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Enantioselective Disposition of Oral Amlodipine in Healthy Volunteers

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ABSTRACT Plasma concentrations of (R)- and (S)-amlodipine were measured after single oral administrations to 18 healthy volunteers of 20 mg amlodipine racemate. The contribution of the pharmacologically active (S)-enantiomer to the concentrations of total amlodipine (sum of enantiomers) was significantly higher than that of the inactive (R)-enantiomer, with mean values of 47% R to 53% S for the C_{max} and 41% R to 59% S for the AUC (range between 24% R:76% S and 50% R:50% S). The oral clearance of the active (S)-form was subject to much less intersubject variation (25% CV) than that of the inactive (R)-form (52% CV). (R)-Amlodipine was more rapidly eliminated from plasma than (S)-amlodipine, with mean terminal half-lives of 34.9 h (R) and 49.6 h (S). The terminal half-lives of total amlodipine (mean 44.2 h) were strongly correlated with—and thus highly predictive for—the half-lives of the (S)-enantiomer. It is proposed that the observed enantioselectivity of oral amlodipine is due to differences in the systemic blood clearance of the enantiomers.

KEY WORDS: (R)-, (S)-, (R, S)-amlodipine, pharmacokinetics, oral dosage, human study

Amlodipine is a calcium channel blocker which is effective as both an antianginal agent in patients with stable angina pectoris and as an antihypertensive agent in patients with mild to moderate hypertension.[1] The high oral bioavailability (64%) and long half-life (42 h) of amlodipine are unique for this class of drugs and allow safe once-a-day treatment with low doses.[2,3]

Like most other calcium blocker agents of the dihydropyridine type, amlodipine is in clinical use as a racemic mixture. As the pharmacological activity resides in the (−)-(S)-enantiomer,[4,5] knowledge of the contribution of this active enantiomer to the observed disposition is a prerequisite for any search of relationships between drug levels and therapeutic response.

No human pharmacokinetic data on the enantiomers of amlodipine have been reported until now. The present communication describes the disposition of the enantiomers in the plasma of healthy subjects after oral doses of racemic amlodipine as the besylate or the maleate salts and was an additional result of a bioequivalence study of these two salts.

MATERIALS AND METHODS

Clinical Procedure

In a randomized balanced crossover study, single doses of amlodipine besylate salt and amlodipine maleate salt, each equivalent to 20 mg amlodipine racemate, were administered in the form of 2 × 10 mg oral capsules. Eighteen healthy male Caucasian subjects between the age of 19 and 45 years (mean 31.4 years), weighing between 60 and 83 kg (mean 74 kg), were included in the study. All were in good health on the basis of history of diseases that could affect the disposition of the study drug.

The subjects were instructed to avoid any drug medication for 2 weeks before and during the study. Tobacco, alcohol, and caffeine-containing beverages were not permitted from 1 day before study entry until the end of the study.

The protocol was approved by the Ethical Review Board of the University of Ulm, Germany. The study was performed in the Clinical Pharmacology Unit of Pfizer Mack Illertissen, Germany, in accordance with the provisions of the Declaration of Helsinki (Revised Tokyo 1978).

The capsules were administered with 150 ml mineral water to the volunteers in the morning after an overnight fast. A washout time of 2 weeks was observed between the two administrations. Five and 9 h after the administration a light carbohydrate meal was given. Blood samples were obtained by venepuncture from the cubital vein prior to dose and at 1, 2, 4, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120, 144, 168, and 192 h after each administration. The heparinized blood was processed to plasma immediately after collection by centrifugation of 1400*g* for 10 min. The plasma was kept frozen in glass tubes at −30°C until the analysis.

Analyses of the Enantiomers of Amlodipine in Plasma

A gas chromatographic method with chiral derivatization and electron capture detection was used.[6] The essential steps

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of the analytical procedure as well as quality parameters are as follows. Plasma was mixed with 10 μl of a methanolic solution of the internal standard (UK-52829-42, the 2,3-dichlorophenyl analogue of amlodipine). Then 6 ml *t*-butyl methyl ether and 2.0 ml of 0.2 *M* borate buffer were added and the mixture was shaken for 40 min on a rotation type mixer. Amlodipine and the internal standard were back-extracted from the separated organic phase into 1 ml citric acid (0.1 *M*). The aqueous extract was separated, alkalized with 1 *M* potassium carbonate solution (2 ml), and shaken with 6 ml *t*-butyl methyl ether for 40 min.

The phases were separated, the aqueous phase was discarded, and the ether phase was mixed by rotation with 100 μl of the chiral derivatization reagent [a dichloromethane solution of (−)-(R)-α-methoxy-α-trifluoromethylphenylacetyl chloride and 4-dimethylaminopyridine] for 20 min to form diastereomers. The samples were then mixed with 1 ml of 1 *M* potassium carbonate solution and shaken for 45 min in a rotating mixer to remove excess reagent. The organic layer was evaporated to dryness under nitrogen and the residue taken up in 80 μl ethyl acetate. Aliquots of 1 μl were analyzed by gas chromatography.

The chromatography column was a 30 m × 0.32 mm (i.d.) fused silicon type DB1 (J&W scientific, USA) with 0.10 μm film thickness. A Dani 6500 gas chromatograph with a 10 m Ci 63 Ni electron capture detector was employed. Hydrogen was used as carrier gas (5 ml/min) and nitrogen served to purge the detector (30 ml/min). The oven temperature was programmed from 65°C during the injection to 290°C at 20°C/min and then kept at 290°C for 25 min. The injector was programmed from 72°C to 295°C at 1300°C/min and then kept at 295°C. Under these conditions the retention time of the diasteromeric derivatives of amlodipine was 22 min, and the peak separation of the diastereomeric derivatives was greater than 85%, both for amlodipine and the internal standard.

The retention times of the derivatives of the enantiomers of amlodipine were identified by separate injection of the diastereomeric products of the purified amlodipine (R)- and (S)-enantiomers (obtained from Pfizer Central Research, Sandwich, UK). (R)-Amlodipine eluted first from the column. Quantitative evaluations were made on the basis of calibration curves, which ranged from single isomer concentrations of 0.02 to 15 ng ml^{-1}, using amlodipine racemate as standard compound.

The accuracy of the measured concentrations and concentration ratios of the enantiomers was assessed with test samples spiked with the purified enantiomers, at R/S ratios varying from 10:0 to 0:10. Approximately 20% of the study samples were analyzed in duplicate. The reproducibility of analyses duplicated within the same analytical batch amounted to 10.1% for R and to 6.2% for S (mean coefficient of variation at enantiomer concentrations ranging from 0.04 to 5.24 ng ml^{-1}). The within-batch precision of the assay ($n = 6$) came to 10.7 and 2.5% for the (R)- and to 11.7 and 2.8% for the (S)-enantiomer, at added concentrations of 0.52 and 5.23 ng ml^{-1}, respectively. Between-batch precision of standard samples was 9.9 and 11.3% (R) and 12.6 and 8.9% (S) at concentrations of 1.14 and 6.82 ng ml^{-1}, respectively; the lower limit of quantification was 0.02 ng ml^{-1} for each enantiomer, which was the lowest calibration point.

Pharmacokinetic and Statistical Calculations

Areas under the curve (AUC) were obtained as the sum of linear trapezoidal area and residual area C/k_{el} (last quantified concentration/elimination rate constant). The contribution of residual areas to the AUCs was from zero to 14% (median 3%) for the (R)- and from zero to 19% (median 6%) for the (S)-enantiomer. The oral clearance was calculated by dividing the dose (10 mg per enantiomer) by the AUC. Hepatic blood clearance was approximated from oral plasma clearance by application of the intrinsic clearance concept,[7] employing the formula $Cl_{Hb}^{-1} = BPR \times Cl_{oral}^{-1} + Q_{Hb}^{-1}$, where *BPR* equals the blood/plasma concentration ratio of the drug and Q_{Hb} the hepatic blood flow. For *BPR*, the average value of 1.74, as observed for total amlodipine (personal communication, M. J. Humphrey, Pfizer Central Research, Sandwich, UK), and for Q_{Hb} a value of 25 ml min^{-1} kg^{-1} were used. Cl_{Hb} was used to obtain estimates of the oral bioavailability from $F = 1 - Cl_{Hb}/Q_{Hb}$. For the application of the intrinsic clearance concept the assumption was made that the dose is completely absorbed and that no extrahepatic metabolism is present.

Termal elimination rate constants k_{el} were calculated by exponential regression of unweighted plasma concentrations employing the algorithm of Marquard.[8] The last 6–10 concentration–time points were used for the regression, depending on the combined criteria of a high coefficient of correlation, low confidence interval of the rate constant, and symmetric distribution of residuals. Coefficients of correlation ranged between 0.949 and 1.000 for R, between 0.962 and 0.999 for S, and between 0.961 and 0.993 for total (R + S)-amlodipine.

Total variances in the AUC, C_{max}, t_{max}, and k_{el} were split into variances due to subjects, treatments (besylate and maleate salt), and enantiomers by ANOVA. The *F*-test was applied to detect significant differences between the effects and confidence bounds on treatment-related differences were calculated,[9] taking the besylate form as standard. ANOVAs were performed in addition with the half-lives of the enantiomers and their R/S ratios, in order to separate intersubject from residual effects, the latter being interpreted as intrasubject effects.

Regression analyses were performed with the following parameters: k_{el} of the single enantiomers versus k_{el} of total amlodipine, and R/S ratio of k_{el} versus the ratio of Cl_{Hb}, the latter regression on the basis of averaged parameters from both administrations.

RESULTS

The (R)- and (S)-enantiomers of amlodipine were sufficiently separated and could be quantified in all study samples. Mean plasma concentration–time curves after administration of amlodipine racemate (as the besylate salt and maleate salt capsules) are shown in Figures 1 and 2. Mean values of the basic pharmacokinetic parameters are listed in Table 1.

On average, the plasma concentrations of (S)-amlodipine were found to be slightly but significantly higher than the concentrations of the (R)-enantiomer, after administration of either the besylate or the maleate salt of racemic amlodipine. The overall enantiomeric ratio R/S in the AUCs was 41:59 (expressed as percentages of the total). The lowest individual

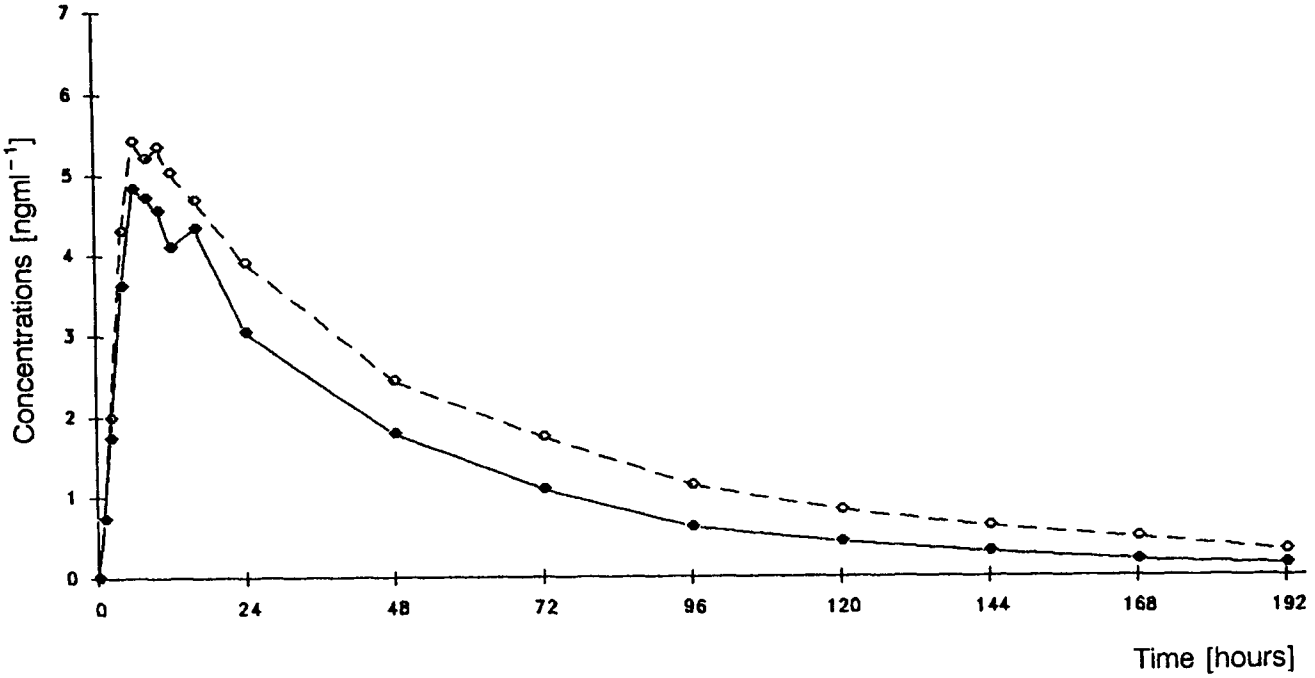

Fig. 1. Mean plasma concentrations of (S)-amlodipine (○) and (R)-amlodipine (●) following oral administration of 20 mg amlodipine racemate as the besylate salt to 18 healthy volunteers.

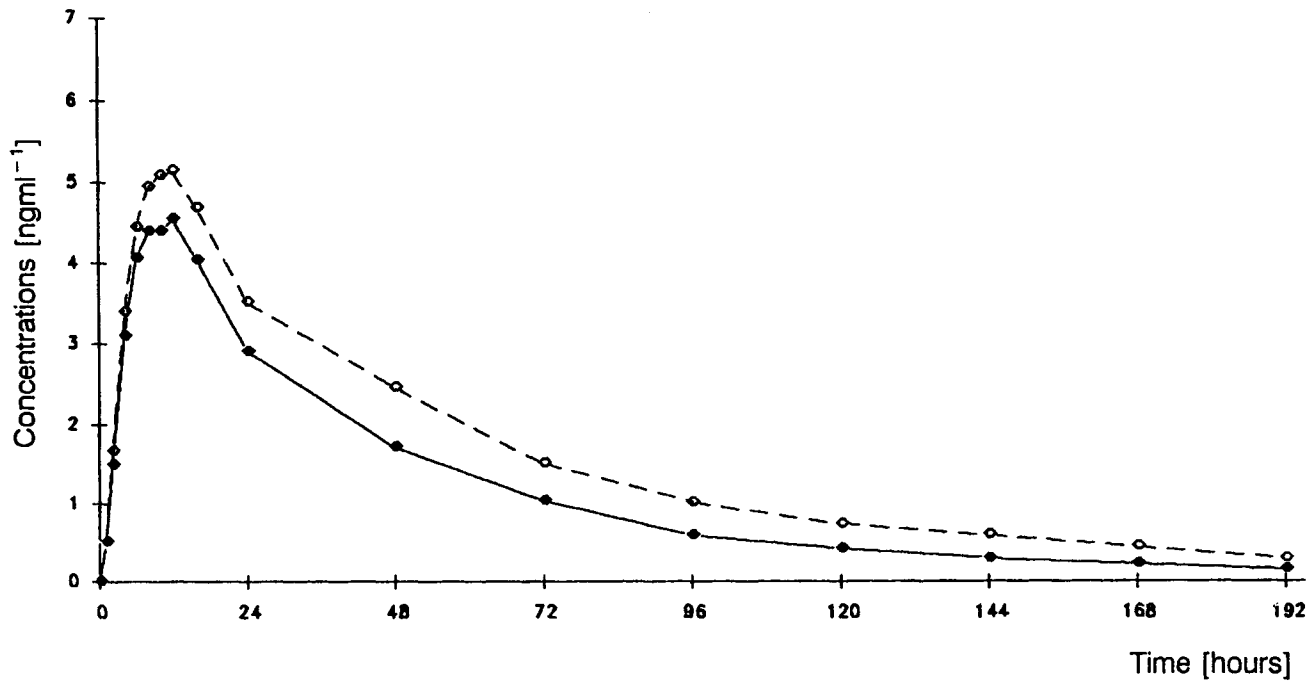

Fig. 2. Mean plasma concentrations of (S)-amlodipine (○) and (R)-amlodipine (●) following oral administration of 20 mg amlodipine racemate as the maleate salt to 18 healthy volunteers.

ratio came to 24:76, the highest was 50:50. In C_{max}, an overall enantiomeric ratio R/S of 47:53 was found. The corresponding difference in mean C_{max} (R 5.29 ng ml^{-1} and S 5.90 ng ml^{-1}) was small but statistically significant. Plasma peak times were almost equal for the two enantiomers.

The terminal elimination rate constants of both enantiomers could be determined with sufficient statistical reliability. Coefficients of correlation of the corresponding regression analyses ranged between 0.949 and 1.000 for R, between 0.962 and 0.999 for S, and between 0.961 and 0.993 for total (R +

TABLE 1. Mean values, standard deviations, and results of statistical tests of variances of pharmacokinetic plasma parameters of amlodipine and its enantiomers, following single oral administration of 20 mg amlodipine racemate as the besylate and maleate salts to 18 healthy volunteers

	Amlodipine enantiomers		Total amlodipine (S + R)	Significance test[a] on differences between	
	S	R		Enantiomers	Salt forms
AUC (h ng ml^{-1})					
Besylate	351 ± 72	240 ± 86	590 ± 144	$p = 0.0001$	$p = 0.252$
Maleate	330 ± 88	236 ± 89	566 ± 166		
C_{max} (ng ml^{-1})					
Besylate	6.13 ± 1.29	5.50 ± 1.36	11.51 ± 2.49	$p = 0.0011$	$p = 0.019$
Maleate	5.66 ± 1.09	5.07 ± 1.48	10.70 ± 2.49		
t_{max} (h)					
Besylate	8.4 ± 3.6	8.7 ± 3.6	8.2 ± 3.1	$p = 0.688$	$p = 0.0002$
Maleate	10.7 ± 3.4	10.9 ± 3.4	10.8 ± 3.4		
Cl_{oral} (ml min^{-1} kg^{-1})					
Besylate	6.9 ± 1.6	11.0 ± 5.3	8.2 ± 2.4	Not tested	Not tested
Maleate	7.3 ± 2.1	11.7 ± 6.8	8.8 ± 3.1		
k_{el} (10^4 h^{-1})					
Besylate	145 ± 36	208 ± 62	163 ± 36	$p = 0.0001$	$p = 0.233$
Maleate	156 ± 48	220 ± 63	170 ± 46		
$t_{0.5}$ (h)					
Besylate	50.6 ± 12.9	35.5 ± 8.2	44.4 ± 9.70	Not tested	Not tested
Maleate	48.7 ± 15.8	34.3 ± 110	44.0 ± 12.8		

[a] F-test of variances (limit of significance; $p < 0.05$).

S)-amlodipine. The elimination of the (S)-enantiomer from plasma was significantly slower than that of the (R). The overall mean values of half-lives (besylate and maleate data combined) amounted to 34.9 h for the (R)- and 40.6 h for the (S)-enantiomer. The intersubject variation in the elimination half-lives showed coefficients of 33.6% (R) and 38.9% (S), while the intrasubject coefficients of variation came to 20.1% (R) and 13.1% (S).

The elimination rate constants of the enantiomers were significantly correlated to the rate constant of total amlodipine after both administrations, the (S)-enantiomer displaying much stronger correlation than the (R)-enantiomer. Figure 3 shows the linear regression of k_{el} (S) after administration of amlodipine besylate. The factor of 0.89, which relates the elimination rate constant of (S)-amlodipine to that of total amlodipine in one part of the study (besylate), was employed to predict the half-lives of (S)-amlodipine in the other part (maleate), by the formula $t_{0.5,S} = t_{0.5,\text{total amlodipine}}/0.89$.

The predicted and actually measured half-lives of the (S)-enantiomer are compared in Table 2. The error in prediction, obtained from the total variance cleared of subject effects by ANOVA, was ±3.8 h (SD) or ±8% (CV). The linear regression of the enantiomeric ratios of k_{el} versus those of the hepatic blood clearance Cl_{Hb} is depicted by Figure 4. The estimates of oral bioavailability obtained through the application of the intrinsic clearance concept amounted to 80% (range 57 to 90%) for the (R)-enantiomer, and to 86% (79 to 91%) for (S), on average. There were significant differences in the C_{max} and t_{max} between the besylate and the maleate salt but not in the AUC or in the k_{el}, suggesting equal bioavailability of the two formulations, but slightly more rapid absorption from the besylate salt. Westlake's 95% confidence intervals for the maleate relative to the besylate were 87–113% for the AUC and 86–114% for C_{max}. The variance due to interaction of treatments and enantiomers was negligibly small (<0.2% of total SSQ).

DISCUSSION

The average plasma concentrations of the active (S)-enantiomer of amlodipine were slightly but statistically significantly higher than those of the inactive (R)-enantiomer, after oral administration of 20 mg racemic amlodipine to healthy volunteers. The mean R/S ratio of the AUCs came to about 40:60, varying from about 25:75 to 50:50 between individuals. Thus, the contribution of the active enantiomer to the AUC of total amlodipine (sum of R and S) depended on subjects and was equal or higher than that of the inactive form. The statistical analyses showed that there was no interaction between treatments (besylate and maleate) and enantiomers in any of the tested pharmacokinetic parameters, indicating that the observed enantioselectivity of amlodipine disposition was independent of the administered type of salt of the racemate.

The rate of absorption, as judged by plasma peak times, was equal for the two enantiomers, while the rate of elimination was higher for R than for S. The mean half-lives of elimination amounted to 34.9 h for the (R)- and 49.6 h for the (S)-form.

The enantioselectivity in the elimination of amlodipine from plasma was not very variable within subjects, but showed more variation between subjects: the coefficients of variation of the ratio R/S of k_{el} were 14.5% (intrasubject) and 30.5%

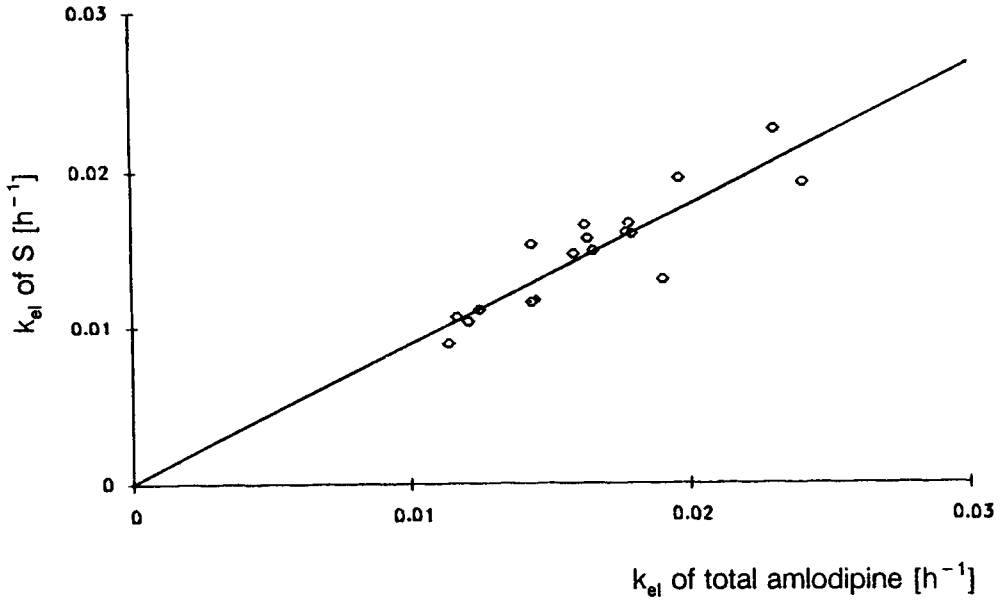

Fig. 3. Correlation of the elimination rate constants of (S)-amlodipine and total (S + R)-amlodipine. Regression line $y = 0.89x$, coefficient of correlation 0.892, significance of the regression $p < 0.00001$.

TABLE 2. Prediction of the half-lives of (S)-amlodipine on the basis of measured half-lives of total (S + R) amlodipine, after administration of 20 mg amlodipine racemate in form of the maleate salt

	$t_{0.5}$ (h)	
Subject no.	Predicted	Measured
1	36.0	26.7
2	49.6	47.5
3	42.8	38.6
4	33.5	34.7
5	55.9	59.8
6	85.6	85.5
7	61.9	56.6
8	41.8	38.9
9	53.7	54.2
10	66.5	62.5
11	50.8	52.1
12	33.7	42.9
13	50.9	38.1
14	65.0	75.3
15	34.5	33.4
16	59.7	57.5
17	32.3	30.0
18	39.6	41.4
Mean	49.7	48.7
SD	14.5	15.8

(intersubject). Due to its slower elimination the active (S)-enantiomer contributes much more to the terminal concentrations of total amlodipine than does the (R)-enantiomer. Consequently, the elimination rate constant of S was highly correlated to that of total amlodipine. We used the slope of the respective regression line obtained in one part of the study to predict the half-lives of S in the other part. There was excellent agreement between predicted and actually measured half-lives. The error in prediction (CV 8%) of the half-lives was even lower than their actual intraindividual variation (CV 13%). Thus, the half-lives of total amlodipine proved to be highly predictive for the half-lives of the active enantiomer. The relationship $t_{0.5,S} = t_{0.5,\text{ total amlodipine}}/0.9$, which was found in this group of subjects after single oral doses of racemic amlodipine, may, therefore, be adapted as a rule for the healthy young male Caucasian population. It remains to be examined if this relationship is also useful in patients in the clinical situation.

The present study does not allow us to quantify fully the contributions of bioavailability, distribution, and systemic clearance to the enantioselective disposition of amlodipine. However, the data can provide some insight into the relative importance of these factors, through application of the intrinsic clearance concept[7] to amlodipine, which is predominantly cleared by hepatic elimination.[3,10]

Assuming complete oral absorption and solely hepatic elimination and ignoring the possibility of chiral inversion as a significant metabolic pathway, the clearance concept predicts a slightly higher oral bioavailability (86%) for the active (S)- than for the inactive (R)-enantiomer (80%). Since the rate of absorption was practically equal for the enantiomers and was rapid compared to the rate of elimination, the relative height of the peak plasma concentrations would be expected to reflect differences in the oral bioavailability between the enantiomers—assuming equal volumes of distribution. Indeed, the average C_{max} of S was about 10% higher than the C_{max} of R. The largest intrasubject difference in the predicted bioavailabilities was that between 57% for R and 80% for S, and this

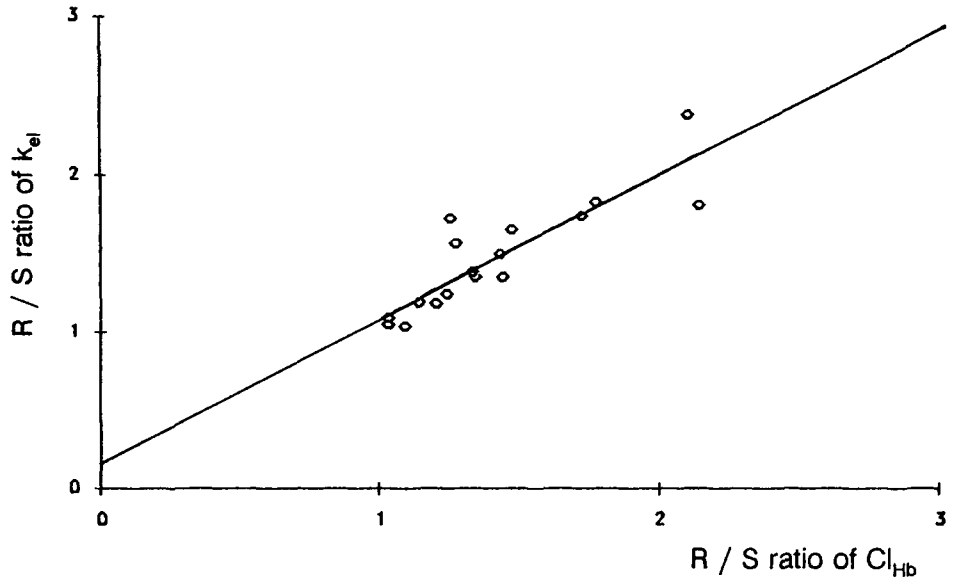

Fig. 4. Correlation of enantiomeric ratios R/S of the elimination rate constant and hepatic blood clearance (means from besylate and maleate parameters). Regression line $y = 0.17 + 0.92x$, coefficient of correlation 0.872, significance of the regression $p < 0.00001$.

corresponded well to the measured C_{max} of 2.80 ng ml^{-1} (R) and 4.21 ng ml^{-1} (S) in this subject.

There was a high correlation of the enantiomeric ratios of elimination rate constant and hepatic blood clearance (Fig. 4). The slope of the regression line was close to 1, which strongly suggests that the two enantiomers of amlodipine have similar volumes of distribution. This agrees with the expectation that it is chiefly nonspecific binding which leads to a distribution volume as large as described for racemic amlodipine.[3]

Taken together, the data suggest that the apparent differences in the pharmacokinetics of the enantiomers of amlodipine are well explained by differences in their systemic clearance. It is pointed out that this interpretation presumes the absence of chiral inversion.

CONCLUSION

The data from the present study provide evidence for the enantioselective disposition of oral amlodipine when administered as a racemate: mean values of C_{max} of the pharmacologically active (S)-enantiomer were slightly higher, and mean AUC values of (S)-amlodipine were markedly higher than the corresponding values for the (R)-enantiomer. It appears that the S/R ratio in AUCs, which was >1 in all subjects, reflects the lower clearance of the (S)-form, which is also evident in the longer half-life of this isomer.

The enantioselective disposition of amlodipine seems to favor the active isomer in other ways. The oral clearance was much less variable between subjects for the (S)- than for the (R)-enantiomer. Furthermore, the individual half-lives of the (S)-form were predictable with high probability from the total amlodipine concentrations, which are usually obtained without enantiospecific analytical methods.

The results of the application of the intrinsic clearance concept to the study data suggest that the observed enantioselectivity in the disposition of amlodipine is primarily due to differences in the systemic blood clearance of (R)- and (S)-amlodipine.

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