Arch Pharm Res Vol 26, No 9, 763-767, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

Pharmacokinetic Behavior and Tissue Distribution of Verapamil and Its Enantiomers in Rats by HPLC

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(Received October 16, 2002)

The differences in pharmacokinetic behavior and tissue distribution of verapamil and its enantiomers were investigated in rats. In high-performance liquid chromatographic method, an achiral ODS column (150 mm × 4.6 mm i.d.) with the mobile phase consisting of methanol-water (73:30, v/v) was used for the determination of the concentration for racemic verapamil, and a Chiralcel OJ column (250 mm×4.6 mm i.d.) with the mixture of *n*-haxane-ethanol-triethylamine (85:15:0.2, v/v/v) as mobile phase was used to determine the concentrations of verapamil enantiomers. A fluorescence detector in the analytical system was set at excitation and emission wavelengths of 275 nm and 315 nm. The differences between enantiomers were apparent in the pharmacokinetics in rats. The area under the concentration-time curve (AUC) of *S*-(-) verapamil was higher than that of *R*-(+) verapamil. The half-distribution time ($T_{1/2(\alpha)}$) of *S*-(-) verapamil which distributing to tissue from blood was shorter than that of *R*-(+) verapamil, but the elimination half-time ($T_{1/2(\beta)}$) was longer in rat following oral administration of racemic verapamil. At 1.3 h after oral administration of racemic verapamil, however, there were no significant differences between enantiomers for the distributions in major tissues such as heart, cerebrum, cerebellum, liver, spleen and kidney.

Key words: Verapamil, Enantiomer separation, Pharmacokinetics, Tissues distribution

INTRODUCTION

Verapamil, a calcium channel antagonist, is clinically useful in the treatment of angina in its several forms, hypertension, some cardiac arrhythmias, and selected vascular disorders (Triggle *et al.*, 1987). With an asymmetrical carbon atom in its molecular structure, it has two enantiomers: *S*-(-) verapamil and *R*-(+) verapamil. The experimental results have shown that the enantiomers have significant differences in the pharmacological effects and the site of action in the body (Amsterdam *et al.*, 1988). For example, the negative dromotropic effect of *S*-(-) verapamil and *R*-(+) verapamil on atrioventricular conduction in man (Echizen *et al.*, 1988) and in dogs (Satoh *et al.*,1980) was different. Until now, however, it has been used as a racemic mixture of equal amounts of verapamil enantiomers in clinic. In recent years, several analytical methods such as GC with nitrogen-phosphorus detection (Ho-Sang Shin *et al.*, 1996) and HPLC with chiral-AGP (acid glycoprotein) as a stationary phase (Sandsteom *et al.*, 1992; Grazia Stagni *et al.*, 1995) have been developed for the separation and determination of verapamil enantiomers in biological samples. In addition, other direct HPLC methods with a cellulose-based reversed-phase (Chiralcel OD-R) (Asafu-Adjaye *et al.*, 1998)and an α_1-AGP column (Brandsteterova *et al.*, 1999) have been used for determination of verapamil and norverapamil enantiomers in urine and in serum samples. Hanada (Hanada *et al.*, 1998) reported the determination of verapamil enantiomers in rat plasma and tissues by HPLC with a cellulose-based reversed-phase column (Chiralpak AD) and fluorescence detection.

In this study, the objectives of this investigation were to determine the feasibility of development of analytical procedure that was applicable in the quantification of the enantiomers of verpamail and to characterize the stereoselective pharmacokinetics. Therefore, the differences of pharmacokinetic behavior and tissue distribution of verapamil and its enantiomers in rat were investigated by using high-performance liquid chromatography method equipped with a ODS column and a Chiralcel OJ column and fluorescence detection.

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MATERIALS AND METHODS

Reagents and materials

Verapamil racemate, *R*-(+) verapamil and *S*-(-) verapamil were purchased from RBI (Natick. MA, USA). Verapamil hydrochloride tablets ware obtained from the local hospital pharmacy (Xi'an, P. R. China). All other chemicals were analytical regent grade unless otherwise indicated. Water was purified with an ion-exchange method and distilled before being used to prepare all solutions. HPLC-grade methanol was obtained from Tedia (Fairfield, OH, USA). Analytical n-hexane and ether was from Xi'an Chemical Regent Plant (Xi'an, P.R.China) and distilled before using.

Rats (male or female, 200±30 g) were supplied by the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, P.R.China)

Apparatus and chromatographic conditions

The chromatographic system consisted of a Shimadzu SPD-10A*vp* pump, Model 7125 injector, an Anastar work station and an RF-535 fluorescence detector (all from Shimadzu, Tokyo, Japan) set at excitation and emission wavelengths of 275 nm and 315 nm. Achiral column was a Kromasil 5 μm ODS column (150 mm×4.6 mm i.d.) with the mobile phase consisting of methanol-water (70:30, v/v) with a flow rate of 1.0 mL · min^{-1} for the determination of total verapamil concentration. The verapamil enantiomers were analyzed by a Chiralcel OJ column (250 mm×4.6 mm i.d.) (Diacel, Tokyo, Japan) with the mobile phase of a mixture of *n*-haxane-ethanol-triethylamine (85:15:0.2, v/v/v) running at 0.5 mL · min^{-1}. All determinations were carried out at 30 using a column heater.

Sample preparation

The rats in this study were fasted overnight (except for water) and then a plastic tube was placed into the auriculocarotid for each. After oral administration of 20 mg · kg^{-1} racemic verapamil hydrochloride tablet, all blood samples (0.5 mL each) were drawn into heparinized tubes containing of an anticoagulant (heparin) in 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24 h, respectively. The samples were then centrifuged at 3000 rpm for 10 min to collect the supernatant and the plasma stored at -20°C until the analysis.

When it was necessary to study the distribution of the drug, the animals were humanely euthanatized after oral administration of 40 mg · kg^{-1} racemic verapamil hydrochloride tablets for 1.3 h (about 3 times of the maximum time (T_{max}) of racemic verapamil in plasma). Tissue samples were then obtained and placed in the normal saline to remove the blood; The tissue was blotted on a filter paper, weighed in wet weight and homogenized in the saline solution. Tissue homogenates were obtained and stored at -20°C until it was time for analysis.

Extraction procedures

To 0.2 mL of plasma or 0.5 mL of tissue homogenate samples were added 0.06 mL of 5 mol · L^{-1} NaOH aqueous. The mixture was shaken and extracted for 10 min with 5 mL of the mixture of *n*-hexane-ether (30:70, v/v) for three times. The supernatant was evaporated to dryness under a stream of nitrogen gas and a shelter from light. The residue was dissolved in 0.25 mL methanol. An aliquot (100 μL) of the solution was injected onto the ODS column to determine racemic verapamil, and another aliquot (100 μL) injected onto the OJ column to separate verapamil enantiomers respectively.

RESULTS AND DISCUSSION

Under the reverse-phase chromatographic condition above, the retention time (t_R) of racemic verapamil was 15.8 min (n>5000) and no peaks of interfering component in the chromatogram (Fig. 1) were apparent. The baseline separation of verapamil enantiomers was obtained under the chiral condition and the retention time was 21.0 min for *S*-(-) verapamil, 27.5 min for *R*-(+) verapamil, and the resolution (*Rs*) between enantiomers was more than 1.5 (Fig. 2).

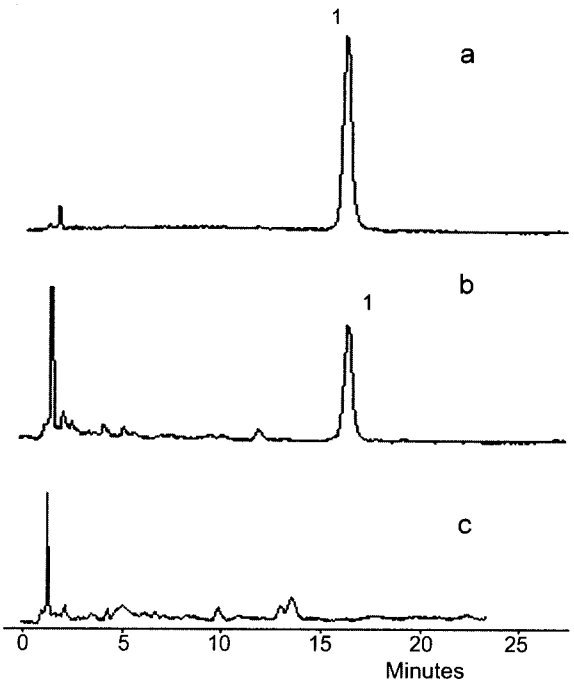

Fig. 1. Typical chromatograms of racemic verapamil in the standard solution (a), in drug-free rat plasma (b), and in rat plasma. 1-Peak of verapamil. Reversed phase liquid chromatographic conditions were a C_{18} column (150 mm×4.6 mm, i.d.) with a mobile phase consisted of methanol-water-triethylamine (70:30:0.2, v/v/v) at the flow rate of 1.0 mL · min^{-1}. The fluorescences detector was set at excitation and emission wavelengths of 275 nm and 315 nm and 30°C.

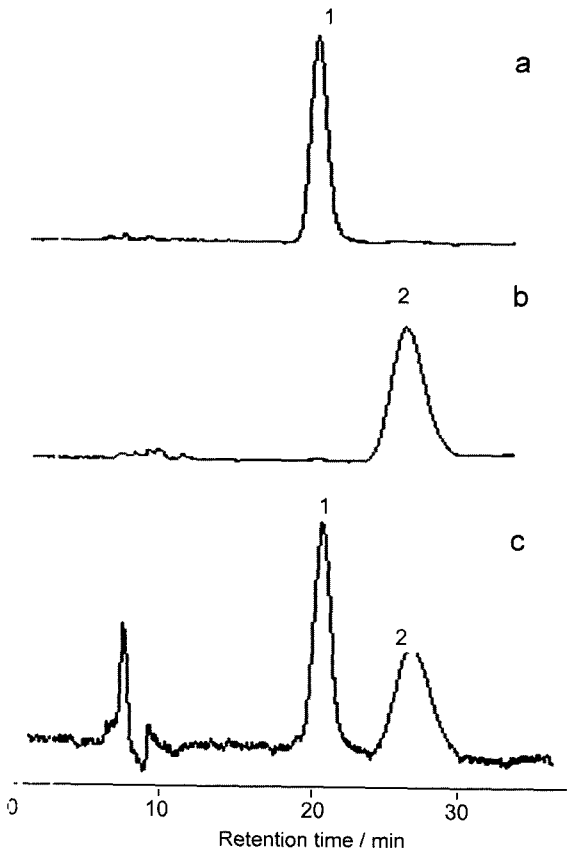

Fig. 1. Typical chromatograms of *S*-(-) verapamil (a), *R*-(+) verapamil (b) in standard solutions and racemic verapamil in rat plasma (c). 1- *S*-(-) verapamil, 2-*R*-(+) verapamil. Chiral separation chromatographic conditions were an Chiralcel OJ column (250 mm×4.6 mm, i.d.) with a mobile phase consisted of *n*-hexane-ethanol-triethylamine (85:15:0.2, v/v/v) at flow rate of 0.5 mL · min^{-1}. The fluorescences detector was set at excitation and emission wavelengths of 275 nm and 315 nm and 30°C.

Standard curves

To generate the standard curves, different amounts of racemic and enantiomeric verapamil standards were added to 0.2 mL of blank plasma and 0.5 mL of blank tissue homogenates (Table I). Then biological samples were extracted by the liquid-liquid method and analyzed by the achiral and chiral systems under the chromatographic conditions above. The standard curves of peak areas (A) versus concentrations (C) were constructed for each sample. The lowest detection limit was 0.2 ng for racemic verapamil and 2 ng for each verapamil enantiomer in rat plasma and tissues (the ratio of signal and noise (S/N) was more than three).

Precision and accuracy

To determine the precision and accuracy of the method, replicate samples of plasma spiked with varying concentration 17, 85, 170 ng of racemic verapamil and 8.5, 42.5, 85 ng of verapamil enantiomers/mL of plasma were determined by the assay procedures. In within-day variability, the average recoveries were 99.3% with RSD of 2.8% for the racemate assay and 99.2% with RSD of 3.1% for the stereoselective assay. In between-day variability, the average recoveries were 98.4% with RSD of 12.1% for the racemate assay and 98.4% with RSD of 10.4% for the stereoselective assay (Table II). These results showed that the method established in this study was adequate for the study on the differences in pharmacokinetics of verapamil and its enantiomers in rats.

Table I. Standard curves, correlation coefficient and linear ranges of racemic and enantiomeric verapamil in biological samples. The chromatographic conditions for assaying each sample in achiral and chiral systems were described above.

biological sample		Standard curve	R^2	linear range (ng ·mL^{-1}or μg/g)
Plasma	(±) verapamil	A=6999C +1458	0.9992	8.5-170
	S-(-) verapamil	A=81765C + 259453	0.9988	4-85
	R-(+) verapamil	A=81043C +341129	0.9985	4-85
heart	(±) verapamil	A=134479C+22251	0.9967	2-20
	S-(-) verapamil	A=57536C+14888	0.9983	1-10
	R-(+) verapamil	A=55544C+13969	0.9924	1-10
liver	(±) verapamil	A=29112C+17189	0.9989	10-50
	S-(-) verapamil	A=6018.4C+1772.8	0.9952	5-25
	R-(+) verapamil	A=5685.3C+3222.8	0.9947	5-25
spleen	(±) verapamil	A=35245C-12030	0.9964	2-20
	S-(-) verapamil	A=42114C+29376	0.9842	1-10
	R-(+) verapamil	A=42246C+25418	0.9833	1-10
kideny	(±) verapamil	A=35246C+5739	0.9997	2-20
	S-(-) verapamil	A=68530C-10983	0.9888	1-10
	R-(+) verapamil	A=67765C-12567	0.9682	1-10
cerebrum	(±) verapamil	A=33108C-5187.9	0.9878	1-10
	S-(-) verapamil	A=58177C+9858.3	0.9928	0.5-5
	R-(+) verapamil	A=56654C+10134	0.9946	0.5-5
cerebellum	(±) verapamil	A=31813C+1061.5	0.9993	0.2-1
	S-(-) verapamil	A=59974C+7812.6	0.9984	0.1-0.5
	R-(+) verapamil	A=58679C+7734.5	0.9985	0.1-0.5

Pharmacokinetics of verapamil in rats

The temporal profiles of concentration of verapamil and its enantiomers were fitted by a 3p87 pharmacokinetic program software in a two-compartmental model. The plasma concentration versus time curves (Fig. 3) and pharmacokinetic parameters (Table III) were obtained. The parameters of verapamil and its enantiomers were statistically analyzed by comparing their means with the t-test ($P<0.05$ and $P<0.01$).

In Table III, the maximum concentration (C_{max}) of *R*-(+)

Table II. Within-day and between-day RSDs and recoveries of racemic verapamil, *S*-(-) verapamil, and *R*-(+) verapamil in rat plasma under the same conditions above (n=5).

Added/ ng · mL^{-1}	Within-day			Between-day		
	Found/ ng · mL^{-1}	RSD/%	Recovery/ %	Found/ ng · mL^{-1}	RSD/%	Recovery/ %
VP						
17	17.2±0.3	3.6	100.9	16.5±0.8	11.7	97.0
85	89.3±1.4	3.9	104.8	86±4.5	12.9	101.2
170	156.5±0.6	1.0	92.3	165±6.1	11.7	97.1
S-VP						
8.5	8.8±2.7	2.7	102.3	8.5±2.1	8.4	99.4
42.5	44.0±6.0	4.4	103.5	41.9±3.6	13.3	98.7
85	78.7±3.4	3.5	92.6	80.5±6.4	10.2	94.7
R-VP						
8.5	8.4±3.7	2.3	98.2	8.1±3.6	10.6	94.7
42.5	45.3±4.3	2.0	106.5	44.1±4.6	8.9	103.6
85	78.0±4.4	3.4	91.8	84.5±6.7	11.4	99.4
Average		3.1	99.2		10.4	98.4

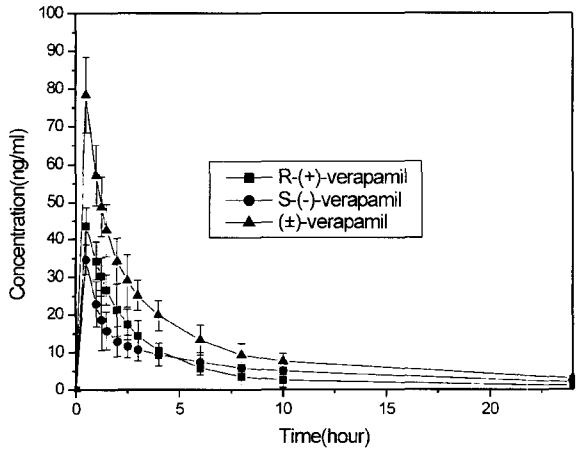

Fig. 3. The mean plasma concentration-time curves of verapamil racemate (▲), *R*-(+) verapamil *S*-(-) verapamil (■) and *S*-(-) verapamil (●) in rat after oral administration of verapamil hydrochloride tablet (20 mg · kg^{-1}, n=3) under the achiral and chiral chromatographic conditions above.

verapamil was 1.25 times as that of *S*-(-) verapamil, consistent with the fact that the area under the concentration-time curve (AUC) for *S*-(-) verapamil was 1.14 times of that obtained with *R*-(+) form. In addition, the time to reach the C_{max} (T_{max}) for the *S* form 1.47 times of that obtained for the *R*-(+) verapamil in rats. In addition, the half time for the distribution phase ($T_{1/2(\alpha)}$) for *R*-(+) verapamil was 2.35 times as that of *S*-(-) verapamil, but the apparent volume of distribution (V_d/F) of *S*-(-) verapamil was 1.11 times as that of *R*-(+) verapamil. The elimination half-time ($T_{1/2(\beta)}$) of *S*-(-) verapamil was 2.12 times as that of *R*-(+) verapamil,

Table III. Pharmacokinetics parameters of verampamil and its enantiomers in rats

parameters	(±) verapamil	*S*-(-) verapamil	*R*-(+) verapamil
C_{max} (ng · mL^{-1})	77.39±2.30	34.78±1.65	43.67±2.08
T_{max} (h)	0.45±0.1	0.53±0.12	0.36±0.14
AUC (ng · h · mL^{-1})	279.77±13.2	159.6±12.1	140.2±13.4
$T_{1/2(\alpha)}$ (h)	1.14±0.02	0.54±0.02	1.27±0.32
V_d/F (L · kg^{-1})	0.31±0.01	0.42±0.01	0.38±0.03
$T_{1/2(\beta)}$ (h)	2.93±0.21	5.68±0.26	2.68±0.34
$CL_{(s)}$/F (mL · min^{-1})	0.14±0.05	0.14±0.01	0.12±0.02

but apparent clearance ($CL_{(s)}$/F) of them was not different between the enantiomers. Therefore, the extent of absorption for *S*-(-) verapamil was apparently higher, the time of distributing to tissue from blood shorter and the elimination time longer than that of *R*-(+) verapamil, indicating that there were pharmacokinetic differences between verapamil enantiomers in rats.

Biodistribution in tissues

Biodistributions of verapamil and its enantiomers in major tissues, such as heart, cerebrum, cerebellum, liver, spleen, and kidney of rat were determined (Fig. 3). The total concentrations (TC) of racemic verapamil were different; For major tissues relevant in drug distribution: TC_{heart}>$TC_{cerebrum}$>$TC_{cerebellum}$, and for major eliminating organs: TC_{liver}>TC_{kidney}>TC_{spleen} at 1.3 h after oral administration of racemic mixture of verapmail. These observations indicated

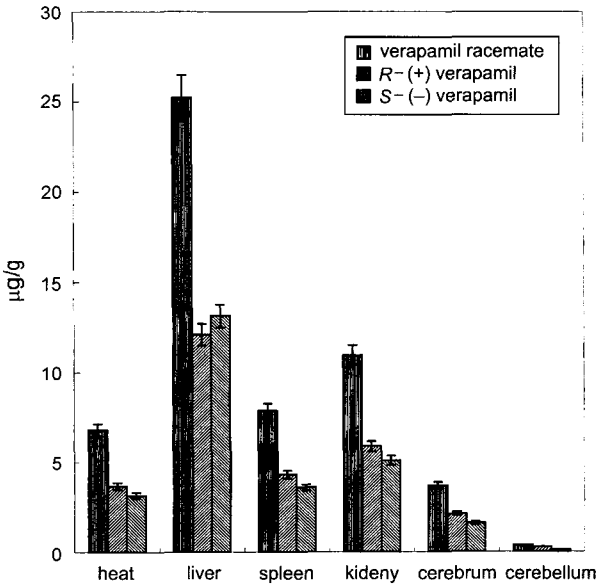

Fig. 4. The mean tissue distributions of racemic verapamil, *R*-(+) verapamil and *S*-(-) verapamil in rat heart, liver, spleen, kidney, cerebrum and cerebellum at 1.30 h after oral administration of verapamil hydrochloride tablet (20 mg · kg^{-1}, n=5).

that the primary organ of racemic verapamil distribution was heart and brain. For verapamil enantiomers, the concentrations of *R*-(+) verapamil were slightly higher than that of *S*-(-) verapamil in all the tissues except in liver, however, there were no significant differences between the enantiomers in all tissues at 1.3 h after oral the administration.

In summary, a high-performance liquid chromatographic method with a Chiralcel OJ column and fluorescence detection was used to study the differences in pharmacokinetic behavior and tissue distribution of verapamil enantiomers in rats. The adsorption, distribution and elimination of *S*-(-) verapamil in rat were apparently different from that of *R*-(+) verapamil. However, there were no significant differences between enantiomers in rat heart, brain, liver, kidney and spleen tissue concentrations at 1.30 h after oral administration.

ACKNOWLEDGEMENT

The authors thank the National Nature Science Foundation of China (39770856 and 30171080) for supporting this study.

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