1.1 ± 0.8 and $4.5 \pm 4.2\%$ at 8 hr after administration, respectively.

Table I Pharmacokinetic parameters of radioactivity in blood and plasma after single oral administration of ^{14}C -felodipine 1 mg/kg to non-fasting dogs

Sex		Pharmacokinetic parameters			
		$T_{\max}^{1)}$ (hr)	$C_{\max}^{2)}$	$T_{1/2}$ (hr)	$AUC_{0-\infty}^{3)}$
Male	Blood	2 (1-2)	1157 $\pm$ 34	5.1 $\pm$ 0.4 ($T_{\max}$ -8hr) 16 $\pm$ 0 (12-48hr) 115 $\pm$ 11 (72-168hr)	23.1 $\pm$ 2.5
	Plasma	2 (1-2)	2228 $\pm$ 78	4.8 $\pm$ 0.2 ($T_{\max}$ -8hr) 16 $\pm$ 0 (12-48hr) 123 $\pm$ 3 (72-168hr)	41.3 $\pm$ 3.6
Female	Blood	2 (1-2)	1272 $\pm$ 97	5.2 $\pm$ 0.7 ($T_{\max}$ -8hr) 16 $\pm$ 1 (12-48hr) 150 $\pm$ 12 (72-168hr)	26.7 $\pm$ 1.4
	Plasma	2 (1-2)	2568 $\pm$ 230	5.0 $\pm$ 0.6 ($T_{\max}$ -8hr) 16 $\pm$ 1 (12-48hr) 117 $\pm$ 3 (72-168hr)	47.3 $\pm$ 4.6

Data are expressed as the mean values $\pm$ S.E. of three animals.

1) : $T_{\max}$ values are expressed the mean and range.

2) : ng equiv. of felodipine/ml

3) : μg equiv. of felodipine $\cdot$ hr/ml

Table II Cumulative excretions of radioactivity in urine and feces after single oral administration of ^{14}C -felodipine 1 mg/kg to non-fasting dogs

Time (hr)	Excretion of radioactivity (% of dose)					
	Male			Female		
	Urine	Feces	Total	Urine	Feces	Total
0-4	21.4 $\pm$ 2.9	—	—	19.0 $\pm$ 0.8	—	—
8	32.6 $\pm$ 3.1	0.4 $\pm$ 0.4	33.0 $\pm$ 2.9	28.7 $\pm$ 3.7	12.6 $\pm$ 8.0	41.2 $\pm$ 9.1
24	47.3 $\pm$ 3.2	31.7 $\pm$ 4.4	79.0 $\pm$ 1.9	48.3 $\pm$ 2.6	23.9 $\pm$ 12.5	72.2 $\pm$ 12.3
48	53.0 $\pm$ 3.2	39.2 $\pm$ 3.6	92.2 $\pm$ 0.4	54.1 $\pm$ 3.4	36.1 $\pm$ 3.7	90.2 $\pm$ 0.6
72	54.2 $\pm$ 3.2	40.6 $\pm$ 3.7	94.9 $\pm$ 0.5	55.1 $\pm$ 3.4	37.8 $\pm$ 3.2	92.9 $\pm$ 0.3
96	54.6 $\pm$ 3.2	41.0 $\pm$ 3.7	95.6 $\pm$ 0.6	55.4 $\pm$ 3.4	38.3 $\pm$ 3.1	93.7 $\pm$ 0.3
120	54.7 $\pm$ 3.2	41.2 $\pm$ 3.7	95.8 $\pm$ 0.6	55.6 $\pm$ 3.5	38.7 $\pm$ 3.2	94.3 $\pm$ 0.2
144	54.8 $\pm$ 3.2	41.3 $\pm$ 3.7	96.0 $\pm$ 0.6	55.7 $\pm$ 3.5	38.8 $\pm$ 3.2	94.5 $\pm$ 0.3
168	54.9 $\pm$ 3.2	41.4 $\pm$ 3.7	96.2 $\pm$ 0.6	55.8 $\pm$ 3.5	38.9 $\pm$ 3.2	94.7 $\pm$ 0.3
Cage washings			0.6 $\pm$ 0.0			0.5 $\pm$ 0.1

Data are expressed as the mean values $\pm$ S.E. for three animals.

— : Not determined.

3. Excretion of Radioactivity in Urine and Feces

The cumulative excretion of radioactivity in the urine and feces, after oral administration of ^{14}C -felodipine 1 mg/kg to male and female dogs, is shown in Table II. In male dogs, the excretion of radioactivity in the urine was 32.6% of the dose within 8hr, 47.3 % within 24hr, 53.0% within 48hr and 54.9% within 168hr. The excretion of radioactivity in the feces was 31.7% of the dose within 24hr, 39.2% within 48hr and 41.4% within 168 hr. Total excretion in the urine and feces was 96.2% of the dose within 168hr after administration and residual radioactivity in the cage washings was 0.6% of the dose at 168 hr.

The urinary and fecal excretion of radioactivity in female dogs was similar to that found in male dogs.

4. Metabolites

The TLC autoradiograms of metabolites in the plasma, urine and feces, after ^{14}C -felodipine 1mg/kg was administered orally to male and female dogs, are shown in Fig. 3~5. Each fraction (metabolite) is named in order of Rf value as follows; DPA₁-DPA₇ in the plasma, DUA₁ in the urine and DFA₁-DFA₆ in the feces on normal phase TLC, and DPB₁-DPB₁₃ in the plasma, DUB₁-DUB₁₀ in the urine and DFB₁-DFB₁₄ in the feces on reverse phase TLC. In co-chromatography with the authentic compounds of the anticipated metabolites, DPB₄, DUB₃ and DFB₆ corresponded to M-III, DPB₃, DUB₂ and DFB₄ corresponded to M-IV, DPB₁₃, DUB₁₀ and DFB₁₄ corresponded to M-V, DFA₅ and DFB₂ corresponded to M-VI (lactone), DPB₆ and DUB₄ corresponded to M-VI (free) and DFA₆ and DFB₁ corresponded to M-VII (lactone). The fraction (metabolite) corresponding to M-II, M-VII (free), M-VIII, M-XI, UM1 and UM2 was not found in any samples. Some metabolites existed in the fraction corresponding to the Rf value of felodipine

Fig. 3 Thin-layer chromatograms of metanol extract from plasma (2hr) after single oral administration of ^{14}C -felodipine 1 mg/kg to a non-fasting male dog

○: Authentic samples of felodipine

TLC system

Normal phase: Silica gel, diethyl ether

Reverse phase: RP-18, acetonitrile: water: acetic acid
(100:100:5)

(felodipine fr.) and M-IV and therefore the relative amounts of the fractions were determined as their admixture.

1) Plasma Metabolites

The relative amounts of metabolites in the plasma are shown in Table III and IV. In male dogs the relative amounts in the felodipine fr. on normal phase TLC was 2.9% of radioactivity in the plasma at 30min, 0.8% at 2hr and a similar level was maintained up to 24hr after administration. Each relative amount of metabolites was less than 2.7%. The relative amount in the felodipine fr. on reverse phase TLC was 4.2% of radioactivity in plasma at 30min, decreased with time, was 2.0% at 2hr and below the limit of detection at 24hr after administration. The relative amounts of DPB₄(M-III), DPB₃

Table III Composition of felodipine and its metabolites in plasma after single oral administration of ^{14}C -felodipine 1 mg/kg to non-fasting male dogs
TLC system ; Silica gel, diethyl ether

Metabolite	% of radioactivity in plasma				
	30 min	2 hr	4 hr	8 hr	24 hr
Felodipine fr. ^{a)}	2.9±0.3	0.8±0.0	0.5**	0.7±0.1	1.2±0.2
DPA 1	1.7±0.2	0.9±0.1	0.8±0.0	1.1±0.1	2.2±0.6
DPA 2	N.D.	0.8±0.1	N.D.	N.D.	N.D.
DPA 3	N.D.	0.9**	N.D.	N.D.	N.D.
DPA 4	N.D.	0.7**	0.5*	0.7±0.0	N.D.
DPA 5	N.D.	N.D.	N.D.	N.D.	2.4±0.7
DPA 6	N.D.	0.6**	1.1±0.1	2.7±0.1	2.3±0.4
DPA 7	1.0±0.1	1.6±0.7	2.7±0.3	1.4±0.4	1.2**
Origin	82.1±2.6	88.4±1.5	84.6±0.8	78.4±1.8	69.1±4.1
Others	3.3±0.3	1.2±0.1	1.4±0.2	1.9±0.3	2.6±0.3
Recovery (%)	91.0±2.6	95.1±0.9	91.1±0.6	87.0±1.9	80.7±3.0

Data are expressed as the mean values ± S.E. for three animals.

N.D. : Not detected, * : Value for one animal.

** : Mean value for two animals. a) : Felodipine fr. ; Fraction corresponding to the Rf value of felodipine (fraction including felodipine).

TLC system ; RP-18, acetonitrile : water : acetic acid (100 : 100 : 5)

Metabolite	% of radioactivity in plasma				
	30 min	2 hr	4 hr	8 hr	24 hr
Felodipine fr. ^{a)}	4.2±0.2	2.0±0.3	N.D.	1.0±0.1	N.D.
DPB 1	7.0±1.1	8.2±1.1	8.7±0.3	3.3±0.3	2.7± 0.8
DPB 2	1.6±0.1	0.8±0.1	N.D.	0.8**	1.2**
DPB 3 (M-IV)	19.7±1.9	14.1±0.4	11.6±0.9	7.6±0.3	9.4± 1.3
DPB 4 (M-III)	41.2±0.3	51.6±1.1	50.4±1.4	52.8±3.3	30.0±10.2
DPB 5	N.D.	N.D.	N.D.	N.D.	5.0**
DPB 6 (M-VI (Free))	3.5±0.5	4.8±0.3	7.0±0.4	8.5±0.2	14.8± 1.5
DPB 7	2.7±0.2	1.3±0.2	1.3±0.3	1.3±0.3	1.3*
DPB 8	4.1±0.5	2.6±0.5	2.9±0.5	3.2±1.0	3.0± 0.3
DPB 9	3.0±0.2	4.3±1.0	3.8±0.4	3.1±0.3	11.0± 5.0
DPB10	0.7**	1.1±0.1	N.D.	N.D.	1.4**
DPB11	0.7**	0.6±0.1	1.0±0.2	1.4±0.3	1.7± 0.1
DPB12	0.6**	0.7±0.0	0.7±0.2	N.D.	N.D.
DPB13 (M-V)	1.1±0.2	2.2±0.4	2.8±0.6	2.8±0.2	1.2**
Origin	0.9±0.1	0.8±0.1	1.0±0.1	0.8*	1.0**
Others	0.7±0.5	0.1±0.1	0.0±0.0	1.2±0.4	1.2± 0.6
Recovery (%)	91.0±2.6	95.1±0.9	91.1±0.6	87.0±1.9	80.7± 3.0

Data are expressed as the mean values ± S.E. for three animals.

N.D. : Not detected, * : Value for one animal.

** : Mean value for two animals. a) : Felodipine fr. ; Fraction corresponding to the Rf value of felodipine (fraction including felodipine).

Table IV Composition of felodipine and its metabolites in plasma after single oral administration of ^{14}C -felodipine 1 mg/kg to non-fasting female dogs
TLC system ; Silica gel, diethyl ether

Metabolite	% of radioactivity in plasma				
	30 min	2 hr	4 hr	8 hr	24 hr
Felodipine fr. ^{a)}	2.0 $\pm$ 0.3	1.0 $\pm$ 0.2	0.7*	1.1**	N.D.
DPA 1	1.0**	N.D.	N.D.	N.D.	1.5**
DPA 2	N.D.	N.D.	N.D.	N.D.	N.D.
DPA 3	0.7**	0.5*	N.D.	N.D.	N.D.
DPA 4	0.9**	0.6**	N.D.	0.8**	N.D.
DPA 5	N.D.	N.D.	N.D.	N.D.	N.D.
DPA 6	N.D.	0.8*	1.1 $\pm$ 0.1	1.9 $\pm$ 0.2	3.7 $\pm$ 0.6
DPA 7	1.1**	0.7*	1.2 $\pm$ 0.1	1.2 $\pm$ 0.1	N.D.
Origin	88.1 $\pm$ 1.2	89.4 $\pm$ 0.8	88.3 $\pm$ 1.3	82.2 $\pm$ 3.6	68.5 $\pm$ 2.3
Others	2.5 $\pm$ 0.5	2.9 $\pm$ 0.6	2.9 $\pm$ 0.5	2.0 $\pm$ 0.3	4.3 $\pm$ 1.3
Recovery (%)	95.0 $\pm$ 0.1	94.4 $\pm$ 0.5	93.7 $\pm$ 1.4	88.4 $\pm$ 3.6	77.5 $\pm$ 0.3

Data are expressed as the mean values $\pm$ S.E. for three animals.

N.D. : Not detected, * : Value for one animal,

** : Mean value for two animals, a) : Felodipine fr. ; Fraction corresponding to the Rf value of felodipine (fraction including felodipine).

TLC system ; RP-18, acetonitrile : water : acetic acid (100 : 100 : 5)

Metabolite	% of radioactivity in plasma				
	30 min	2 hr	4 hr	8 hr	24 hr
Felodipine fr. ^{a)}	3.2 $\pm$ 0.5	2.1**	1.3 $\pm$ 0.1	1.5 $\pm$ 0.2	1.9 $\pm$ 0.0
DPB 1	8.5 $\pm$ 0.2	7.9 $\pm$ 1.1	5.2 $\pm$ 0.2 ^{b)}	3.3 $\pm$ 0.2	1.5**
DPB 2	1.4 $\pm$ 0.1	N.D.	1.1**	N.D.	0.9 $\pm$ 0.0
DPB 3 (M-IV)	22.3 $\pm$ 0.9	15.7 $\pm$ 1.5	13.2 $\pm$ 1.5	8.9 $\pm$ 0.7	8.2 $\pm$ 1.3
DPB 4 (M-III)	43.5 $\pm$ 1.3	50.1 $\pm$ 1.5	54.6 $\pm$ 3.8	53.2 $\pm$ 2.1	36.0 $\pm$ 1.4
DPB 5	2.1 $\pm$ 0.7	N.D.	N.D.	N.D.	N.D.
DPB 6 (M-VI (Free))	2.7 $\pm$ 0.2	4.5 $\pm$ 0.4	6.2 $\pm$ 0.3	11.4 $\pm$ 0.9 ^{c)}	13.0 $\pm$ 1.7
DPB 7	2.0 $\pm$ 0.2	1.8 $\pm$ 0.3	1.7 $\pm$ 0.1	2.3 $\pm$ 0.9	1.8 $\pm$ 0.3
DPB 8	2.5 $\pm$ 0.6	3.1 $\pm$ 1.3	1.8**	2.3**	7.6 $\pm$ 3.5
DPB 9	3.5 $\pm$ 0.4	4.4 $\pm$ 0.8	3.7 $\pm$ 0.3	2.2 $\pm$ 0.6	2.4**
DPB10	N.D.	1.3 $\pm$ 0.2	N.D.	N.D.	N.D.
DPB11	1.2 $\pm$ 0.1	0.5*	1.6**	N.D.	1.6 $\pm$ 0.4
DPB12	1.4*	0.8 $\pm$ 0.2	1.3**	0.8*	N.D.
DPB13 (M-V)	1.1**	1.5 $\pm$ 0.6	2.4 $\pm$ 0.5	2.0 $\pm$ 0.1	1.2**
Origin	0.7 $\pm$ 0.1	0.9 $\pm$ 0.1	1.2*	0.6*	0.9*
Others	0.4 $\pm$ 0.3	0.7 $\pm$ 0.2	1.2 $\pm$ 1.0	1.7 $\pm$ 1.1	2.7 $\pm$ 0.4
Recovery (%)	95.0 $\pm$ 0.1	94.4 $\pm$ 0.5	93.7 $\pm$ 1.4	88.4 $\pm$ 3.6	77.5 $\pm$ 0.3

Data are expressed as the mean values $\pm$ S.E. for three animals.

N.D. : Not detected, * : Value for one animal,

** : Mean value for two animals, a) : Felodipine fr. ; Fraction corresponding to the Rf value of felodipine (fraction including felodipine).

b) : $p < 0.05$, compared with DPB 1 (4 hr) in Table III (Male).

c) : $p < 0.05$, compared with DPB 6 (M-VI (Free)) (8hr) in Table III (Male).

Fig. 4 Thin-layer chromatograms of metanol extract from urine (0-24hr) after single oral administration of ^{14}C -felodipine 1 mg/kg to a non-fasting male dog

○: Authentic samples of felodipine

TLC system

Normal phase: Silica gel, diethyl ether

Reverse phase: RP-18, acetonitrile: water: acetic acid (100:100:5)

(M-IV), $\text{DPB}_6(\text{M-V (Free)})$ and $\text{DPB}_{13}(\text{M-V})$ were 30.0~52.8, 7.6~19.7, 3.5~14.8 and 1.1~2.8%, respectively, up to 24hr after administration. The relative amounts of unknown metabolites DPB_1 and DPB_9 were 2.7~8.7 and 3.0~11.0%, respectively. Each relative amount of the other metabolite was less than 5.0%.

The relative amounts of plasma metabolites in female dogs (Table IV) on normal and reverse phase TLC were similar to those obtained in male dogs.

2) Urinary Metabolites

The relative amounts of metabolites in the urine are shown in Table V. In male dogs the relative amount of DUA_1 on normal phase TLC was 1.9% of radioactivity in the urine but the relative amount in the felodipine fr. was below the detection limit. The

Table V Composition of felodipine and its metabolites in urine (0-24 hr) after single oral administration of ^{14}C -felodipine 1 mg/kg to non-fasting dogs

TLC system ; Silica gel, diethyl ether

Metabolite	% of radioactivity in urine	
	Male	Female
Felodipine fr. ^{a)}	N.D.	N.D.
DUA 1	1.9 $\pm$ 0.1	2.2 $\pm$ 0.3
Origin	93.3 $\pm$ 2.8	95.0 $\pm$ 0.4
Others	0.2 $\pm$ 0.1	0.7 $\pm$ 0.1
Recovery (%)	95.4 $\pm$ 2.9	97.9 $\pm$ 0.3

TLC system ; RP-18, acetonitrile : water : acetic acid (100 : 100 : 5)

Metabolite	% of radioactivity in urine	
	Male	Female
Felodipine fr. ^{a)}	1.5 $\pm$ 0.2	1.3 $\pm$ 0.2
DUB 1	7.3 $\pm$ 0.6	7.8 $\pm$ 0.8
DUB 2 (M-V)	11.7 $\pm$ 0.7	12.4 $\pm$ 0.5
DUB 3 (M-III)	42.7 $\pm$ 1.4	44.5 $\pm$ 0.8
DUB 4 (M-VI (Free))	3.8 $\pm$ 0.3	3.5 $\pm$ 0.3
DUB 5	4.0 $\pm$ 0.4	4.0 $\pm$ 0.3
DUB 6	3.9 $\pm$ 0.2	4.6 $\pm$ 0.6
DUB 7	2.5 $\pm$ 0.2	2.4 $\pm$ 0.1
DFB 8	3.1 $\pm$ 0.2	3.2 $\pm$ 0.2
DFB 9	3.4 $\pm$ 0.5	2.7 $\pm$ 0.3
DUB10 (M-V)	9.4 $\pm$ 0.5	10.2 $\pm$ 1.3
Origin	1.6 $\pm$ 0.2	1.4 $\pm$ 0.1
Others	0.6 $\pm$ 0.1	0.0 $\pm$ 0.0
Recovery (%)	95.4 $\pm$ 2.9	97.9 $\pm$ 0.3

Data are expressed as the mean values $\pm$ S.E. for three animals.

N.D. : Not detected.

a) : Felodipine fr. ; Fraction corresponding to the Rf value of felodipine (fraction including felodipine).

relative amount in the felodipine fr. on reverse phase TLC was 1.5% of radioactivity in urine. The relative amounts of major metabolites DUB₃(M-III), DUB₂(M-V), DUB₁₀(M-V) and DUB₁ were 42.7, 11.7, 9.4 and 7.3%, respectively. The relative amounts of DUB₅, DUB₆, DUB₄(M-VI, (free)), DUB₉, DUB₈ and DUB₇ were 2.5~4.0%. In a preliminary study (not published), hardly any conjugates were found in the urine.

The relative amounts of urinary metabolites in female dogs on normal and reverse phase TLC were similar to those found in male dogs.

3) Fecal Metabolites

The relative amounts of metabolites in the feces are shown in Table VI. In male dogs, the relative amounts in the felodipine fr., DFA₆(M-VII(lactone)), DFA₅(M-VI(lactone)), DFA₄ and DFA₃ on normal phase were 32.1, 13.1, 8.7, 2.2 and 1.0% of radioactivity in the feces, respectively. The relative amount in the felodipine fr. on reverse phase TLC

Fig. 5 Thin-layer chromatograms of metanol extract from feces (0-48hr) after single oral administration of ^{14}C -felodipine 1 mg/kg to a non-fasting male dog

○ ○ : Authentic samples of felodipine

TLC system

Normal phase : Silica gel, diethyl ether

Reverse phase : RP-18, acetonitrile : water : acetic acid (100 : 100 : 5)

was 28.4%. The relative amounts of $\text{DFB}_1(\text{M-VI}(\text{lactone}))$, $\text{DFB}_4(\text{M-IV})$ and $\text{DFB}_2(\text{M-V}(\text{lactone}))$ were 23.5, 8.3 and 6.7%, respectively, and the relative amounts of $\text{DFB}_6(\text{M-III})$, DFB_{12} , DFB_8 , DFB_3 , DFB_7 , DFB_{10} , DFB_9 , DFB_5 , $\text{DFB}_{14}(\text{M-V})$ and DFB_{13} were 0.9~4.3%.

The relative amounts of fecal metabolites in female dogs on normal and reverse phase TLC were similar to those obtained in male dogs.

DISCUSSION

In our previous study^{1,4)}, a sex difference was observed in the radioactivity concentration in the blood and the relative amounts of major plasma metabolites containing the

Table VI Composition of felodipine and its metabolites in feces (0-48 hr) after single oral administration of ^{14}C -felodipine 1 mg/kg to non-fasting dogs

TLC system ; Silica gel, diethyl ether

Metabolite	% of radioactivity in feces	
	Male	Female
Felodipine fr. ^{a)}	32.1±11.1	27.6±16.1
DFA 1	N.D.	1.2± 0.3
DFA 2	N.D.	1.5± 0.2
DFA 3	1.0± 0.2	1.1± 0.2
DFA 4	2.2± 0.8	1.9± 0.1
DFA 5 (M-VI (Lactone))	8.7± 0.7	6.2± 1.3
DFA 6 (M-VII (Lactone))	13.1± 0.3	11.4± 4.1
Origin	33.3± 5.6	40.8± 9.8
Others	4.0± 0.5	1.5± 0.1 ^{d)}
Recovery (%)	102.8±10.8 ^{b)}	101.1±11.5 ^{b)}

TLC system ; RP-18, acetonitrile : water : acetic acid (100 : 100 : 5)

Metabolite	% of radioactivity in feces	
	Male	Female
Felodipine fr. ^{a)}	28.4±12.3	24.5±18.3
DFB 1 (M-VII (Lactone))	23.5± 0.9	23.1± 6.3
DFB 2 (M-VI (Lactone))	6.7± 0.3	7.0± 1.6
DFB 3	3.0± 1.0	2.5± 0.7
DFB 4 (M-IV)	8.3± 2.5	4.3± 1.0
DFB 5	1.6± 0.2	N.D.
DFB 6 (M-III)	4.3± 1.5	6.5± 1.1
DFB 7	2.8± 0.6	3.8± 1.6
DFB 8	3.5± 0.4	5.0± 1.1 ^{c)}
DFB 9	2.1**	11.7± 3.8
DFB10	2.6± 0.5	
DFB11	1.6± 0.6	
DFB12	4.3**	
DFB13	0.9± 0.0	1.1± 0.2
DFB14 (M-V)	1.5± 0.1	2.0± 0.6
Origin	1.4± 0.3	2.1**
Others	0.3± 0.1 ^{b)}	0.3± 0.2 ^{b)}
Recovery (%)	102.8±10.8	101.1±11.5

Data are expressed as the mean values ± S.E. for three animals.

N.D. : Not detected.

a) : Felodipine fr. ; Fraction corresponding to the R_f value of felodipine (fraction including felodipine). b) : Composition was calculated as 100% recovery. c) : Mixture of DFB₉, DFB₁₀, DFB₁₁, and DFB₁₂. **) : Mean value for two animals.

d) : p<0.05, compared with Male.

Fig. 6 The estimated metabolic pathways of felodipine in dogs

UM-1 and UM-2 were found as major metabolites in rat plasma and bile, and their structures are also shown in a square.

dihydropyridine ring after oral administration of ¹⁴C-felodipine to rats, which may have been due to differences in hepatic drug metabolizing enzyme activity, especially the rate of oxidation of the dihydropyridine ring to the pyridine ring of dihydropyridines. A sex difference in the concentration of radioactivity in the blood has also been reported after administration of nifedipine⁵⁾ and nilvadipine⁶⁾ to rats. Metabolites containing a dihydropyridine ring have also been reported after administration of nilvadipine⁷⁾, benidipine⁸⁾ and nicardipine^{9,10)}.

In this study, the concentrations of radioactivity in the blood and plasma, the transfer of radioactivity into blood cells, the excretion of radioactivity in the urine and feces and the metabolism were investigated in dogs to determine whether the sex differences found in rats are species specific.

UM1 and UM2 of rat major metabolites^{1,4)} were not found in dog plasma after oral administration of ¹⁴C-felodipine 1 mg/kg and M-III and M-IV were major metabolites. The results suggest that ester hydrolysis of the side chain of felodipine is dominant compared with the oxidation of the dihydropyridine ring of felodipine in rats, and vice versa in dogs.

Hardly any radioactivity in the felodipine fr. was found in rat feces¹⁾ but radioactivity was found in dog feces, indicating that unabsorbed felodipine may be excreted as unchanged drug.

In addition to M-III and M-IV, M-V (free) was found in the plasma, M-V and M-VI (free) in the urine and M-VII (lactone) and M-V (lactone) in the feces. M-III, M-IV and M-V were major metabolites in dog urine, which corresponded to the results obtained by Weidolf¹¹⁾ et al. The estimated metabolic pathways of felodipine in dogs is shown in Fig. 6, based on both their results¹¹⁾ and the results we obtained.

The relative amounts of urinary metabolites in male dogs were similar to those found in male rats¹⁾, suggesting that species differences in hepatic metabolism hardly affected excretion. The transfer of radioactivity into blood cells was low in dogs as in male rats³⁾.

The radioactivity concentrations in the blood and plasma, the excretion of radioactivity in the urine and feces and the transfer of radioactivity into blood cells in female dogs were similar to those in male dogs. UM1 and UM2 were not found in dog plasma of either sex and the sex difference found in rats¹⁾ was not present in dogs. The relative amounts of felodipine fr. and metabolites in the urine and feces of female dogs were similar to those found in males, indicating no difference between the sexes.

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