Use of pseudoracemic nitrendipine to elucidate the metabolic steps responsible for stereoselective disposition of nitrendipine enantiomers

V. MAST, C. FISCHER, G. MIKUS & M. EICHELBAUM

Dr Margarete Fischer-Bosch-Institut für Klinische Pharmakologie, Auerbachstrasse 112, D-7000 Stuttgart 50, Germany

- 1 The pharmacokinetics, protein binding, bioavailability and metabolism of (+)-R- and (–)-S-nitrendipine were studied in six healthy subjects following random oral administration of 20 mg (+)-R-, 20 mg (–)-S- and 20 mg R,S- nitrendipine (pseudoracemic mixture of 10 mg [$^{13}\text{C}_4$]-(+)-R- and 10 mg (–)-S-enantiomer).
- 2 After administration of the enantiomers pronounced differences in AUC (R: 29.9 ± 20.1 ; S: 123.8 ± 63.7 ng ml $^{-1}$ h; $P < 0.05$), bioavailability (R: $10.7 \pm 7.4\%$; S: $44.6 \pm 23.1\%$; $P < 0.05$) and $C_{\max}$ (R: 14.4 ± 7.7 ; S: 72.5 ± 40.5 ng ml $^{-1}$; $P < 0.05$) were observed between R- and S-nitrendipine. When racemic nitrendipine was given bioavailability and dose normalized AUC and $C_{\max}$ values of the S-enantiomer were not different from the values after S-nitrendipine-administration. In contrast, bioavailability (R: 10.7% R,S: 22.1%) and dose normalized AUC (R: 15.0; R,S: 29.5 ng ml $^{-1}$ h and $C_{\max}$ (R: 7.2; R,S: 16.8 ng ml $^{-1}$) of R-nitrendipine were doubled following R,S- as compared with R-nitrendipine administration. $t_{1/2}$ (R: 9.8; S: 9.1 h) and $t_{\max}$ were not different between the enantiomers nor were the values different after administration of the enantiomers or racemate. The fraction unbound in serum of R-nitrendipine was 0.0098 ± 0.0032 (s.d.) and that of S-nitrendipine was 0.0083 ± 0.0015 (s.d.).
- 3 The AUC values of the major pyridine metabolite M1 were similar after administration of R- and S-nitrendipine (S: 114.7 ± 48.5 ; R: 71.7 ± 29.9 ng ml $^{-1}$ h). After racemic nitrendipine dose normalized AUC values were not different from those observed after administration of the enantiomers.
- 4 The cumulative urinary excretion of the four major pyridine metabolites M2a, M2b, M3a and M3b was significantly higher after S-nitrendipine. The % of dose which was recovered in urine within 48 h was $63.2 \pm 6.6\%$ (administration of racemate)/ $69.0 \pm 5.9\%$ (administration of S-enantiomer) for the S-enantiomer and $42.0 \pm 5.1\%$ (R,S-nitrendipine)/ $43.8 \pm 7.6\%$ (R-enantiomer) for the R-enantiomer, respectively. Formation of metabolite M2a, which is generated by cleavage of the ethyl ester bond and oxidation of the 1,4-dihydropyridine ring to pyridine, exhibited stereoselectivity. On average $43.9 \pm 3.9\%$ of dose was formed from S-nitrendipine as compared with $24.7 \pm 2.2\%$ from R-nitrendipine following administration of the racemate.
- 5 Substantial differences in the metabolism, bioavailability and disposition of R- and S-nitrendipine were observed. The first-pass metabolism of nitrendipine is stereoselective affecting mainly the less potent R-enantiomer. The bioavailability 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. With regard to the metabolic pathways responsible for stereoselective first-pass metabolism, it is unlikely that oxidation of nitrendipine to the pyridine metabolite M1 is involved since no substantial

differences in the AUC of this metabolite were observed after administration of R- and S-nitrendipine. The hypothesis that formation of M1 metabolite is the primary metabolic step in the biotransformation of nitrendipine and that this is followed by cleavage of the ester bonds to carboxylic monoesters is not compatible with the findings of this study. The marked stereoselectivity in the formation of metabolite M2a indicates that a substantial proportion of the dose is cleaved prior to oxidation of the 1,4-dihydropyridine ring.

Keywords nitrendipine enantiomers stereoselectivity pharmacokinetics first-pass metabolism bioavailability

Introduction

Nitrendipine (3-ethyl 5-methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl) 3,5-pyridine dicarboxylate) is a calcium antagonist with potent vasodilating properties used in the treatment of hypertension (Goa & Sorkin, 1987; Hulthen *et al.*, 1984; Kazda *et al.*, 1984; Lehmann *et al.*, 1983; Sarmiento *et al.*, 1986a, b).

With the exception of nifedipine, which is an achiral drug, all currently used 1,4-dihydropyridine Ca^{2+} antagonists are racemates. Pronounced differences in pharmacological activity can exist between enantiomers since a certain spatial orientation of functional groups is required for optimal binding to receptors (Easson & Stedman, 1933). Since the processes of drug distribution, metabolism and excretion also involve an interaction between the drug molecule and chiral biological macromolecules it is anticipated that these processes may also be stereoselective. Depending on whether the more active or less active enantiomer is preferentially affected there may be amplification or attenuation of *in vivo* as compared to *in vitro* drug potency (Ariens *et al.*, 1988; Drayer, 1986; Lam, 1988; Tucker & Lennard, 1990; Williams & Lee, 1985). Therefore, in order to obtain meaningful pharmacodynamic and pharmacokinetic data for a racemate it is mandatory to study the individual enantiomers.

Most published data on the pharmacokinetics, bioavailability, metabolism and pharmacological actions of nitrendipine have been based on total plasma drug concentrations not taking account of stereoisomeric composition (Ankermann *et al.*, 1989; Aronoff & Sloan, 1985; Dylewicz *et al.*, 1987; Kann *et al.*, 1984; Kendall *et al.*, 1987; Krol *et al.*, 1987; Lasseter *et al.*, 1984; Lettieri *et al.*, 1987; Mikus *et al.*, 1987; Raemsch *et al.*, 1986; Raemsch & Sommer, 1984; Soons *et al.*, 1989).

Studies with other chiral 1,4-dihydropyridine calcium antagonists have demonstrated stereoselectivity in their action and disposition (Hof *et al.*, 1985; Niwa *et al.*, 1988; Soons *et al.*, 1990; Towart *et al.*, 1982). Data on this aspect of the clinical pharmacology of nitrendipine are scarce. S-nitrendipine is 7 to 8 times more potent than the R-enantiomer in inhibiting thromboxane A_2 induced coronary vasoconstriction and in lowering blood pressure in spontaneously hypertensive rats (Eltze *et al.*, 1990). Similar differences in cardiovascular and biochemical effects of S- and R-nitrendipine have been observed in healthy volunteers (Mikus *et al.*, 1989).

Despite its almost complete absorption after oral administration the bioavailability of nitrendipine is only 10 to 30% due to extensive presystemic elimination. It is not known if this first-pass metabolism is stereoselective. The metabolism of calcium antagonists with the 1,4-dihydropyridine structure involves oxidation of the ring to the corresponding pyridine, cleavage of the ester bonds or hydroxylation of the methyl groups and conversion to cyclic lactones (Raemsch *et al.*, 1986). It has been suggested that oxidation of the 1,4-dihydropyridine ring to the pyridine metabolite is the primary metabolic step. This metabolite is then further metabolized by cleavage of the ester bonds to the corresponding carboxylic monoesters (Guengerich *et al.*, 1988). In the case of chiral 1,4-dihydropyridines oxidation transforms a chiral drug into achiral pyridine metabolites. Assuming that this major biotransformation pathway exhibits stereoselectivity, the chiral – achiral transformation leading to achiral metabolites would obviate identification of stereoselectivity in this pathway after administration of the racemate. Administration of the individual enantiomers to study the pharmacokinetics and metabolism of the enantiomers has limitations. Because of both pharmacodynamic and pharmacokinetic enantiomer – enantiomer interactions disposition may be different after administration of the individual enantiomers and the racemate (Tucker & Lennard, 1990). However, by using a racemate in which one enantiomer is labelled with a stable isotope (a so-called pseudoracemate) an unequivocal allocation of metabolites formed from the respective enantiomers is possible. We have studied stereoselectivity in the disposition and metabolism of (+)-R- and (–)-S-nitrendipine after oral administration of the individual enantiomers and the pseudoracemate.

Methods

Subjects

Six healthy normotensive subjects (three female, three male, age 22 to 32 years, body weight 52–85 kg) were recruited for the study. All subjects gave written consent to participate in the study which was approved by the

local Ethics Committee. A complete physical examination and appropriate laboratory tests were performed before the study. All subjects were nonsmokers. They were not taking any drugs with the exception of subject 5 who was taking an oral contraceptive.

Drug administration, blood and urine sampling

The study commenced between 07.00 h and 07.30 h. The subjects fasted over night. Prior to drug administration they rested for at least 30 min in the supine position for basal recording of haemodynamic and biochemical parameters. Thereafter the subjects received in random order orally the following drugs dissolved in 100 ml tap water containing 5% v/v ethanol:

1. 20 mg (+)-R-nitrendipine
2. 20 mg (–)-S-nitrendipine
3. 20 mg R,S-nitrendipine as a pseudoracemic mixture consisting of 10 mg (+)-R-nitrendipine which was labelled with four ^{13}C -atoms ($[^{13}\text{C}_4]$ -R) and 10 mg unlabelled (–)-S-nitrendipine.

The optical purity of the R- and S-nitrendipine enantiomers, which were obtained by synthesis was greater than 99% as determined by chiral stationary phase h.p.l.c. (see below). The isotope content of $[^{13}\text{C}_4]$ -R-nitrendipine was 99.4%. The position of the four ^{13}C -atoms is shown in Figure 1.

In a pilot study one subject received 10 mg of labelled and unlabelled R-nitrendipine simultaneously to determine any isotope effect.

Venous blood samples were withdrawn into glass tubes which were protected from light with aluminum foil before and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 9, 11, 12, 14, 15, 24, 25 and 30 h after drug administration. Blood samples were centrifuged at 3000 g for 10 min, and the separated serum was stored at -20°C until analysis. Urine was collected over 48 h in two fractions (0–24, 24–48 h) and a 100 ml aliquot of each fraction was stored in light protected glass bottles at -20°C until analysis.

Protein binding

The protein binding of R- and S-nitrendipine was determined by equilibrium dialysis at 37°C . Aliquots of serum (1 ml) obtained at $t_{\max}$ from each subject after administration of the racemate were dialysed against

potassium phosphate buffer (0.05 M, pH 7.4). The protein binding was determined in two samples from each subject on each study day. The samples were incubated for 4 h by which time equilibrium was reached between serum and buffer.

Drug and metabolite analysis

Nitrendipine and ($^{13}\text{C}_4$)-nitrendipine in serum were measured by GC/MS (selected ion monitoring of the molecular ion in negative ion chemical ionization mode) using racemic $[^2\text{H}_8]$ -nitrendipine as internal standard (Fischer *et al.*, 1986). The isotope content of the deuterated internal standard was 99.8%.

The major nitrendipine serum metabolite M1 (3-carboethoxy, 5-carbomethoxy-2,6-dimethyl-4-(3-nitrophenyl)-pyridine) was measured simultaneously with nitrendipine using the corresponding M1 nifedipine metabolite (3-carbomethoxy, 5-carbomethoxy-2,6-dimethyl-4-(2-nitrophenyl)-pyridine) as internal standard. The four major urinary nitrendipine metabolites M2a (3-carboxy 5-carbomethoxy-2,6-dimethyl-4-(3-nitrophenyl)-pyridine), M2b (3-carboethoxy, 5-carboxy-2,6-dimethyl-4-(3-nitrophenyl)-pyridine), M3a (3-carboxy 5-carbomethoxy-2-hydroxy methyl, 6-methyl-4-(3-nitrophenyl)-pyridine) and M3b (3-carboethoxy, 5-carboxy-2-methyl, 6-hydroxymethyl-4-(3-nitrophenyl)-pyridine) were measured by GC/MS (Krol *et al.*, 1987) using nifedipine metabolite M2 (3-carboxy 5-carbomethoxy, 2,6-dimethyl-4-(2-nitrophenyl)-pyridine) and $[^2\text{H}_8]$ -nitrendipine as internal standards (for structures see Figure 2). Calibration curves were constructed using 0.5 ml aliquots of blank urine to which the metabolites M2a (range 50 to 2000 ng), M2b (range 12.5 to 500 ng), M3a (range 12.5 to 500 ng) and M3b (range 25 to 100 ng) together with the internal standards were added. The enzymatic hydrolysis, extraction and derivatization procedure was performed as described by Krol *et al.* (1987). The 100% relative abundance ions in negative ion chemical ionization mode were the molecular ions M^- 344, 358, 328 and 342 for M2a methylester, M2b methylester, M3a and M3b lactones, respectively, and were used for selected ion monitoring.

To exclude chiral conversion or racemization serum samples following the administration of 20 mg of R- or S-nitrendipine were analyzed for their R- and S-drug concentrations. To 0.5 ml serum, 1 ng racemic $[^2\text{H}_8]$ -nitrendipine was added as internal standard. The samples were extracted as described for the GC/MS method. The R- and S-enantiomers were separated by chiral stationary phase h.p.l.c. The R- and S-fractions were collected and thereafter their concentrations measured by GC/MS. A Daicel OJ analytical column (tris (4-methylbenzoate) cellulose, Daicel Chemical Industries, Ltd, Japan), 25 cm $\times$ 0.46 cm i.d., mean silica particle size 10 μm , with a precolumn filled with the same material (5 cm $\times$ 0.46 cm i.d.) was used. The mobile phase was a mixture of n-hexane: ethanol, 85:15 (v/v) at a flow rate of 0.3 ml min^{-1} . The h.p.l.c. system was kept at 22°C . Retention times were 22.5 and 26.3 min for (–)-S- and (+)-R-nitrendipine, respectively, with a resolution factor $R = 1.21$ and a separation factor $\alpha = 1.24$. Within- and between-day precision of the stereospecific assay at the

Figure 1 Position of the ^{13}C -atoms in $[^{13}\text{C}_4]$ -nitrendipine.
* = ^{13}C label.

lower limit of assay was $\pm 10\%$ s.d. The limit of assay was 0.3 ng ml^{-1} per enantiomer following extraction of 0.5 ml of serum, separation of the enantiomers and measurement of the separated fractions by GC/MS.

Standard compounds were kindly provided by Drs Heuck and Rämisch (Bayer AG, Wuppertal, Germany).

Pharmacokinetics and statistical analysis

The serum nitrendipine concentration-time data were fitted by a multiexponential equation:

$$C(t) = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} - (C_1 + C_2) e^{-k_a t}$$

where $C(t)$ is the serum concentration; C_1 , C_2 , λ_1 and λ_2 are macroconstants, k_a is the apparent absorption rate constant and t is the time of blood sample. Nonlinear least squares repression was performed using the LSNLR programme (Peck & Barrett, 1979). The data were weighted by the reciprocal of the squares of the individual concentrations. The terminal half-life ($t_{1/2,z}$) was determined from $(\ln 2)/\lambda_2$. The AUC was calculated using the linear trapezoidal rule and the segment to infinity as the last measured concentration divided by λ_2 . The oral clearance (CL_o) was calculated from D/AUC .

The absolute bioavailability was calculated using the relationship (Mast, 1988):

$$1/F = 0.826 \times CL_o + 0.03$$

The M1-nitrendipine serum concentration-time data were analysed in the above manner and $t_{1/2,z}$ and AUC were determined. $C_{\max}$ and $t_{\max}$ values were derived by visual inspection of experimental data. Comparison of the pharmacokinetic parameters and cumulative urinary excretion of metabolites M2a, M2b, M3a and M3b after administration of the enantiomers and racemates was

carried out using student's paired t -test. A P value of less than 0.05 was considered to be statistically significant.

Results

No difference in the serum concentration-time curves of R-nitrendipine was observed when equimolar amounts of labelled and unlabelled enantiomer were administered. Thus an isotope effect on the disposition of R-nitrendipine can be excluded. The serum samples obtained after administration of R- or S-nitrendipine contained less than 0.7% of the opposite enantiomer and therefore chiral conversion or racemization can be discounted.

Following the separate administration of R- and S-nitrendipine significant differences in all pharmacokinetic parameters except for $t_{1/2}$ and $t_{\max}$ were observed (Table 1). AUC, bioavailability and $C_{\max}$ values were on average four times greater for the S-enantiomer. Substantial interindividual variations were seen. After administration of the racemate, the dose normalized values for S-nitrendipine were not significantly different from the values obtained after S-nitrendipine. In contrast, R-nitrendipine pharmacokinetics were changed such that AUC, F and $C_{\max}$ values increased twofold when the racemate was given (Table 2). No significant differences in $t_{1/2}$ were observed for R- and S-nitrendipine when administered as enantiomer or racemate (Tables 1 and 2).

The serum concentrations of the M1 metabolite formed from the R-enantiomer always exceeded R-nitrendipine concentrations whereas S-nitrendipine concentrations were always greater than M1 metabolite ones derived from the S-enantiomer. The M1-AUC values of the S-enantiomer were higher than the corresponding values

Table 1 Pharmacokinetic parameters of (+)-R- and (–)-S-nitrendipine following separate oral administration of 20 mg of each enantiomer. Data are given as mean $\pm$ s.d.

Subject		$t_{\max}$ (min)	$C_{\max}$ (ng ml ⁻¹)	$t_{1/2}$ (h)	AUC (μg ml ⁻¹ h)	CL_o (l min ⁻¹)	F (%)
1	R	30	12.5	10.4	20.5	16.7	7.2
	S	30	55.4	12.2	83.1	4.0	30.0
2	R	60	15.3	10.6	65.1	5.1	23.6
	S	45	66.1	10.7	217.2	1.5	79.8
3	R	30	12.9	8.6	16.5	20.3	5.9
	S	30	45.9	7.9	85.8	3.8	31.6
4	R	15	12.9	9.5	23.6	15.5	7.8
	S	30	122.9	8.9	129.9	2.6	45.9
5	R	30	28.2	9.3	42.3	7.9	15.3
	S	30	120.2	6.5	177.6	1.9	62.5
6	R	30	4.5	10.3	11.8	28.3	4.3
	S	30	24.3	8.6	49.4	6.8	17.7
R		32.5 ^a $\pm$ 14.7	14.4 ^b $\pm$ 7.7	9.8 ^a	29.9 ^b $\pm$ 20.1	15.6 ^b $\pm$ 8.4	10.7 ^b $\pm$ 7.4
S		32.5 $\pm$ 6.1	72.5 $\pm$ 40.5	9.1*	123.8 $\pm$ 63.7	3.4 $\pm$ 1.9	44.6 $\pm$ 23.1

^a: not significantly different between R- and S-nitrendipine.

^b: $P < 0.05$ between R and S-nitrendipine.

*harmonic mean, $t_{\max}$ = time of maximum serum drug concentration, $C_{\max}$ = maximum serum drug concentration, $t_{1/2}$ = terminal elimination half-life, AUC = area under the serum drug concentration-time curve, CL_o = oral clearance, F = bioavailability.

Table 2 Pharmacokinetic parameters of (+)-R- and (–)-S-nitrendipine after oral administration of 20 mg R,S-nitrendipine (pseudoracemate; 10 mg [$^{13}\text{C}_4$]- (+)-R-nitrendipine and 10 mg of S-nitrendipine). Data are given as mean $\pm$ s.d.

Subject		$t_{\max}$ (min)	$C_{\max}$ (ng ml $^{-1}$)	$t_{1/2}$ (h)	AUC (ng ml $^{-1}$ h)	CL_0 (l min $^{-1}$)	F (%)	fu
1	R	30	14.4	7.8	18.0	9.2	13.1	0.007
	S	30	36.3	8.6	51.0	3.3	36.3	0.007
2	R	30	29.7	6.2	60.1	2.8	42.7	0.008
	S	45	47.2	8.6	124.6	1.3	90.6	0.008
3	R	30	16.6	8.4	23.2	7.1	16.9	0.008
	S	30	31.2	7.6	45.9	3.6	33.3	0.007
4	R	15	12.8	6.8	22.0	7.6	15.9	0.016
	S	15	28.0	6.9	49.2	2.9	41.2	0.011
5	R	45	13.1	9.4	30.2	5.5	21.9	0.010
	S	45	30.2	9.1	79.9	2.1	56.7	0.008
6	R	30	14.2	6.2	23.4	7.1	16.9	0.010
	S	30	20.9	6.2	33.0	5.1	23.6	0.009
R		30.0 ^a $\pm$ 9.5	16.8 ^{b,c} $\pm$ 6.5 7.2 ^{c,d} $\pm$ 3.9	7.5 ^{*a}	29.5 ^b $\pm$ 15.5 15.0 ^{c,d} $\pm$ 10.0	6.6 ^{b,c} $\pm$ 2.0 15.6 $\pm$ 8.4 ^{c,e}	21.2 ^{b,c} $\pm$ 10.9 10.7 $\pm$ 7.4 ^{c,e}	0.0098 $\pm$ 0.0032
S		32.3 ^a $\pm$ 11.3	32.3 $\pm$ 8.8 36.3 ^{a,d} $\pm$ 20.3	7.7 ^{*a}	63.9 $\pm$ 33.5 61.9 ^{a,d} $\pm$ 31.9	3.1 ^a $\pm$ 1.3 3.4 $\pm$ 1.9 ^{a,e}	46.9 ^a $\pm$ 24.0 44.6 $\pm$ 23.1 ^{a,e}	0.0083 $\pm$ 0.0015

^a: not significantly different between R- and S-nitrendipine or when administered as enantiomer or racemate.

^b: $P < 0.05$ between R- and S-nitrendipine.

^c: $P < 0.05$ when administered as enantiomer or racemate.

^d: dose normalized (10 mg) values after administration of the enantiomers.

^e: values after administration of the enantiomer.

*harmonic mean, $t_{\max}$ = time of maximum serum drug concentration, $C_{\max}$ = maximum serum drug concentration, $t_{1/2}$ = terminal elimination half-life, AUC = area under the serum drug concentration-time curve, CL_0 = oral clearance, F = bioavailability; fu = fraction unbound.

Table 3 Pharmacokinetic parameters of the M1 metabolite of nitrendipine following oral administration of 20 mg of (–)-S-nitrendipine, 20 mg (+)-R- nitrendipine and 20 mg of R,S-nitrendipine (pseudoracemate: 10 mg (–)-S- and 10 mg [$^{13}\text{C}_4$]-(+)-R-nitrendipine). Data are given as mean $\pm$ s.d.

Parameter	Administration of enantiomers		Administration of racemate	
	20 mg (–)-S	20 mg (+)-R	10 mg (–)-S	10 mg (+)-R
$t_{\max}$ (min)	35.5 $\pm$ 6.1 ^a	30.0 $\pm$ 9.5	27.5 $\pm$ 6.1 ^a	25.0 $\pm$ 7.7
$C_{\max}$ (ng ml $^{-1}$)	65.0 $\pm$ 28.4 ^a	37.4 $\pm$ 22.9	30.3 $\pm$ 6.9 ^a 32.5 $\pm$ 14.2 ^{b,c}	30.3 $\pm$ 6.8 18.7 $\pm$ 11.5 ^{b,c}
$t_{1/2}$ (h)	10.8 ^{*a}	11.1 [*]	9.8 ^{*a}	8.5 [*]
AUC (ng ml $^{-1}$ h)	114.7 $\pm$ 48.5 ^a	71.7 $\pm$ 29.9 ^a	55.8 $\pm$ 17.3 ^a 57.4 $\pm$ 24.2 ^{b,c}	44.7 $\pm$ 11.7 ^a 35.9 $\pm$ 15.0 ^{b,c}

*harmonic mean.

^a not significantly different between R- and S-nitrendipine.

^b dose normalized (10 mg) values after administration of the enantiomers.

^c not significantly different when administered as enantiomer or racemate.

of the R-enantiomer but this difference did not reach statistical significance. The same was true for dose normalized M1-AUC values when the racemate was given (Table 3).

Protein binding

Both enantiomers are highly bound to plasma proteins. The fraction unbound was on average 0.0098 ± 0.0032 for the R-enantiomer and 0.0083 ± 0.0015 for the S-enantiomer with no significant differences in protein binding between the two isomers (Table 2).

Metabolism

Less than 0.1% of the dose was excreted in urine as nitrendipine and its M1-metabolite. Following administration of the enantiomers the cumulative urinary excretion of the four metabolites measured averaged $69.0 \pm 5.9\%$ of dose for S-nitrendipine and $43.8 \pm 7.6\%$ for R-nitrendipine. Almost identical values were observed after administration of the racemate (Table 4). The percentage of the dose recovered as metabolites was significantly lower for R-nitrendipine. Comparison of cumulative urinary excretion of metabolites for the

Table 4 Cumulative urinary excretion of four major nitrendipine metabolites over 48 h as percent of dose administered following oral administration of 20 mg R-, 20 mg S- and 20 mg R,S-pseudoracemate. For chemical structures see Figure 2. Data are given as mean $\pm$ s.d.

Metabolite	Administration of enantiomers		Administration of racemate		S/R ratio
	20 mg R	20 mg S	10 mg R	10 mg S	
M2a	26.9 ^b	45.6	24.7 ^b	43.9	1.78
s.d.	5.4	7.2	2.2	4.1	
M2b	11.3 ^a	15.3	10.8 ^a	13.3	1.23
s.d.	3.7	5.3	4.4	6.2	
M3a	2.9 ^b	4.1	2.6 ^b	3.3	1.27
s.d.	0.7	0.9	0.5	0.5	
M3b	2.6 ^a	3.3	2.5 ^a	2.7	1.08
s.d.	0.8	0.7	0.3	0.3	
Sum	43.8 ^b	69.0	42.0 ^{b,c}	63.2 ^c	
s.d.	7.6	5.9	5.1	6.6	

^a not significantly different between R- and S-nitrendipine

^b $P < 0.05$ between R- and S-nitrendipine

^c cumulative urinary excretion not significantly different when the enantiomer or racemate was administered

respective enantiomers showed no difference when the enantiomer or racemate had been administered (Table 4). With regard to the metabolites responsible for these differences in cumulative urinary excretion a greater percentage of the dose was excreted as metabolite M2a from S-nitrendipine as compared with R-nitrendipine both after administration of the enantiomers and of racemate. Smaller but significant differences were observed for metabolite M3a whereas the recoveries of metabolites M2b and M3b showed no stereoselectivity (Table 4).

Discussion

25% of all drugs prescribed in Germany are racemates. Similar data have been reported for other countries (Roth & Kleemann, 1982; Simonyi, 1984). In spite of the widespread therapeutic use of racemates data on the activity and disposition of the enantiomers are sparse. Like other racemic drugs with a low oral bioavailability due to extensive presystemic elimination the first-pass metabolism of nitrendipine is stereoselective. Presystemic elimination affects preferentially the less potent R-enantiomer and as a consequence its bioavailability is substantially lower than that of S-nitrendipine. The oral S/R serum concentration ratio is on average 2.0 ranging from 1.5 to 2.5. These data are in agreement with our own previous data (Mikus *et al.*, 1989) and the results of Soons *et al.* (1990).

The differences in bioavailability of R-nitrendipine observed after administration of the R-enantiomer vs the racemate reveals another aspect of stereoselective drug disposition which has not been addressed in great detail, i.e. metabolic enantiomer-enantiomer interaction. As a consequence of such an interaction the disposition of enantiomers may be different depending on whether the individual enantiomer or the racemate is admini-

stered. There are three possible mechanisms to explain the observed interaction.

1) Both enantiomers are metabolized at different rates by the same enzyme and compete for the active site. In such a situation a mutual competitive inhibition occurs and both enantiomers are affected when the racemate is administered.

2) The metabolism of both enantiomers is catalyzed by the same enzyme but only one enantiomer acts as a competitive inhibitor. Under such circumstances the metabolism of only one enantiomer is impaired when the racemate is given.

3) One enantiomer is metabolized by an enzyme with the other acting as an inhibitor but not being a substrate for this enzyme.

Since only the first-pass metabolism of R-nitrendipine was affected following oral administration of racemic nitrendipine the data indicate that S-nitrendipine acts as a competitive inhibitor of the metabolism of R-nitrendipine. R-nitrendipine seems not to inhibit S-nitrendipine metabolism as the bioavailability of S-nitrendipine was identical both after administration of S-nitrendipine and the racemate. Thus it is likely that the second mechanism is operating although the third mechanism cannot be ruled out. Only characterization of the cytochrome P-450 isoenzymes involved in the biotransformation of the nitrendipine enantiomers and *in vitro* inhibition studies will distinguish which of the latter two mechanisms is responsible for the observed *in vivo* enantiomer-enantiomer interaction.

With regard to the metabolic steps responsible for the observed stereoselectivity in first-pass metabolism some questions related to the mechanism and sequence of the formation of the metabolites arise. It has been proposed that oxidation of the 1,4-dihydropyridine ring to the pyridine metabolite M1 constitutes the primary metabolic step in the biotransformation of 1,4-dihydropyridine calcium antagonists (Guengerich, 1987). This M1 metabolite is then further biotransformed by cleavage of the ester bonds to the corresponding carboxylic acid metabolites M2a and M2b or by hydroxylation of the methyl groups and ready conversion to cyclic lactones of the corresponding metabolites M3a and M3b, (see Figure 2) (Guengerich *et al.*, 1988).

In the metabolism of nilvadipine a certain degree of stereoselectivity in the formation of the M1 metabolite has been observed (Niwa *et al.*, 1988). The findings of the present study, however, indicate that this is not the case for nitrendipine. If the formation of the M1 metabolite is the primary metabolic step and the stereoselectivity of this step is responsible for the preferential first-pass metabolism of the R-enantiomer one would expect a greater AUC for this metabolite following R-nitrendipine. The AUCs of the M1 metabolite, however, were of the same order of magnitude for both enantiomers indicating that the rate of formation and amounts of metabolite M1 formed are not different for R- and S-nitrendipine. Therefore it is unlikely that this metabolic step is responsible for the stereoselective first-pass metabolism. The use of a racemate in which the R-enantiomer was labelled with the stable ¹³C isotope in the present study allowed an unequivocal identification how much of the respective metabolites are formed from

Figure 2 Metabolic pathways of nitrendipine. The metabolites M4a and M4b shown in brackets have not been found in urine, but are formed *in vitro* by human liver microsomes. The asterisk denotes the chiral carbon atom. The numbers indicate the S/R ratio of the respective metabolites following oral administration of pseudoracemic nitrendipine. In the case of metabolite M1 this ratio is based on the AUCs, whereas for all other metabolites the ratio was calculated from cumulative urinary excretion of the corresponding metabolites.

R- and S-nitrendipine. The experimental findings of this study showing that much more metabolite M2a is formed from S-nitrendipine although the formation of metabolite M1 was very similar for the R- and S-enantiomer is not compatible with the assumption that the formation of metabolite M2a proceeds primarily and solely via metabolite M1. Whereas the formation of metabolite M1, which involves the transformation of a chiral substrate to an achiral metabolite, can be stereoselective the further metabolism of the achiral metabolite M1 to the achiral metabolite M2a cannot be stereoselective. Thus if M1 metabolite formation exhibits no stereoselectivity, no differences in M2a formation from R- and S-nitrendipine are possible.

Therefore an additional alternate and stereoselective pathway in the formation of metabolite M2a has to be assumed. The observed stereoselectivity in M2a formation suggests that a proportion of the dose undergoes as the primary metabolic reaction not the formation of M1 but the cleavage of the ester bond yielding a carboxylic acid metabolite with the 1,4-dihydropyridine structure (M4a) and which retains the centre of chirality (Figure 2). This chiral metabolite is then further oxidized to the

corresponding achiral carboxylic acid M2a metabolite. Since metabolite M4a, which serves as substrate for the latter reaction, is chiral both metabolic steps can be stereoselective. The findings of the present study demonstrating that much more of the M2a and to a minor extent of the M3a metabolite are formed from S-nitrendipine are in agreement with this hypothesis and indicate stereoselectivity of these pathways for compounds of the S-configuration. However, provided that these two metabolic steps are solely responsible for the observed stereoselectivity, the experimental data demonstrating preferential stereoselective first-pass metabolism of R-nitrendipine are not compatible with this hypothesis, since one would expect a greater cumulative urinary excretion of these two metabolites following administration of the R-enantiomer.

Recent studies on the *in vitro* metabolism of racemic nitrendipine in rat liver microsomes have demonstrated that besides M1, M2a, M2b, M3a and M3b the corresponding metabolites of M2a and M2b with 1,4-dihydropyridine structure were formed (M4a and M4b, Figure 2) (Böcker *et al.*, 1990). Our own studies on the *in vitro* metabolism of R- and S-nitrendipine in human liver

microsomes have shown that, in addition to the known metabolites, the chiral monocarboxylic methyl (M4a) and monocarboxylic ethyl esters (M4b) are formed. The intrinsic clearance of metabolite M1 was identical for R- and S-nitrendipine. However, intrinsic clearance of M4a was on average three times higher for R-nitrendipine. The opposite stereoselectivity was observed for M2a formation, the intrinsic clearance being two times higher for S-nitrendipine (Fischer & Janicki, 1990 and unpublished results).

Thus the metabolism of nitrendipine enantiomers involve two major metabolic pathways only one of which is stereoselective. The pathway which involves the oxidation of the chiral 1,4-dihydropyridine ring to the achiral M1 pyridine metabolite and its further metabolism to achiral monocarboxylic esters is not stereoselective. The other pathway, which transforms a chiral 1,4-dihydropyridine ring by cleavage of the ester bond into a chiral monocarboxylic ester which is further oxidised to an achiral monocarboxylic ester, is stereoselective but the two steps involved exhibit opposite stereoselectivity. Since no carboxylic acid metabolites with the 1,4-dihydropyridine structure could be identified in urine these metabolites once formed *in vivo* must be oxidized very rapidly to the corresponding achiral metabolites. However, these data explain only partly the observed *in vivo* differences. Because the cumulative urinary excretion of the metabolites M2a, M2b, M3a and M3b is substantially lower following administration of R-nitrendipine this suggests that other yet unidentified metabolites are being formed in man and that the proportion of the dose which is metabolized via these routes

is greater for the R-enantiomer. In support of this assumption are recent data from Scherling & Karl (1990) who have identified 17 metabolites, including several with the 1,4-dihydropyridine structure, in dogs.

In conclusion the present data refute the hypothesis that oxidation of the 1,4-dihydropyridine ring to pyridine is the sole primary metabolic reaction in the formation of metabolites with a monocarboxylic ester structure. While this assumption seems to be valid for the achiral 1,4-dihydropyridine nifedipine and some chiral dihydropyridine compounds like nilvadipine, this is certainly not the case for nitrendipine. Since the formation of the pyridine metabolite M1 was not stereoselective, the observed stereoselectivity in the biotransformation of nitrendipine suggests that a substantial part of the dose is metabolized by cleavage of the ester groups prior to oxidation of the 1,4-dihydropyridine ring. In agreement with this assumption are data of Frost *et al.* (1990) on nisoldipine indicating only a marginal enantioselectivity in the oxidation to the pyridine analogue, whereas side chain hydroxylation exhibited marked stereoselectivity. In the present study the use of a pseudoracemate was essential to establish unequivocally the metabolic fate of the R- and S-enantiomers of nitrendipine.

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