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PHARMACOKINETICS OF THE ENANTIOMERS OF VERAPAMIL AFTER INTRAVENOUS AND ORAL ADMINISTRATION OF RACEMIC VERAPAMIL IN A RAT MODEL

M. MASOOD BHATTI AND ROBERT T. FOSTER*

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Canada

ABSTRACT

Verapamil is a chiral calcium channel blocking drug which is useful clinically as the racemate in treating hypertension and arrhythmia. The published pharmacokinetic data for verapamil enantiomers in the rat model are limited. Utilizing a stereospecific high-performance liquid chromatographic (HPLC) assay, the enantiomeric disposition of verapamil is reported after intravenous ($1{\cdot}0\,mg\,kg^{-1}$) and oral ($10\,mg\,kg^{-1}$) administration of racemic verapamil to the rat model. After intravenous administration the systemic clearance of R-verapamil was significantly greater than that of S-verapamil; $34{\cdot}9 \pm 7$ against $23{\cdot}7 \pm 3{\cdot}7\,mL\,min^{-1}\,kg^{-1}$ (mean ± SD), respectively. After oral administration, the clearance of R-verapamil was significantly greater than that of S-verapamil, 889 ± 294 against $351 \pm 109\,mL\,min^{-1}\,kg^{-1}$, respectively. The apparent oral bioavailability of S-verapamil was greater than that of R-verapamil, $0{\cdot}074 \pm 0{\cdot}031$ against $0{\cdot}041 \pm 0{\cdot}011$, respectively. These data suggest that the disposition of verapamil in the rat is stereoselective; verapamil undergoes extensive stereoselective first-pass clearance after oral administration and the direction of stereoselectivity in plasma is opposite to that observed in the human. ©1997 by John Wiley & Sons, Ltd.

KEY WORDS: verapamil enantiomers; rat; pharmacokinetics; bioavailability

INTRODUCTION

The calcium channel blocking drug verapamil is an effective agent in the treatment of supraventricular tachyarrhythmias, angina pectoris, and hypertension.[1] Verapamil is administered as a racemate; the two enantiomers differ considerably in their pharmacological potency with the S-enantiomer being 10–20 times more potent than the R-enantiomer in terms of negative dromotropic effect on atrioventricular conduction in man,[2] in the dog,[3] and in the rabbit.[4] The pharmacokinetics of verapamil enantiomers also differ from each other after intravenous (i.v.) and oral (p.o.) doses in both humans[5] and animals.[4,6–10] Although the kinetics of the individual enantiomers of verapamil in human

*To whom correspondence should be addressed at the Faculty of Pharmacy, University of Alberta, Edmonton, Alberta, Canada T6G 2N8.

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have been extensively studied, a review of the literature reveals that there is a scarcity of stereospecific studies of verapamil in animals.[4,10] In view of the increasing interest in interspecies scaling up of absorption[11,12] and disposition kinetics[13–15] and of the findings that the rat may serve as a good model for the human in p.o. absorption,[10] it was decided to study, in detail, the kinetics of verapamil enantiomers in male Sprague–Dawley rats.

Studies conducted on the kinetics of racemic verapamil in rats,[6,8,9] measuring racemic verapamil, indicated that, similar to the case in man, verapamil undergoes extensive hepatic metabolism with a high hepatic extraction ratio. Additionally, studies using the rat liver microsomal fraction[16] or isolated perfused rat liver[17] demonstrated stereoselectivity in different metabolic pathways of verapamil. However, the kinetics of the individual enantiomers of verapamil, after the administration of racemic verapamil in rats, are unknown. The major objective of the present investigation was to evaluate the kinetics of the verapamil enantiomers following i.v. and p.o. administration in the male Sprague–Dawley rat. These data will be used to contrast the disposition of verapamil to that observed in humans.

MATERIALS AND METHODS

Chemicals

Racemic verapamil hydrochloride was purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.). Internal standard, (+)-naproxen chloride (I.S.), was synthesized in our laboratory by reacting thionyl chloride with (+)-naproxen and purified by repeated recrystallization. Thionyl chloride and (+)-naproxen were obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Verapamil hydrochloride solutions (1·0 mg mL^{-1} for intravenous administration and 10 mg mL^{-1} for oral administration) were prepared by dissolving the drug in normal saline. All other chemicals and reagents were HPLC or analytical grade.

Surgery and animal maintenance

Male Sprague–Dawley rats weighing approximately between 200 and 300 g were used for the study. A total of 12 rats were catheterized with silastic tubing (0·025 in i.d. × 0·037 in o.d.; Dow Corning, Midland, MI, U.S.A.) at the right jugular vein. Immediately prior to surgery, rats were anaesthetized *via* intraperitoneal sodium pentobarbital (MTC Pharmaceutical Cambridge, Ontario, Canada). The animals were allowed to recover overnight prior to the experiment and were individually stored in 18 in × 9·5 in × 8 in polycarbonate rodent cages. The animals were fasted for at least 12 h before the administration of verapamil dose.

Drug administration and sample collection

One group of six rats received racemic verapamil i.v. and the other group of six rats received racemic verapamil p.o. dosage. Since the R- and S-verapamil were individually detected, this was a parallel group design study. Racemic verapamil dissolved in normal saline was administered, 0·5 mg kg^{-1} of each enantiomer *via* the jugular vein cannula in the case of i.v. administration and 5 mg kg^{-1} of each enantiomer *via* feeding tube in the case of p.o. administration. After administration of the verapamil dose, the cannula or the feeding tube was flushed with approximately 1·0 mL normal saline. Blood (0·25 mL) was collected from the jugular vein cannula just prior to, and at 0·033, 0·166, 0·33, 0·5, 0·75, 1, 1·5, 2, 3, and 5 h after, drug administration. Between each blood sample collection 0·2 mL normal (0·9%) saline was administered *via* the jugular vein cannula as fluid replacement, and the cannula was heparinized (10 U mL^{-1}). Blood samples were centrifuged and the plasma portion was separated and immediately frozen at $-20\,^{\circ}$C until analysed. Animals were given water *ad libitum* throughout the study and food was withheld only during the 2 h period immediately following drug administration.

Stereospecific HPLC analysis of verapamil

Concentrations of S- and R-verapamil in plasma were determined utilizing a stereospecific high-performance liquid chromatographic (HPLC) method developed in our laboratory.[18] Briefly, after the addition of I.S., plasma containing the verapamil was extracted at a basic pH. After evaporation of the organic layer, verapamil and I.S. were reconstituted in mobile phase and injected into the HPLC. The enantiomers were separated at ambient temperature using a single 4·6 mm × 250 mm amylose-carbamate-packed chiral column (ChiralPak AD) with hexane–isopropyl alcohol–triethylamine (90:9·9:0·1, v/v) as the mobile phase, pumped at 1·2 mL min^{-1}. The enantiomers of verapamil were then quantified by fluorescence detection using the wavelengths 272 and 317 nm for the excitation and emission respectively. The coefficient of variation of the assay for control plasma standards was 1·5–9·0%. The lower limit of sensitivity was 5 ng mL^{-1} of each enantiomer.

Pharmacokinetic drug analysis

The area under the plasma concentration–time curve ($AUC_{0-\infty}$) was calculated by the trapezoidal rule using a Lagran computer software program.[19] The area from the last concentration point (C_{last}) to infinity was calculated as C_{last}/λ_n, where λ_n was the terminal elimination rate constant, calculated by a least-squares regression line through data points in the terminal

elimination phase. Systemic clearance (Cl_s) was calculated as $D_{i.v.}/AUC_{0-\infty}$, where $D_{i.v.}$ was the enantiomeric dose administered i.v. and $AUC_{0-\infty}$ was the corresponding area under the plasma enantiomer concentration–time curve. Similarly, oral clearance (CL_o) was calculated as $D_{oral}/AUC_{0-\infty}$ where D_{oral} was the enantiomeric dose administered orally and $AUC_{0-\infty}$ was the corresponding area under the plasma enantiomer concentration–time curve. The apparent volume of distribution ($V_{d\beta}$) was calculated by dividing the corresponding Cl_s by λ_n. The absolute bioavailability (F) was calculated as $(dose_{i.v.}\,AUC_{oral})/(dose_{oral}\,AUC_{i.v.})$ and the extraction ratio (E) was calculated by subtracting F from one.

Statistical analysis

Comparisons between the S- and R-verapamil concentrations observed in rats administered the racemate were assessed utilizing a Student's t test for paired data. All t tests were two-tailed, with the level of significance pre-set at $\alpha = 0{\cdot}05$. Results are expressed as mean ± SD.

RESULTS

Intravenous pharmacokinetics

The mean plasma concentration–time profiles of R- and S-verapamil after i.v. administration of racemic verapamil are shown in Figure 1(a). The plasma concentration declined after drug administration in a biphasic fashion. At almost every time point the plasma concentration of R-verapamil was approximately 40–50% that of S-verapamil. The i.v. pharmacokinetic data derived from Figure 1 are summarized in Table 1. The systemic clearance of R-verapamil was on average 48% greater than that of S-verapamil (40·9 against 27·7 mL min^{-1} kg^{-1}). The apparent volume of distribution of R-verapamil was also significantly greater than that of S-verapamil (5·30 against 3·34 L kg^{-1}). The higher Cl_s and $V_{d\beta}$ of R-verapamil had a counteracting effect on the elimination half-life such that there were no differences between the enantiomers in this pharmacokinetic parameter.

Oral pharmacokinetics

The mean plasma concentration–time profiles of R- and S-verapamil after p.o. administration of racemic verapamil are shown in Figure 1(b) and the corresponding pharmacokinetic parameters are summarized in Table 2. As is apparent from Figure 1(b), the oral clearance of R-verapamil was of the order of 2·5 times that of S-verapamil, indicating that presystemic elimination of racemic verapamil is selective for the R-enantiomer. As was seen in the i.v.

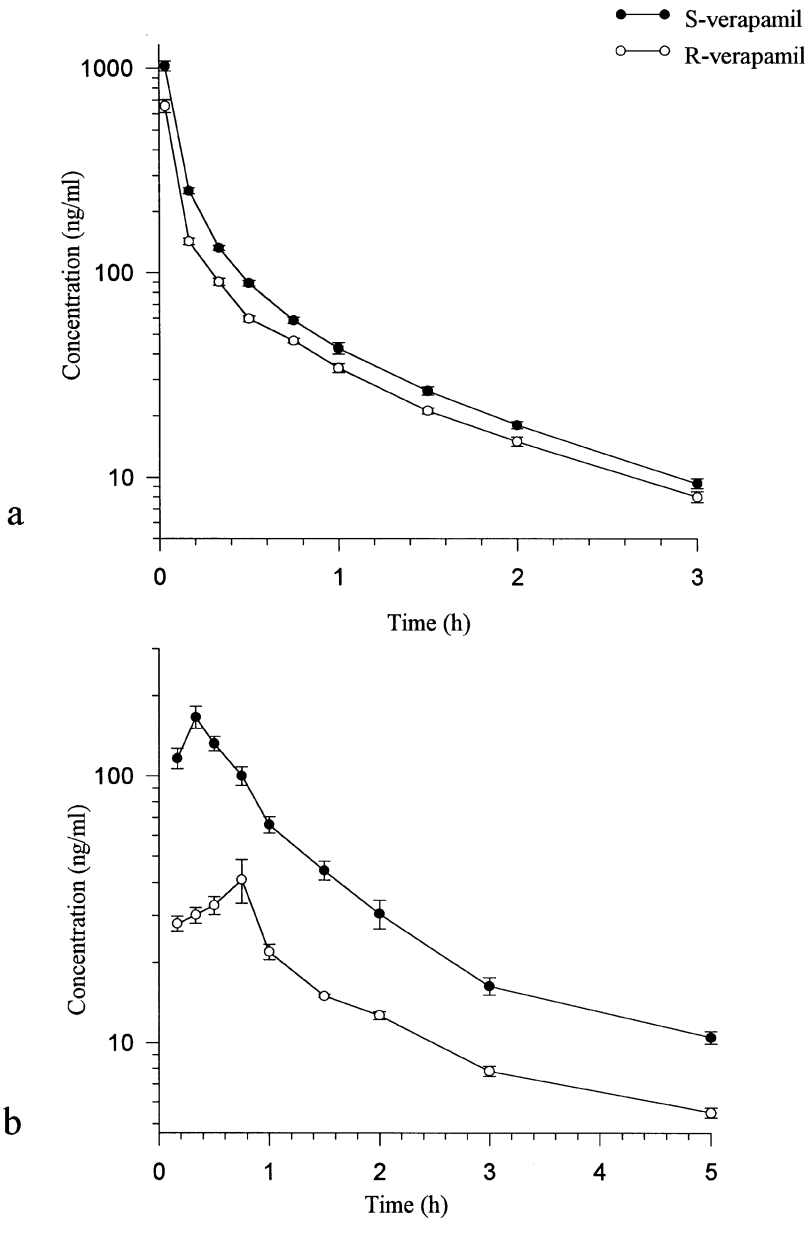

Figure 1. (a) The plasma concentration–time profile for enantiomers of verapamil after i.v. administration of racemic verapamil: ○, R-verapamil; ●, S-verapamil. Each point is the group mean ± SD. (b) The plasma concentration–time profile for enantiomers of verapamil after p.o. administration of racemic verapamil: ○, R-verapamil; ●, S-verapamil. Each point is the group mean ± SD

Table 1. Pharmacokinetic parameters of verapamil enantiomers after i.v. administration of racemic verapamil in rats. Data are expressed as mean ± SD

Rat	Weight (g)	$AUC_{0-\infty}$ ($\mu g\,h\,L^{-1}$)		Cl_t ($mL\,min^{-1}\,kg^{-1}$)		$t_{1/2}$ (h)		$V_{d\beta}$ ($L\,kg^{-1}$)	
		S	R	S	R	S	R	S	R
1	210	300	198	23·3	35·3	0·53	0·85	1·27	3·09
2	210	260	157	26·8	44·4	0·63	0·64	1·74	2·93
3	215	267	194	26·7	36·9	0·82	0·94	2·15	3·49
4	210	284	184	24·6	38·0	2·01	1·78	5·10	6·99
5	220	303	229	24·2	31·9	2·32	2·82	5·52	8·89
6	220	439	314	16·7	23·3	2·57	2·76	4·24	6·36
Mean	214	309[a]	212	23·7[a]	34·9	1·48	1·63	3·34[a]	5·30
SD	4·9	65·8	54·8	3·7	7·0	0·91	0·97	1·83	2·47

[a]Significantly different from corresponding antipode.

data, the elimination half-lives of the two enantiomers were similar, being around 1·7–2·4 h. The oral bioavailability of R-verapamil was 4·1%, while that of S-verapamil was 7·4%. The extraction ratio of R-verapamil was significantly greater than that of S-verapamil.

DISCUSSION

In this study, the pharmacokinetics and bioavailability of the enantiomers of verapamil were studied in the rat after single i.v. and p.o. dosing of racemic verapamil. The method used to quantitate the plasma concentration of the individual enantiomers of verapamil involved a stereospecific HPLC assay using a single chiral column.

Both the systemic and oral clearances of R-verapamil were significantly higher than those of S-verapamil in the rat. These findings are in contrast to the reports of the i.v.[20] and p.o.[21] kinetics of the verapamil enantiomers in man. After intravenous administration of a pseudoracemic mixture (50% dideuterated R-verapamil–50% unlabelled S-verapamil) in man, Eichelbaum *et al.*[20] found the systemic plasma clearance of S-verapamil was 80% greater than that of R-verapamil. In contrast, in the present study, using a rat model, the systemic clearance of the R-verapamil was 48% greater than that of S-verapamil. As the hepatic blood flow was the same for the both enantiomers, the higher systemic clearance for the R-verapamil reflects a higher extraction ratio. This was confirmed by the results of the p.o. dosing experiments, where the oral clearance, which reflects the intrinsic ability of the liver to clear the drug, of R-verapamil was over 2·5 times that of S-verapamil. Needless to say, the determination of hepatic intrinsic clearance in this manner assumes

Table 2. Pharmacokinetic parameters of verapamil enantiometers after p.o. administration of racemic verapamil in rats. Data are expressed as mean ± SD

	Weight	$AUC_{0-\infty}$ ($\mu g\,h\,L^{-1}$)		Cl_{oral} ($mL\,min^{-1}\,kg^{-1}$)		$t_{1/2}$ (h)		C_{max} ($ng\,mL^{-1}$)		t_{max} ($ng\,mL^{-1}$)		F		E	
Rat	(g)	S	R	S	R	S	R	S	R	S	R	S	R	S	R
1	215	204	90	352	792	0·95	2·29	212	47·2	20	20	0·066	0·044	0·933	0·955
2	215	359	79	200	907	1·31	1·95	286	42·2	20	20	0·134	0·048	0·865	0·951
3	215	213	51	328	1400	1·55	1·23	218	30·9	20	20	0·081	0·026	0·918	0·973
4	210	133	104	527	673	2·25	3·71	74	30·6	20	45	0·046	0·056	0·953	0·943
5	220	185	73	396	998	2·56	2·45	69	30·2	30	30	0·060	0·032	0·939	0·967
6	210	232	123	301	566	1·31	2·74	192	34·5	10	45	0·055	0·041	0·944	0·958
Mean	214	221[a]	87	351[a]	889	1·65	2·39	175[a]	35·9	20	30	0·074[a]	0·041	0·925[a]	0·958
SD	3·7	75	25	109	294	0·62	0·82	86	7·1	6·3	12	0·031	0·011	0·031	0·011

[a]Significantly different from corresponding antipode.

complete absorption from the gut. This was further reflected in the approximately twice as high oral bioavailability of S-verapamil over that of R-verapamil (7·5 against 4·1%). Man also shows a stereoselective presystemic uptake of verapamil.[20,21] However, in man the S-enantiomer presystemic uptake is greater (four to five times) than that of the R-enantiomer. The oral bioavailability (S-verapamil, $0{\cdot}074 \pm 0{\cdot}031$; R-verapamil, $0{\cdot}041 \pm 0{\cdot}011$) observed in the present study is comparable with hepatic availability values (S-verapamil, $0{\cdot}069 \pm 0{\cdot}030$; R-verapamil, $0{\cdot}046 \pm 0{\cdot}025$) reported by Mehvar *et al.*[17] in an isolated perfused rat liver study. These findings suggested that the liver is most likely responsible for stereoselective presystemic uptake of verapamil enantiomers in rat. Although, in an *in vitro* study, Koch and Palicharla[22] found that verapamil is also metabolized by intestinal flora in the rat caecal contents, it is somewhat doubtful that a significant portion of the dose reaches the colon, after the oral administration of verapamil solution.

In an *in vitro* study Nelson *et al.*[16] compared the verapamil metabolism between humans and rats using liver microsomal fraction from both species. In their study they found considerable similarities in the metabolic pathways of the two species and concluded that similar sets of cytochrome P-450 isozymes may be responsible for this biotransformation. They also suggested that the different stereoselectivities observed in the two species may be due to the structural differences in these enzymes and/or due to free fraction of the drug.

In addition to the stereoselective clearances, the apparent volume of distribution for the enantiomers of verapamil exhibited stereoselectivity in the rat. The volume of distribution values of R-verapamil were approximately 58% greater than those of S-verapamil. In man, the opposite situation occurs both qualitatively and quantitatively.[23] The physiological relationship that relates a drug's apparent volume of distribution to physiological volumes, the binding in plasma, and the binding in tissues, first proposed by Gillette,[24] is

$$V_d = V_p + V_t(f_p/f_t)$$

where V_p is the plasma volume (approximately 5% body weight), V_t is the tissue volume (approximately 55% body weight), and f_p and f_t are the free fractions in the plasma and tissues, respectively. Recent work done in our laboratory[25] and others[26] recounted a higher f_p value of R-verapamil than S-verapamil in rats. Based on this relationship, it is possible to speculate that the larger apparent volume of distribution of R-verapamil can be accounted for its higher free fraction in the plasma. Interestingly, the same rationale can be used to explain the higher volume of distribution for S-verapamil in the human,[27] where the f_p of S-verapamil is higher than those of R-verapamil.

The pharmacokinetics of enantiomers of verapamil have also been studied in dogs and rabbits.[4,10] Bai *et al.*[10] reported stereoselective disposition of verapamil in dogs and found that the Cl_s, Cl_o and $V_{d\beta}$ values of S-verapamil were greater than those of R-verapamil and that the oral bioavailability of

R-verapamil was 14 times greater than that of S-verapamil. These differences between the bioavailabilities of the enantiomers in dogs were much greater than that found (fourfold) in man. In contrast to the case in the human, dog and rat, Giacomini *et al*.[4] reported that the pharmacokinetics of verapamil enantiomers were not stereoselective in rabbits. In the latter study they also determined that there was no difference in the protein binding of the enantiomers of verapamil. These findings further support the notion that stereoselective plasma protein binding of verapamil enantiomers may be the origin of the differences in their apparent volumes of distribution.

In conclusion, the present study found that, similar to the case in the human, verapamil is stereoselectively metabolized after p.o. and i.v. administration by the rat and the apparent oral bioavailabilities of both enantiomers are low. The stereoselectivity in the systemic and presystemic clearance is of the same order of magnitude as in the human but the enantiomeric ratio differs. It is suggested that a likely explanation for the higher apparent volume of distribution of R-verapamil may be its lower plasma protein binding.

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