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Short communication

Enantioselective pharmacokinetics of lercanidipine in healthy volunteers

Valquiria Aparecida Polisel Jabor[a], Eduardo Barbosa Coelho[b], Vera Lucia Lanchote[a,*]

[a] *Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil*
[b] *Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil*

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Abstract

Objective: The enantioselective kinetic disposition of lercanidipine, a dihydropyridine type of third-generation calcium antagonist, was investigated in six healthy male volunteers following a single 20 mg racemic oral dose.
Methods: Serial plasma samples were obtained from 0 to 24 h after drug administration. Lercanidipine enantiomers were analysed using a chiral LC–MS–MS method.
Results: The following differences ($p < 0.05$, Wilcoxon test) between (*S*) and (*R*) enantiomers were found (median): C_{max} 2.071 ng mL^{-1} versus 1.681 ng mL^{-1}; AUC$^{0-24}$ 12.352 ng h mL^{-1} versus 10.063 ng h mL^{-1} and Cl/*f* 732.16 L h^{-1} versus 1891.84 L h^{-1}. The AUC$^{0-\infty}$ values for (*S*)-LER were 1.21-fold higher than those for (*R*)-LER.
Conclusion: The pharmacokinetics of LER was enantioselective in healthy volunteers following a single dose of 20 mg of the unlabeled racemic drug.

Keywords: Lercanidipine; Enantiomers; Pharmacokinetics; Healthy volunteers; Calcium antagonists; Dihydropyridines

1. Introduction

Calcium antagonists have been widely used for the treatment of cardiovascular disease, such as angina pectoris, arrhythmias and arterial hypertension [5]. Lercarnidipine (LER), a racemic mixture, is a third-generation dihydropyridine calcium antagonist used in the treatment of hypertension (Fig. 1). The dihydropyridine calcium antagonists promote systemic vasodilatation by a reversible blockade of voltage-dependent Ca^{2+} influx through L-type channels in the cell membrane. (*S*)-LER has 100- to 200-fold greater affinity than the (*R*)-enantiomer for the L-type calcium channel. LER has a bulky *bis*-phenylalkylamine side chain, which makes it highly lipophilic and results in its accumulation in the lipid bilayer of cell membranes in the arterial wall compartment. Consequently, the drug redistribution from this tissue is slow and is a rate-limiting step, which promotes a slow onset of action and a long duration of action [1,2]. Compared to other dyhidropyridines, LER has a weaker negative inotropic activity and a more vascular selectivity than amlodipine, felodipine, nitrendipine and nifedipine [1].

The pharmacokinectics of (*S*)-LER has been evaluated in healthy volunteers, in elderly and non-elderly patients with hypertension, and in patients with renal or hepatic impairment. Patients from these studies were investigated after receiving a single 10 or 20 mg dose of [^{14}C]-labeled *rac*-LER as a solution. The maximum plasma concentrations of (*S*)-LER were reached within 2–3 h and the area under the plasma concentration–time curves were not linearly related to the dose, indicating a saturable first-pass metabolism. The absorption of (*S*)-LER increases after the ingestion of a high-fat meal. LER is highly bound to plasma protein (>98%) in humans. Its volume of distribution of 2–2.5 L/kg was determined in healthy volunteers after intravenous infusion of 2 mg. LER is extensively metabolized by CYP 3A4 to

* Corresponding author. Tel.: +55 16 602 4699; fax: +55 16 633 1936.
E-mail address: lanchote@fcfrp.usp.br (V.L. Lanchote).

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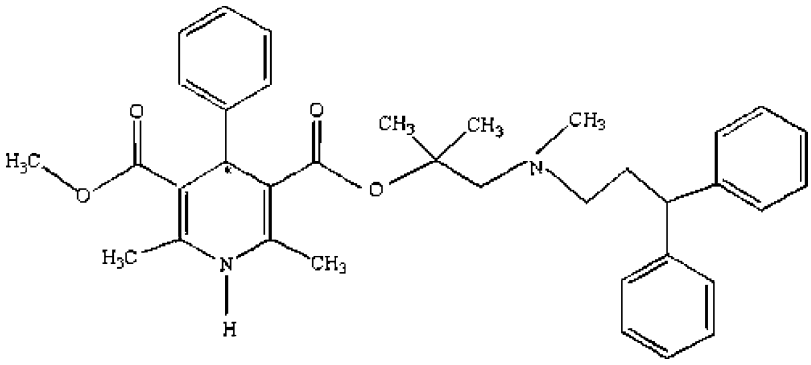

Fig. 1. Chemical structure of lercanidipine. (*) denotes the chiral center.

inactive pyridine derivatives. Terminal elimination half-lives of 2.8–3.7 h have been reported in healthy volunteers and patients with hypertension [4].

A crossover study involving a single administration of either 10 mg of (*R*)- or (*S*)-LER or 20 mg of *rac*-LER as a solution demonstrated no in vivo enantiomer interconversion [6].

Recently, a highly sensitive LC–MS–MS method to quantify plasma concentrations as low as 0.025 ng mL^{-1} of each LER enantiomer in humans was developed by our group [3]. This method permits pharmacokinetic studies without the use of radioactive labeled compounds.

In the present study, we describe the enantioselective pharmacokinetics of LER in healthy volunteers after a single 20 mg oral dose of a marketed available *rac*-LER formulation (Zanidip® 10 mg tablets) and compare the data with those obtained in a previous study using [^{14}C]-labeled LER.

2. Methods

2.1. Subjects

Six healthy male volunteers, age 25–45 years, weight 70–83 kg, were investigated after clinical and physical examinations. The study was approved by the Ethics Committee of the University Hospital, Faculty of Medicine of Ribeirão Preto (University of São Paulo, São Paulo, Brazil) and conducted in accordance with good clinical practice. Each volunteer gave written consent to participate after receiving written and verbal information describing the study. The volunteers were required to abstain from any medication and alcohol for 15 days prior to the study.

2.2. Study design

All volunteers received a single oral dose of 20 mg *rac*-LER (Zanidip® 10 mg tablets, Medley, Campinas, Brazil) with 200 mL of water after an overnight fast. Three and twelve hours after dosing, a light breakfast and dinner, respectively, were given to each volunteer.

Serial blood samples (10 mL) were collected via an intravenous catheter at predose ($t = 0$ h) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12 and 24 h postdose into heparinized tubes (Liquemine® 5000 IU, Roche). The samples were centrifuged (10 min at $2000 \times g$), and plasma was separated and stored at −70 °C until chromatographic analysis.

2.3. Plasma drug concentrations

Plasma concentrations of (*R*)- and (*S*)-LER were determined by an enantioselective LC–MS–MS method using an electrospray interface in the positive ion mode previously described by Jabor et al. [3]. Briefly, the internal standard (25 μL 0.66 μg mL^{-1} amiodarone solution) and sodium hydroxide solution (50 μL 0.1 mol L^{-1}) were added to the plasma samples (1.0 mL) and the LER enantiomers were extracted with a 4.5 mL mixture of hexane–isopropanol (99:1, v/v) by shaking in a vortex mixer for 2 min. After centrifugation, the organic phases were collected and evaporated to dryness and the residues were dissolved in 50 μL hexane–ethanol (95:5, v/v) plus 0.1% (v/v) diethylamine. Aliquots (20 μL) of the final extracts were analysed by LC–MS–MS on a Chiralpak® AD, 250 mm × 4.6 mm i.d. column with 10 μm particle size (Chiral Technologies Inc., Exton, PA, USA), with a guard column LiChrospher® 100 RP-18, 4 mm × 4 mm i.d., 5 μm (Merck, Darmstadt, Germany) and a mobile phase of a mixture of hexane–ethanol–diethylamine (95:5:0.1, v/v/v) at a flow-rate at 1.3 mL min^{-1}. MS–MS detection was carried out by a post-column infusion consisting of the addition of a 10 mmol L^{-1} ammonium acetate aqueous solution in ethanol (5:95, v/v). For MRM the ammonium adducts $[M + NH_4]^+$ and their respective product ions were monitored in two functions, 612.40 > 100.10 (0.0–5.0 min) for LER enantiomers and 646.30 > 100.30 (5.0–8.0 min) for the IS. Absolute recoveries up to 70% were obtained for both LER enantiomers. Limit of quantitation was 0.025 ng mL^{-1} for each LER enantiomer. Linearity of the method cover the range of 0.025 to 0.025–50 ng mL^{-1} for each LER enantiomer. The precision and accuracy shows coefficient of variation and reletive errors less than 15%.

2.4. Pharmacokinetic analysis

The enantioselective kinetic disposition of (*R*)- and (*S*)-LER following a single oral administration was analysed using a bicompartment model.

The maximum plasma concentration of each LER enantiomer (C_{max}) and the time to reach C_{max} (t_{max}) were determined directly from the observed plasma concentration–time original data. The distribution half-life ($t_{1/2}\alpha$) was determined after correction of the respective phase by method of residuals, whereas the elimination half-life ($t_{1/2}\beta$) was directly determined by the graphic method (log *c* versus *t*). The distribution (α) and elimination (β) rate constants were calculated using the $0.693/t_{1/2}\alpha$ and $0.693/t_{1/2}\beta$ equations, respectively. The area under the LER enantiomer concentration–time curve from time zero to the last point measured (AUC^{0-24}) was calculated using the linear trapezoid method and extrapolated to infinity ($AUC^{0-\infty}$). The apparent total clearance (Cl/*f*) and volume of distribution (Vd/f) were calculated from $Cl/f = dose/AUC^{0-\infty}$ and $Vd/f = Cl/\beta$ equations, respectively, where *f* is the fraction of dose reaching the systemic circulation as unchanged drug. Data were analysed statistically by the Wilcoxon test for paired nonparametric values, with the level of significance set at $p < 0.05$. The data are expressed as median and mean values (confidence intervals, 95% CI).

3. Results and discussion

The present study reports for the first time the pharmacokinetics of both LER enantiomers following administration of the drug in the form of a tablet.

The kinetic disposition data reported by Testa et al. [6] and McClellan and Jarvis [4] are related to the administration of [^{14}C]-labeled LER and the pharmacokinetic parameters were described only for the eutomer (*S*)-LER, based on administration of either the racemate or the single enantiomer.

The mean plasma concentration–time profiles of (*S*)- and (*R*)-LER for healthy male volunteers receiving a single oral administration of 20 mg *rac*-LER after an overnight fast are shown in Fig. 2. It can be observed that the levels of (*S*)-LER were always higher than those of (*R*)-LER.

The values for the assessed LER pharmacokinetic parameters are summarized in Table 1. The kinetic disposition of LER is enantioselective with C_{max}, AUC and Cl/*f* values significantly ($p < 0.05$) different between the enantiomers. The decay of plasma levels of (*S*)- and (*R*)-LER was biphasic, with distribution half-lives of 0.55 and 0.60 h and elimination half-lives of 9.30 and 7.50 h, respectively. The maximum plasma concentrations (C_{max}) were 2.07 and 1.68 ng mL^{-1} for (*S*)- and (*R*)-LER, respectively, and the time to reach C_{max} (t_{max}) was 1.25 h for both enantiomers. The $AUC^{0-\infty}$ values were 13.68 ((*S*)-LER) and 11.24 ((*R*)-LER) ng h mL^{-1}. Similar values for C_{max} (3.2 ng mL^{-1}) and AUC (9.05 ng h mL^{-1}) for (*S*)-LER were reported by McClellan and Jarvis [4] following a single 20 mg dose of racemic LER

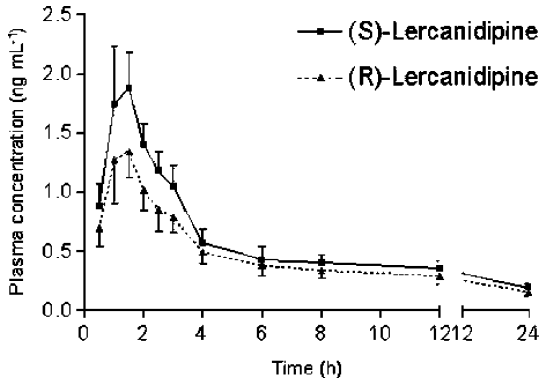

Fig. 2. Plasma concentration–time curves for (*S*)- and (*R*)-lercanidipine after a 20 mg oral dose of racemic lercanidipine administered to six healthy male volunteers. Data are expressed as mean ± S.E.M.

administered to male volunteers. In addition, Testa et al. [6], in a crossover study of LER in tablet form or in soft gelatin capsules containing the drug in solution, detected 87% as relative AUC and a slightly shifted t_{max} (1.5 h) for the tablet form.

The present results show that the $AUC^{0-\infty}$ values for (*S*)-LER were 1.21-fold higher than those for (*R*)-LER. Similar results were obtained by Testa et al. [6] following administration of 20 mg of the racemate. Considering that no in vivo enantiomer interconversion was reported by Testa et al. [6], the higher plasma levels of (*S*)-LER than of (*R*)-LER could be due to the enantioselectivity in the first-pass metabolism and/or to enantioselectivity in plasma protein binding. There

Table 1
Enantioselective kinetic disposition of lercanidipine in healthy male volunteers ($n = 6$)

	(*S*)-LER	(*R*)-LER
C_{max} (ng mL^{-1})	2.07	1.68*
	2.37 (1.35–3.39)	1.62 (0.89–2.35)
t_{max} (h)	1.25	1.25
	1.21 (0.75–1.50)	1.21 (0.75–1.50)
$t_{1/2}\alpha$ (h)	0.55	0.60
	0.62 (0.50–1.00)	0.80 (0.40–1.80)
$t_{1/2}\beta$ (h)	9.30	7.50
	8.45 (6.20–10.00)	7.80 (6.00–10.00)
$AUC^{0-\infty}$ (ng h mL^{-1})	13.68	11.24*
	13.25 (7.60–18.90)	10.79 (6.10–15.48)
Cl/*f* (L h^{-1})	732.16	891.84*
	899.61 (423.25–1376.00)	1128.30 (456.86–1799.70)
$AUC^{0-\infty}$ (*S*)/(*R*)	1.21	
	1.24 (1.09–1.40)	

Data are reported as median and mean (CI 95%). C_{max}: maximum plasma concentration; t_{max}: time to reach C_{max}; $t_{1/2}\alpha$ = distribution half-life; $t_{1/2}\beta$: elimination half-life; $AUC^{0-\infty}$: area under the plasma concentration–time curve; Cl/*f*: apparent total clearance.

* $p < 0.05$, Wilcoxon test.

are no data regarding protein binding or bioavailability for the individual enantiomers.

The data obtained in the present investigation are related to the administration of unlabeled 20 mg LER as racemate. The results could not be compared to the data obtained with the administration of 10 mg of each of the two enantiomers due to saturation of the first-pass metabolism. In their crossover study investigating pharmacokinetic linearity following a single LER dose of 10, 20 and 40 mg, Testa et al. [6] obtained AUC ratios of 1:4:18, respectively.

In conclusion, the pharmacokinetics of LER was enantioselective in healthy volunteers following a single 20 mg dose of the unlabeled racemic drug.

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References

[1] L. Guarneri, P. Angelico, M. Ibba, E. Poggesi, C. Taddei, A. Leonardi, R. Testa, Arzneim. Forsch./Drug Res. 46 (1996) 15.
[2] L.G. Herbette, M. Vecchiarelli, A. Leonardi, J. Cardiovasc. Pharmacol. 29 (Suppl. 1) (1997) S19.
[3] V.A.P. Jabor, E. Coelho, D.R. Ifa, P.S. Bonato, Santos NAG, V.L. Lanchote, J. Chromatogr. B 796 (2003) 429.
[4] K.J. McClellan, B. Jarvis, Drugs 60 (2000) 1123.
[5] F.H. Messerli, Am. J. Hypertens. 15 (2002) 94S.
[6] R. Testa, A. Leonardi, A. Tajana, E. Riscassi, R. Magliocca, A. Sartani, Cardiovasc. Drug Rev. 15 (1997) 187.