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Review

Stereoselective pharmacokinetics of dihydropyridine calcium antagonists

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Abstract

Many dihydropyridine calcium antagonists are widely used for the treatment of angina and hypertension, and many more are under development. Most of these drugs have one or more chiral centre, and the pharmacological activity between the enantiomers for these drugs is known to be markedly different. First, the stereospecific assay methods for these drugs in plasma or serum are reviewed with emphasis on chiral stationary phase high-performance liquid chromatography for their determination. Next, the stereoselective pharmacokinetics of these drugs (nilvadipine, nitrendipine, felodipine, nimodipine, manidipine, benidipine and nisoldipine) in animals, healthy subjects and patients with hepatic disease is reviewed. Enantiomer–enantiomer interaction, enantiomeric inversion and the stereochemical aspects of pharmacokinetic drug interactions in these drugs are also described.

Contents

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1. Introduction

Calcium antagonists are currently one of the major classes of cardiovascular drugs, and are valuable and widely used in the treatment of essential hypertension and angina. The development of calcium antagonists has been somewhat unusual, as the three agents that are now in extensive clinical use, verapamil (phenylalkyl amine derivative), diltiazem (benzodiazepine derivative) and nifedipine [1,4-dihydropyridine (DHP) derivative], are chemically unrelated, sharing only the property of blocking calcium slow channels, while otherwise producing disparate pharmacological effects [1,2]. Most of the research on new calcium antagonists during the last decade has principally concerned various derivatives based of the DHP structure; much less has been directed towards the discovery and development of new phenlyalklylamine and benzodiazepine derivatives. Many DHP calcium antagonists have been designed to modify both physico-chemical (photochemical stability, water solubility) and pharmacokinetic properties of the DHP structure while retaining the capacity to block selectively the potential-operated calcium channel. Except for nifedipine, the carbon in position 4 of the DHP ring of all these drugs exhibits chirality due to asymmetric ester moieties. Most of these drugs are used as a racemic mixture, and the pharmacological effects of the enantiomers of these drugs can be different. In nilvadipine, for example, the (*S*)-(+)-enantiomer is about 100 times more potent in relaxing potassium-induced contractions of isolated dog coronary arteries than the (R)-(−)-enantiomer. In the dihydropyridine derivative 202-719 [3,4], furthermore, the (+)-enantiomer is a pure inhibitor of calcium entry, whereas the (−)-enantiomer enhances calcium entry into cells. Thus its enantiomers show opposite actions.

The active processes which are receptor, enzyme or binding dependent, such as renal and biliary secretion, protein binding or drug metabolism, could all be expected to show stereoselectivity. In fact, stereoselectivity in the pharmacokinetics of many drugs has been reported and reviewed [5–12]. Likewise, the pharmacokinetics of racemic DHP calcium antagonists in animals and man have been extensively described and reviewed [13–15]. The common characteristics of these drugs with the exception of amlodipine include extensive first-pass hepatic extraction after oral administration, high clearance and extensive binding to plasma proteins, but stereoselective pharmacokinetic data were not available for these drugs.

In 1987, Tokuma et al. [16] first reported the stereoselective pharmacokinetics in man of a DHP calcium antagonist, nilvadipine. It was shown that the plasma concentrations of the pharmacological more potent (*S*)-enantiomer were about three times higher than those of the (*R*)-enantiomer. Thereafter, the stereochemical aspects of the pharmacokinetics of nilvadipine and other DHP calcium antagonists were extensively studied. This review attempts to bring up to date the information on the stereospecific assay methods for the plasma or serum and the stereoselective pharmacokinetics in animals and man of these drugs. The structures of the selected DHP calcium antagonists covered in this review are shown in Fig. 1. The criteria for inclusion is that data on stereoselective pharmacokinetics in man are available.

2. Analytical methods

2.1. Chiral stationary phase high-performance liquid chromatography

Chiral resolution of enantiomers by high-performance liquid chromatography (HPLC) is generally approached in three ways [17,18]: (1) derivatization to corresponding diastereomers using a chiral reagent, followed by achiral (conventional) stationary phase chromatography; (2) addition of chiral reagent to the mobile phase, followed by achiral stationary phase chromatography; (3) separation of the enantiomers on a chiral stationary phase (CSP). Many kinds of CSP columns are now commercially available and they have rapidly become major tools in the analysis of enantiomers [18–20]. It is now possible to separate the anantiomers of DHP cal-

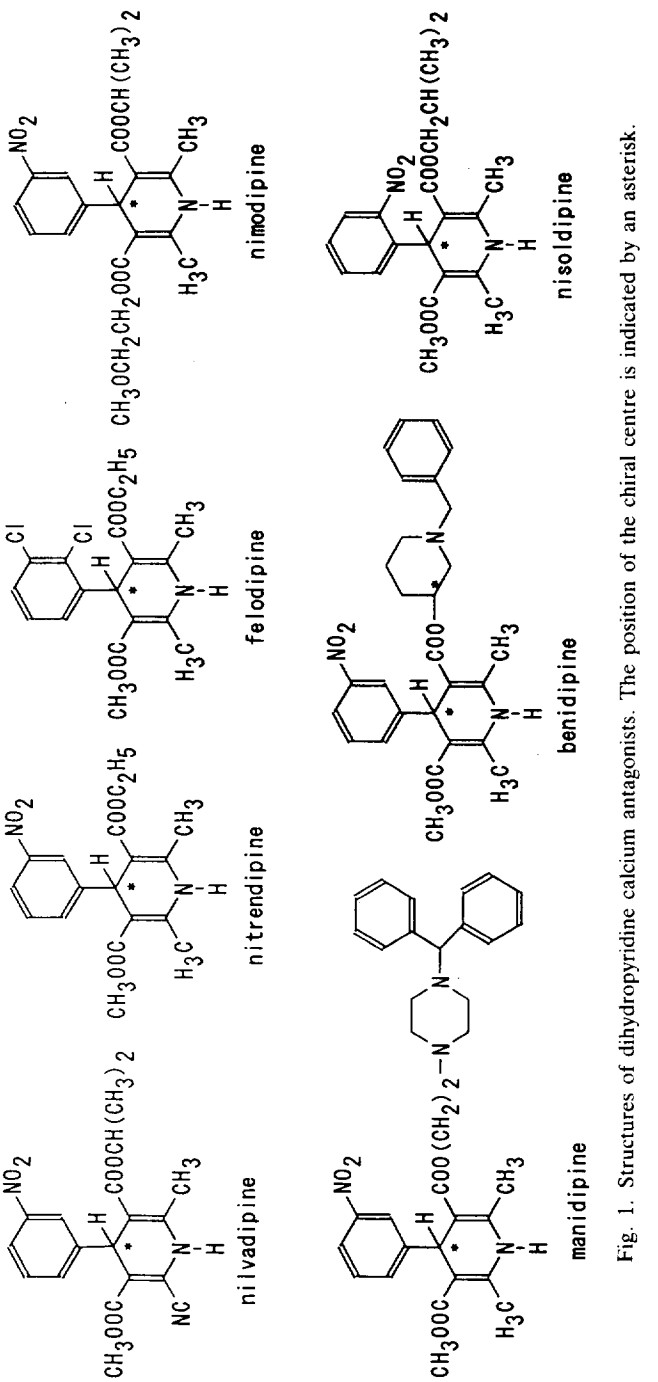

Fig. 1. Structures of dihydropyridine calcium antagonists. The position of the chiral centre is indicated by an asterisk.

cium antagonists with acid glycoprotein [21], polysaccharide phenylcarbamates [22–24] and Pirkle-type [25] CSP-HPLC. Nakagawa et al. [26] used a CSP column for the stereospecific determination of nilvadipine in dog plasma, with a limit of determination of 1 ng/ml (Table 1). However, the limit of quantification was insufficient to assess the kinetic parameters in man, because the concentrations of DHP calcium antagonists in man would be low in relation to the dose applied (2–40 mg), its rapid presystemic elimination and its high apparent volume of distribution [13–15]. Gas chromatography–mass spectrometry (GC–MS) with detection in the electron-capture negative-ion chemical ionization (NICI) mode and gas chromatography–electron capture detection (GC–ECD) using a capillary column have the sensitivity required to obtain the values necessary for human pharmacokinetic studies because concentrations as low as 0.01–0.4 ng/ml can be measured with DHP compounds that possess a phenyl group with a high electron affinity substituent (NO_2, Cl, etc.), as in nilvadipine [27,28], nitrendipine [29], felodipine [30], nimodipine [31,32] and nicardipine [33].

Tokuma et al. [16] were the first to report the off-line measurement of a DHP calcium antagonist, nilvadipine, which was separated into enatiomers with a CSP-HPLC system and subsequently quantified by GC–NICI-MS. A HPLC stationary phase that consisted of a chiral poly(triphenylmethyl methacrylate)polymer [Chiralpak OT(+)] was used. This method has also been applied to the sensitive stereospecific determination of nimodipine [34] (Table 1). Soons et al. [35] applied CSP-HPLC combined with GC–ECD to the stereospecific determination of DHP calcium antagonists. A variety of racemic drugs such as nitrendipine and felodipine could be separated and the method applied to serum. This method has also been applied to the stereospecific determination of benidipine [36]. Another successful application of CSP-HPLC combined with sensitive column-switching HPLC [37,38] to the stereospecific determination of manidipine in human serum was reported by Yamaguchi et al. [39]. These methods using CSP-HPLC would be very useful in the study of the pharmacokinetics, metabolism and relationships between kinetics and effects of the enatiomers, as there is no need to synthesize and administer a racemate in which one enantiomer is labelled with a stable isotope (so-called pseudo-racemate) or pure enatiomers.

Table 1
Stereospecific determination for dihydropyridine calcium antagonists in plasma or serum

Drug	Method	Sensitivity (ng/ml)	Ref.
Nilvadipine	Chiral HPLC and off-line GC–NICI–MS	0.025	16
	Chiral HPLC	1	26
Nitrendipine	Chiral HPLC and off-line GC–ECD	0.2	57
	Pseudo-racemate and GC–NICI-MS	0.3	40
Felodipine	Chiral HPLC and off-line GC–ECD	0.1	36
	Pseudo-racemate and GC–NICI-MS	0.3	41
Nimodipine	Chiral HPLC and off-line GC–NICI-MS	0.1	34
Manidipine	Chiral HPLC and off-line HPLC	0.2	39
Benidipine	Chiral HPLC and off-line GC–ECD	–[a]	36
Nisoldipine	Pseudo-racemate and GC–NICI-MS	–[a]	42

[a]Data not reported.

2.2. Stable isotope technique

The use of individual enantiomers in the study of the pharmacokinetics and metabolism of the enantiomers has limitations. Because of both pharmacodynamic and pharmacokinetic enantiomer–enantiomer interactions, the disposition may be different after administration of the individual enantiomers and racemate [8,9,11]. However, by using a pseudo-racemate in combination with GC–MS, an unequivocal allocation of the respective enantiomers and metabolites formed from the respective enantiomers is possible. This technique relies on the detection of the mass difference between the isotope-labelled and unlabelled drug by MS. This method has been used in the stereospecific determination of nitrendipine [40], felodipine [41] and nisoldipine [42] in plasma or serum (Table 1).

The use of drugs labelled with stable isotopes in combination with GC–MS measurement has been proved to be a powerful tool for simultaneously assessing the disposition and bioavailability of a drug that is administered at same time either by different routes [intravenous (i.v.) versus oral] or two different preparations using the same route of administration (tablet versus solution). This method has been successfully used in both absolute and relative bioavailability studies [43]. Fischer et al. [44] investigated whether a stable labelled drug together with an internal standard labelled differently could be used to combine the two techniques CSP-HPLC and GC–MS, and allow the simultaneous administration of racemic labelled drug i.v. and the racemic unlabelled drug orally. This combination allowed the simultaneous assessment of the absolute bioavailability of the enantiomers. The authors have performed studies with the two DHP calcium antagonists nimodipine and nitrendipine.

3. Species differences in stereoselective pharmacokinetics

Tokuma et al. [16,45] examined the pharmacokinetics of nilvadipine enantiomers in healthy subjects, dogs and male and female rats after an oral administration of racemic nilvadipine (Table 2), and showed that AUC and C_{max} were higher

Table 2
Pharmacokinetic parameters of (*S*)- and (*R*)-nilvadipine after oral administration of racemic nilvadipine

Species	Dose		C_{max} (ng/ml)	t_{max} (h)	AUC (ng h/ml)	CL_o (ml/min · kg)	$t_{1/2}$ (h)
Man	6 mg	(*S*)-Nilvadipine	4.11 ± 1.41	1.0 ± 0.0	11.8 ± 2.3	75.5 ± 12.2	4.21 ± 0.53
		(*R*)-Nilvadipine	1.52 ± 0.53	1.0 ± 0.0	3.95 ± 0.87	229 ± 36	3.58 ± 0.21
		S/*R* ratio	2.78 ± 0.28	1.0 ± 0.0	3.07 ± 0.30	0.34 ± 0.04	1.16 ± 0.09
Dog	1 mg/kg	(*S*)-Nilvadipine	102 ± 23	0.5 ± 0.0	392 ± 50	22.1 ± 2.3	6.46 ± 1.01
		(*R*)-Nilvadipine	34.6 ± 9.1	0.5 ± 0.0	105 ± 16	84.8 ± 12.1	7.44 ± 0.87
		S/*R* ratio	3.13 ± 0.32	1.0 ± 0.0	3.83 ± 0.35	0.27 ± 0.03	0.85 ± 0.05
Male rat	10 mg/kg	(*S*)-Nilvadipine	14.1	0.5	24.8	3360	–[a]
		(*R*)-Nilvadipine	23.6	0.25	41.8	1990	–[a]
		S/*R* ratio	0.60	2.0	0.59	1.69	
Female rat	10 mg/kg	(*S*)-Nilvadipine	144	0.25	264	316	2.78
		(*R*)-Nilvadipine	159	0.25	276	302	2.87
		S/*R* ratio	0.91	1.0	0.96	1.05	0.97

Values are means ± S.E. ($n = 4$).
[a]Could not be determined.

for more potent (*S*)-(+)-nilvadipine in man and dogs, were lower for the (*S*)-enantiomer in male rats and were almost equal for the (*S*)- and (*R*)-enantiomers in female rats. The *S*/*R* ratios of the apparent oral clearance (CL_o) were 0.34, 0.27, 1.69 and 1.05 in man, dogs, male rats and female rats, respectively. The values of $t_{1/2}$ in all species were similar for the (*S*)- and (*R*)-enantiomers. Thus, the stereoselective disposition of nilvadipine was species-dependent and sex-related in rats.

Assuming complete absorption and only liver elimination in the case of nilvadipine [46,47], CL_o based on plasma concentration is represented by the product of free fraction of plasma protein binding (f_p) and intrinsic hepatic clearance of free drug [48]. In other words, stereoselectivity of the CL_o is ascribed to the stereoselective differences in plasma protein binding and/or liver metabolism, and therefore stereoselectivity for the plasma protein binding and pyridine formation in the liver microsomes of nilvadipine have been examined [49,50]. The aromatization of nilvadipine and other DHP calcium antagonists to the correspond pyridine has been consistently reported as a primary metabolic step [14,47,51]. In dogs, because the f_p of (*S*)-nilvadipine was only about half that of the (*R*)-enantiomer, the stereoselectivity in plasma protein binding would seem to contribute at least partially to the stereoselective difference in the CL_o. However, the *S*/*R* ratios of the f_p values in man and male and female rats were close to unity. Kinetic parameters for oxidation reaction of (*S*)- and (*R*)-nilvadipine to the pyridine analogue by liver microsomes are shown in Table 3. When the substrate concentration is far below K_m, intrinsic hepatic clearance is proportional to V_{max}/K_m [52,53]. The *S*/*R* ratios of V_{max}/K_m in man, dogs, male rats and female rats were 0.49, 0.69, 1.59 and 1.23, respectively. The *S*/*R* ratios of the product of f_p and V_{max}/K_m were 0.54, 0.35, 1.81 and 1.21, respectively; these ratios were similar to those of CL_o in vivo. From these results, it is speculated that the differences between (*S*)- and (*R*)-nilvadipine in CL_o for man and male rats are mainly due to the differences in the pyridine formation in the liver microsomes, whereas the difference in dogs is due both to the difference in pyridine formation in the liver microsomes and plasma protein binding. Eriksson et al. [54] have also shown that the oxidative metabolism of felodipine is stereoselective in rats, dogs and human liver microsomes. This difference is expected to give a higher bioavailability of the more potent (*S*)-enantiomer in man, whereas the opposite is expected for rats and dogs. This prediction agreed with in vivo observations in dogs [41] and man [55]. These findings for nilvadipine and felodipine

Table 3
Kinetic parameters for oxidation reaction of (*S*)- and (*R*)-nilvadipine to its pyridine analogue by liver microsomes

Species		V_{max} (nmol/mg protein · min)	K_m (μM)	V_{max}/K_m (ml/mg protein · min)	*S*/*R* ratio for V_{max}/K_m
Man (A,B,C)	(*S*)-Nilvadipine	0.927,0.423, 4.71	19.6,40.6, 32.7	0.0473,0.0104, 0.144	0.49 ± 0.03
	(*R*)-Nilvadipine	0.996,0.392, 4.46	10.5,16.1, 16.8	0.0949,0.0243, 0.265	(0.50,0.43,0.54)
Dog	(*S*)-Nilvadipine	3.02 ± 0.15	21.9 ± 1.3	0.139 ± 0.012	0.69 ± 0.2
	(*R*)-Nilvadipine	2.45 ± 0.15	12.2 ± 0.1	0.201 ± 0.012	
Male rat	(*S*)-Nilvadipine	7.48 ± 0.29	11.2 ± 0.2	0.668 ± 0.021	1.59 ± 0.12
	(*R*)-Nilvadipine	3.37 ± 0.04	8.06 ± 0.72	0.426 ± 0.041	
Female rat	(*S*)-Nilvadipine	0.392 ± 0.017	2.99 ± 0.16	0.131 ± 0.003	1.23 ± 0.10
	(*R*)-Nilvadipine	0.222 ± 0.020	2.10 ± 0.28	0.108 ± 0.009	

Values are individual values for man or means ± S.E. for dogs and rats ($n = 3$).

illustrate that in vitro data are useful for predictions or explanations of in vivo observations.

4. Stereoselectivity in human pharmacokinetics

The pharmacokinetics of nilvadipine enantiomers after oral administration of 6 mg of racemic nilvadipine to four healthy subjects have been studied (Fig. 2) [16]. The plasma levels of (*S*)- and (*R*)-nilvadipine in four subjects peaked at 1 h, and the C_{max} and *AUC* of the more potent (*S*)-enantiomer were 2.0–3.2 and 2.4–3.6 times, respectively, higher than those of its optical antipode. The $t_{1/2}$ values were mostly similar. Similar $t_{1/2}$s of the enantiomers regardless of the differential CL_o values have been reported with most DHP antagonists [34,36,42,55–57].

Soons and Breimer [57] examined the pharmacokinetics of nitrendipine enantiomers after 40 μg/kg i.v. and 20 mg oral administration of racemic nitrendipine to healthy subjects. Upon oral administration the bioavailability (*F*) of the more potent (*S*)-enantiomer (13%) was 75% higher than that of the (*R*)-enantiomer (7.9%). The *AUC* and C_{max} for the (*S*)-enantiomer were 90% and 77% higher, respectively, than those for the (*R*)-enantiomer. After i.v. administration, the *AUC* was slightly (7%) higher than that of the (*R*)-enantiomer, but $t_{1/2}$ and V_{dss} were similar. Thus, the stereoselective disposition of nitrendipine in man was dosing route dependent, as reported with high clearance and all hepatically eliminated drugs such as verapamil [58] and propranolol [59]. This is in line with theories on the hepatic elimination of high-clearance drugs [60,61]. A difference in stereoselective pharmacokinetics according to dosing route has also been reported with nisoldipine in man [42] and nilvadipine in dogs [45].

For DHPs of which the stereoselective pharmacokinetics in man and absolute configuration have been reported, the stereoselectivity of plasma concentrations after oral administration of racemate and pharmacological activity are summarized in Table 4. For DHPs (nilvadipine, nitrendipine, felodipine, manidipine and benidipine) which have a methyl ester moiety (Fig. 1), the (*S*)-enantiomers were pharmacologically more potent than the (*R*)-enantiomers and the plasma or serum concentrations of the (*S*)-enantiomers were higher than those of the (*R*)-enantiomers. Although nisoldipine has a methyl ester moiety and the *AUC* of the more potent (+)-enantiomer is reported to be 6.3 times higher than that of (−)-enantiomer [42], data on its absolute configuration are not available. For nimodipine, which has no methyl ester moiety, the (*S*)-enantiomer was pharmacologically more potent but its concentrations were lower. The similarity for stereoselective pharmacokinetics in man of DHP calcium antagonists except for nimodipine suggests that there are identical or closely related rate-limiting steps in the metabolism. The similarity can be explained by the rate-limiting of a single cytochrome P-450 en-

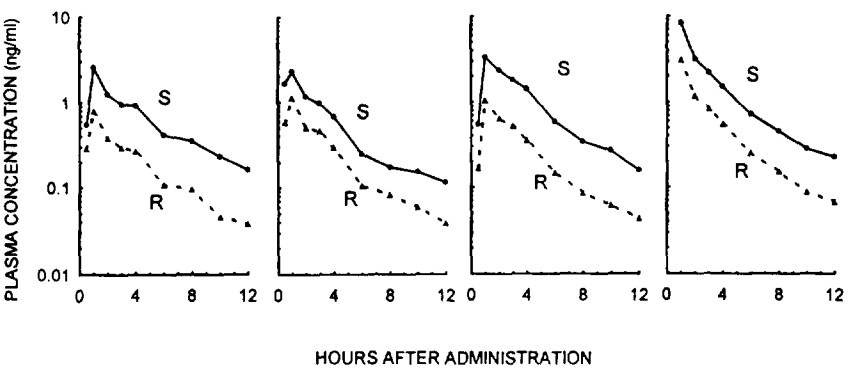

Fig. 2. Plasma concentrations of (*S*)- and (*R*)-nilvadipine in healthy subjects after oral administration of 6 mg of racemic nilvadipine.

Table 4
S/R ratios for plasma or serum enantiomer concentrations in man after oral administration of racemate and pharmacological activity in dihydropyridine calcium antagonists

Drug	Plasma or serum concentration	Pharmacological activity	Ref.
Nilvadipine	3.1 (*AUC*)	100	16
Nitrendipine	1.9,2.2 (*AUC*)	8,30	55,57,62,63
Felodipine	2.4 (*AUC*)	3–5,12–13	41,55,63
Nimodipine	0.15 (*AUC*)	5	34,64,81
Manidipine	2 (concentration)	30–80	56,65
Benidipine	1.9 (C_{max})	30–100	36

zyme in vivo and similar stereoselectivity of the enzyme for these structurally related drugs. Experiments in vitro confirm the presence of a single human cytochrome P-450 enzyme (P-450 IIIA4) which has by far the highest activity towards most if not all DHP calcium antagonists [66–68].

5. Inter-individual variability in stereoselective pharmacokinetics

Most clinically used DHP calcium antagonists exhibit wide inter-individual variability in pharmacokinetics after oral administration [69–72], which is caused by extensive and variable presystemic elimination in the liver [73,74]. Wide inter-individual variability in the ratio between plasma levels of the enantiomers has been reported in alprenolol [75] and mephenytoin [76]. For mephenytoin, stereoselective disposition kinetics have been reported to be related to the oxidation phenotype. The subjects in this study were phenotyped as extensive metabolizers (EM) or poor metabolizers (PM) of mephenytoin. In the EM group, the CL_0 of (*S*)-mephenytoin was about 170 times that of (*R*)-mephenytoin, whereas in the PM group the CL_0 of the enantiomers were apparently similar. Tokuma et al. [77] examined the plasma levels of the enantiomers 1 h (at or near C_{max}) after oral administration of 4 mg of racemic nilvadipine to sixteen healthy subjects. The variability in the *S/R* ratios [relative standard deviation (R.S.D.) = 21%] of enantiomer concentrations was much less than in the plasma concentration of the enantiomers [R.S.D. = 61% and 66% for the (*S*)- and (*R*)-enantiomers respectively]. Soons et al. [55] investigated the stereoselective pharmacokinetics of the administration of 20 mg each of felodipine and nitrendipine in a randomized cross-over study in twelve healthy subjects. The variability in the *S/R* ratio for plasma concentrations of felodipine enantiomers (R.S.D. = 12%) and nitrendipine enantiomers (R.S.D. = 30%) was much less than the variability in the *AUC* of felodipine enantiomers [R.S.D. = 45% for the (*S*)- and (*R*)-enantiomers] and nitrendipine enantiomers (R.S.D. = 80% and 65%). For these DHP calcium antagonists, the variability in stereoselectivity therefore represents only a minor contribution to the observed variability in the pharmacokinetics of racemates, and is only a minor contributor to the variability in the plasma concentrations of the active enantiomers.

6. Enantiomer–enantiomer interaction

Nakagawa et al. [26] studied the pharmacokinetics of (*S*)- and (*R*)-nilvadipine in six beagle dogs following oral administration of 0.5 mg/kg of (*S*)-, 0.5 mg/kg of (*R*)- and 1 mg/kg of racemic nilvadipine (Fig. 3). After administration of each enantiomer, a pronounced difference in *AUC*s (*S*, 270; *R*, 70.7 ng h/ml) was observed between the (*S*)- and (*R*)-enantiomers. When the racemate was given, the *AUC*s (*S*, 234; *R*, 78.0 ng h/ml) of the (*S*)- and (*R*)-

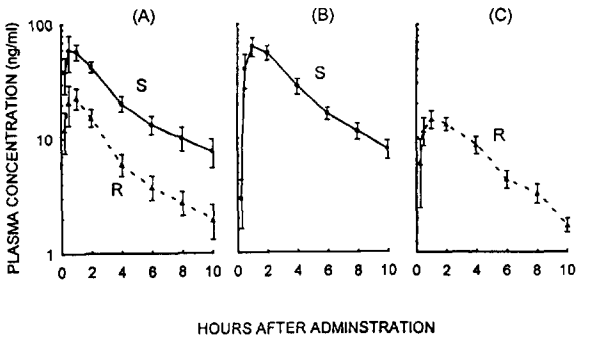

Fig. 3. Plasma concentrations of (*S*)- and (*R*)-nilvadipine in dogs after oral administration of (A) 1.0 mg/kg of racemic, (B) 0.5 mg/kg of (*S*)- and (C) 0.5 mg/kg of (*R*)-nilvadipine. Values are means ± S.E.

enantiomers were not different from the values after administration of either enantiomer, suggesting that there is no enantiomer–enantiomer interaction for the pharmacokinetics of nilvadipine enantiomers in dogs. The $t_{1/2}$ and t_{max} values were not different between the enantiomers, nor were the values different after administration of each enantiomer or racemate. The antipode was not detected in plasma after oral administration of each enantiomer, which indicates that enantiomeric inversion does not occur. Experiments involving the administration of separate enantiomers of nitrendipine to man [40] and of manidipine to dogs [39] also did not reveal any sign of their inter-conversion.

Mast et al. [40] have reported the pharmacokinetics of (*S*)- and (*R*)-nitrendipine in six healthy subjects following random oral administration of 20 mg each of (*S*)-, (*R*)- and racemic nitrendipine {pseudo-racemic mixture of 10 mg of unlabelled (*S*)- and 10 mg of [$^{13}C_4$]-(*R*)-enantiomer}. After administration of each enantiomer, pronounced differences in the *AUC* (*S*, 123.8; *R*, 29.9 ng h/ml), *F* (*S*, 44.6; *R*, 10.7%) and C_{max} (*S*, 72.5; *R*, 14.4 ng/ml) were observed between the (*S*)- and (*R*)-enantiomers. When the racemate was given, the *F* and dose-normalized *AUC* and C_{max} values for the (*S*)-enantiomer were not different from the values after administration of the (*S*)-enantiomer. In contrast, the *F* (*R*, 10.7%; racemate, 22.1%) and dose-normalized *AUC* (*R*, 15.0; racemate, 29.5 ng h/ml) and C_{max} (*R*, 7.2; racemate, 16.8 ng/ml) of the (*R*)-enantiomer were doubled following administration of the racemate as compared with the (*R*)-enantiomer. The $t_{1/2}$ and t_{max} values were not different between the enantiomers, nor were the values different after administration of each enantiomer or racemate. The *F* and dose-normalized *AUC* of (*R*)-nitrendipine doubled after administration of the racemate as compared with (*R*)-nitrendipine, suggesting a metabolic enantiomer–enantiomer interaction with the (*S*)-enantiomer acting as an inhibitor of (*R*)-nitrendipine metabolism.

7. Stereoselective pharmacokinetics in hepatic disease

The DHP calcium antagonists are high-clearance drugs and metabolized almost entirely by the liver [13–15,47,78,79]. Hepatic cirrhosis, with its decreases of functional liver mass and reduction of hepatic blood flow, would be expected to be associated with changes in the pharmacokinetics of these drugs. As first-pass metabolism is a major characteristic of the DHP calcium antagonists, a decrease in this factor in hepatic disease would be expected to alter their oral bioavailability, and result in higher plasma concentrations and lower CL_o. Various studies have confirmed that chronic cirrhosis can influence the elimination of racemic DHP calcium antagonists, although other chronic or acute liver diseases are associated with no or only slight effects on pharmacokinetics [15,80].

Two studies of the stereoselective pharmacokinetics of DHP calcium antagonists in patients with liver cirrhosis have been reported. Fisher et al. [81] studied the absolute bioavailability of racemic nimodipine in eight patients with liver cirrhosis (2 mg of the stable isotope-labelled racemic analogue simultaneously administered i.v. with 30 mg orally), and found that F was 3.6% for the (*S*)-enantiomer and 20.3% for the (*R*)-enantiomer in healthy subjects and 42.1% for the (*S*)-enantiomer and 67.3% for the (*R*)-enantiomer in the patients. These results revealed a dramatic increase in *F*

in patients with liver cirrhosis. The CL_o values of the enantiomers were significantly reduced and consequently the AUCs were significantly greater when compared with the healthy subjects in the control group. The stereoselectivity of CL_o in these patients was reduced to an S/R ratio of 1.8 compared with 6.9 in healthy subjects; the $t_{1/2}$ values were increased about 1.5-fold. In the other study, Mettang et al. [82] investigated the stereoselective disposition of nitrendipine in six patients with liver cirrhosis and six age-matched healthy subjects who received 20-mg of racemic nitrendipine. CL_o was 3.6 l/min for the (*S*)-enantiomer and 9.6 l/min for the (*R*)-enantiomer in the healthy subjects, and 0.72 l/min for the (*S*)-enantiomer and 1.33 l/min for the (*R*)-enantiomer in the patients. The stereoselectivity of CL_o in the patients was reduced to an R/S ratio of 1.8 compared with 2.7; the $t_{1/2}$ values were increased about twofold. Thus, liver cirrhosis had a profound impact on the pharamacokinetics and stereoselectivity of nimodipine and nitrendipine. In the case of racemic drugs with extensive stereoselective first-pass metabolism such as DHP calcium antagonists, the aspect of altered stereoselective disposition has to be considered.

8. Stereochemical aspects of pharmacokinetic drug interaction

Cimetidine is a well known inhibitor of the microsomal cytochrome P-450 mixed-function oxidase system [83], whereas certain flavonoids present in grapefruit juice have also been shown to inhibit cytochrome P-450 IIIA4 activity [84]. P-450 IIIA4 catalyzes the oxidation of DHP ring to form the corresponding pyridine metabolite [66–68]. This oxidation is a predominant metabolic step that determines the extent of first-pass extraction of DHPs [14,47,51]. The interaction with cimetidine has been reported for most racemic DHP calcium antagonists such as nilvadipine, nitrendipine, felodipine, nifedipine, nimodipine and nisoldipine [13–15,85–87]. The interaction with grapefruit juice has also been reported for racemic nitrendipine, felodipine, nifedipine and nisoldipine [88–91]. A few studies with cimetidine have been published in which the stereochemical aspects of pharmacokinetic interactions with verapamil, metoprolol, warfarin and flurbiprofen were evaluated [92–95]. Soons et al. [96] investigated the effects of grapefruit juice (150 ml at −15, −10, −1/4, +5 and +10 h) and cimetidine (200 mg at the same times) on the stereoselective pharmacokinetics of oral administration of 20 mg of racemic nitrendipine in a placebo-controlled crossover study in nine healthy subjects. In all subjects the AUC of racemic nitrendipine was increased by grapefruit juice (by 106%) and cimetidine treatment (by 154%). Comparable results were obtained for the C_{max} of the racemic drug and for the AUC and C_{max} of (*S*)- and (*R*)-nitrendipine. There was a highly significant difference in the AUC and C_{max} between the enantiomers within all treatments: grapefruit juice had no effect on this stereoselectivity, but cimetidine increased the S/R ratio of the AUC (2.25) by 20% compared with placebo treatment (1.89). The $t_{1/2}$ and t_{max} values were not different within and between treatments. Cimetidine treatment resulted in a small but significant increase in the S/R ratio of AUC, indicating a more pronounced inhibition of presystemic metabolism of the (*S*)-enantiomer. Grapefruit juice did not show this effect.

9. Conclusions

The stereoselective pharmacokinetics of DHP calcium antagonists (nilvadipine, nitrendipine, felodipine, nimodipine, benidipine and nisoldipine) in animals and man have been extensively reported, and is reviewed. The stereoselective pharmacokinetics of nilvadipine have been studied by giving its racemate to man, dogs and male and female rats. After oral administration to man and dogs, the AUCs of the pharmacologically more potent (*S*)-enantiomer were 3–4 times those of the (*R*)-enantiomer, whereas in male and female rats the AUCs of the (*S*)-enantiomer were 0.59 and 0.96 times those of the (*R*)-enantiomer, respectively. Thus the stereoselective pharmacokinetics of nilvadipine

was species-dependent and sex-related in rats. The stereoselectivity of in vivo kinetics was explained by the stereoselectivity of the aromatization of nilvadipine to the corresponding pyridine in the liver microsomes and plasma protein binding. For DHPs (nilvadipine, nitrendipine, felodipine, manidipine and benidipine), which have a methyl ester moiety, these (*S*)-enantiomers were pharmacologically more potent than the (*R*)-enantiomers and the plasma or serum concentrations of the (*S*)-enantiomers after oral administration to man were higher than those of the (*R*)-enantiomers. Similarity of the stereoselectivity of pharmacokinetics in man and the pharmacological activity of DHP calcium antagonists was observed. Most clinically used DHP calcium antagonists exhibit wide inter-individual variability in pharmacokinetics after oral administration of racemates. The variability in the *S*/*R* for plasma enantiomer concentrations in man of nilvadipine, nitrendipine and felodipine was much less than the variability in the plasma concentration of the enantiomers. After oral administration of racemic nitrendipine to man, the plasma concentrations of the (*S*)-enantiomer were not different from those after administration of the (*S*)-enantiomer alone. In contrast, the plasma concentrations of the (*R*)-enantiomer were doubled following administration of the racemate as compared with the (*R*)-enantiomer alone. This suggests a metabolic enantiomer–enantiomer interaction with the (*S*)-enantiomer acting as an inhibitor of (*R*)-nitrendipine metabolism. Liver cirrhosis had a profound impact on the pharmacokinetics and stereoselectivity of nimodipine and nitrendipine. In the case of racemic drugs with extensive stereoselective first-pass metabolism such as DHP calcium antagonists, the aspect of altered stereoselective disposition has to be considered.

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Symbols and abbreviations

AUC	Area under the plasma or serum concentration–time curve
CL_o	Apparent oral clearance
CL	Total body clearance
C_{max}	Maximum plasma or serum concentration
CSP	Chiral stationary phase
DHP	Dihydropyridine
ECD	Electron-capture detection
EM	Extensive metabolizer
F	Extent of bioavailability
f_p	Free fraction of plasma protein binding
GC	Gas chromatography
HPLC	High-performance liquid chromatography
i.v.	Intravenous or intravenously
K_m	Michaelis–Menten constant
MS	Mass spectrometry
NICI	Negative-ion chemical ionization
R.S.D.	Relative standard deviation
S.D.	Standard deviation
S.E.	Standard error
t_{max}	Time to reach maximum concentration
$t_{1/2}$	Terminal half-life
V_{dss}	Volume of distribution at steady state
V_{max}	Maximum rate of metabolism

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