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PHARMACOKINETICS AND DISPOSITION

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Dynamic and kinetic disposition of nisoldipine enantiomers in hypertensive patients presenting with type-2 diabetes mellitus

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Abstract *Objective*: Nisoldipine (N) is a dihydropyridine calcium antagonist marketed as a racemic mixture and used for the treatment of hypertension. In the present study, we investigated the influence of type-2 diabetes mellitus (DM) on the enantioselective pharmacokinetic and dynamic parameters of N. *Methods*: Seventeen hypertensive patients, nine of them with DM, were investigated in a cross-over study with administration of rac-N as coat-core tablets (20 mg day^{-1}) or placebo for 15 days each. Serial blood samples (0–24 h) were collected on the 15th day, and 24-h ambulatory blood pressure (BP) monitoring was simultaneously evaluated. N enantiomers in plasma samples were analysed using chiral high-performance liquid chromatography combined with gas chromatography/mass spectrometry. The enantiomeric ratios differing from one were evaluated using the Wilcoxon test, and the results are reported as means with the 95% confidence intervals. A lidocaine (L) test was carried out as an in vivo marker of CYP3A4 (and CYP1A2) activities. *Results*: The following differences were observed between the (+)-N and (–)-N enantiomers, respectively, in the patients presenting with DM (means and ranges): C_{max} 3.9 (1.7–6.1) ng ml^{-1} versus 0.7 (0.4–1.0) ng ml^{-1}, AUC_{0-24} 51.5 (29.0–74.0) ng ml^{-1} h versus 9.4 (5.9–12.8) ng ml^{-1} h, and Cl/f 3.6 (1.9–5.4) l h^{-1} kg^{-1} versus 18.7 (11.7–25.7) l h^{-1} kg^{-1}. The Cl/f value of (+)-N was lower (Mann-Whitney test) in patients with DM: 6.0 (4.3–7.5) l h^{-1} kg^{-1} versus 3.6 (1.9–5.4) l h^{-1} kg^{-1}. The same observation was made for the (–)-N, with Cl/f reaching 38.8 (26.8–51.0) l h^{-1} kg^{-1} and 18.7 (11.7–25.7) l h^{-1} kg^{-1} for the non-diabetic and DM groups, respectively. The L test resulted in higher ratios ($P < 0.05$) of plasma L/MEGX concentrations (30 min after i.v. L) for DM (11.1 vs 18.6). N significantly reduced systolic and diastolic BP ($P < 0.05$, Wilcoxon test) in all patients investigated relative to placebo. No differences in BP reduction were observed between diabetic and non-diabetic patients. N significantly increased noradrenaline concentrations in plasma of both patient groups. The data also demonstrated that the plasma concentrations of noradrenaline 30 min after N administration were lower ($P < 0.05$) in diabetic (mean 2.86 pmol ml^{-1}) than in non-diabetic patients (4.80 pmol ml^{-1}).
Conclusions: The present data permit us to infer that type-2 diabetes mellitus alters the kinetic disposition of the (+)-N eutomer and (–)-N distomer, presumably due to a lower activity of CYP3A4, although it does not modify the clinical effect brought about by the reduction in BP.

Keywords Nisoldipine · Diabetes · Hypertension

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Introduction

Nisoldipine [(±) 3-isobutyl-5-methyl-1,4-dihydro-2, 6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylate] (Fig. 1), a calcium antagonist of the 1,4-dihydropyridine class, is clinically used as an anti-angina and vasodilating agent [1, 2, 3, 4, 5]. The drug is administered by the peroral route in the form of coat core tablets in a single daily dose [6, 7]. Nisoldipine is a chiral drug commercially sold as racemate, with still undefined absolute configuration of the (+) and (–) enantiomers [6, 7]. The antihypertensive activity of the (+)-nisoldipine enantiomer in rats is about 20 times higher than that of the (–) antipode [6].

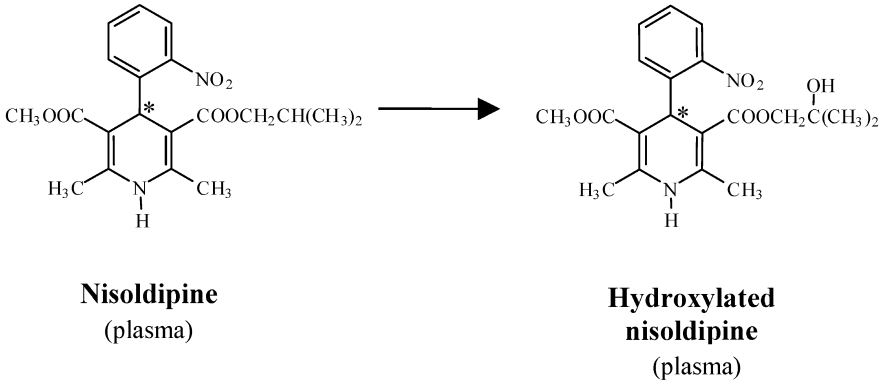

Fig. 1 Structures of nisoldipine and its active metabolite

Stereoselectivity in the clinical pharmacokinetics of nisoldipine was first described after i.v. or p.o. administration to healthy volunteers of the pseudoracemate containing 4×13 C-labelled (+)-nisoldipine and non-labelled (–)-nisoldipine [8]. Intravascular administration of pseudoracemic or racemic nisoldipine does not result in relevant differences in the plasma concentrations of the enantiomers [6, 7, 8]. Heinig et al. [6] investigated stereoselectivity in the pharmacokinetics of nisoldipine administered as racemate by the oral route as a controlled-release formulation to healthy volunteers and to elderly hypertensive patients. The ratios of the (+)/(–) plasma concentrations varied from 5 to 13 times, respectively, in elderly hypertensive patients (multiple dose) and in healthy volunteers (single dose), with the ratio being higher during the period from 2.5 h to 4 h (15.4 to 16.2) and lower between 7 h and 14 h (6.8 to 9.2) after administration.

The absolute bioavailability of nisoldipine in the form of coat core tablets is low (3.7–8.4%) because of its marked presystemic elimination [9, 10]. Nisoldipine is strongly bound to plasma proteins (>99%) with a high volume of distribution (2.7–5.9 l kg^{-1}) indicating extensive distribution towards the tissues [10, 11]. The systemic clearance of 0.83–1.17 l min^{-1} characterises a drug with a high hepatic extraction rate and with clearance mainly depending on hepatic blood flow [11]. Reported values for the apparent terminal half-life have ranged between 9.1 h to 14 h when the controlled-release formulation is administered [11].

Nisoldipine is mainly eliminated by metabolism. Oxidation of 1,4-dihydropyridine (nisoldipine) to pyridine, catalysed by CYP3A4, is the major metabolic route [12]. Hydroxylation of the isobutyl ester of nisoldipine or of the pyridine metabolite represents the second major metabolic pathway [11, 13]. Among the metabolites identified in human plasma, only the hydroxylated isobutyl ester of nisoldipine maintains the function of 1,4-dihydropyridine and presents a pharmacological activity qualitatively similar to that of nisoldipine in experimental animals [11].

CYP3A4 represents 30–40% of the total CYP present in human liver. CYP3A4 is involved in the metabolism of nisoldipine and of a large number of therapeutic agents, among them lidocaine, cyclosporine, erythromycin, midazolam, triazolam, and nifedipine. The endogenous substrates include steroids such as testosterone, cortisol, progesterone, androstanediol, dihydroepiandrosterol-3-sulfate, and estradiol. CYP3A4 activity can be induced in vivo by corticosteroids such as dexamethasone, anticonvulsants such as phenobarbital, phenytoin and carbamazepine, and antimicrobial agents such as rifampicin and rifapentin, and can be inhibited by erythromycin, clarithromycin, troleandomycin, gestodene, ritonavir, and fluvoxamine [14, 15].

CYP3A activity varies up to ten times among patients considered non-induced. Intersubject variability in CYP3A4 activity and expression currently cannot be attributed to a major genetic polymorphism and therefore information about the in vivo activity of the isoform is relevant for a rational adjustment of the dosage regimen for drugs that are CYP3A4 substrates [15]. Deethylation of lidocaine, which depends on CYP3A4 (and also on CYP1A2), results in the formation of the major monoethylglycinaxilidide (MEGX) metabolite [16]. The MEGX metabolite is deethylated and hydrolysed to 4-hydroxy-xylodine, the main urinary metabolite. In human liver microsomes, lidocaine is also hydroxylated to 3-hydroxy-lidocaine [17, 18]. Lidocaine is a drug of medium to high hepatic extraction (0.6 to 0.8) with oral clearance depending both on enzymatic activity and hepatic blood flow [19, 20]. However, when lidocaine is administered intravascularly, the plasma concentrations of the MEGX metabolite primarily reflect the activity of CYP3A4 and CYP1A2 together. The MEGX test is used in clinical practice as a hepatic function test, particularly for donors of liver for transplant and also as an indicator of progressive hepatic disease [21, 22].

Hypertension is an extremely common co-morbid condition in diabetes, affecting more than 50% of patients with diabetes mellitus (DM). In recent years, clinical trials have demonstrated the effectiveness of aggressive treatment of hypertension in reducing macrovascular and microvascular diabetes complications. Thus, a target blood pressure (BP) goal of lower than 130/80 mmHg is now recommended. It must be noted that many patients will require three or more drugs,

including calcium antagonists of the dihydropyridine class, to achieve BP control [23].

Clinical and experimental studies have demonstrated that DM differentially modifies the expression of cytochrome P_{450} (CYP) isoforms [24]. DM experimentally induced in male rats tends to suppress the expression of CYP1A2, CYP2C11, CYP2C13 and CYP3A2 and to induce the expression of CYP2A1, CYP2B1, CYP2C12, CYP4A1 and CYP2E1 [25, 26]. Matzke et al. [27] reported that the metabolism of antipyrine is increased in patients with type-1 diabetes and suggested this to be due to an increase in CYP1A2 activity. Hannon-Fletcher et al. [28] reported that in patients with insulin-dependent diabetes, the expression of CYP2E1 in peripheral blood lymphocytes is elevated.

The objective of the present study was to assess the effect of type-2 DM on the pharmacokinetics of the (+) and (–)-nisoldipine enantiomers in hypertensive patients treated with controlled-release racemic nisoldipine using lidocaine as a marker of in vivo CYP3A4 and CYP1A2 activities. Pharmacodynamic parameters such as plasma noradrenaline concentration, heart rate and systolic and diastolic BP were also investigated.

Subjects and methods

Subjects

The study was conducted on 17 patients with systemic arterial hypertension (defined as an office BP ≥140/90 mmHg) with or without type-2 DM; Table 1. Glycaemia was assessed by plasma glucose concentrations using standard methods. Informed consent was obtained from all patients. The study was approved by the research ethics committee of the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil. The clinical characteristics of the subjects investigated, with liver and renal function within the normal range, are shown in Table 1. Subjects were separated into two groups according to their plasma glucose levels:

- Non-diabetic: plasma glucose concentrations < 126 mg dl^{-1}, $n = 8$
- Diabetic: plasma glucose concentrations > 126 mg dl^{-1}, $n = 9$

Study design

The study was single blind and placebo controlled and was carried out in two phases: some patients started the investigation with a placebo (phase I) followed by administration of with nisoldipine (phase II), and other patients started the study directly with nisoldipine administration (phase II) followed by a placebo (phase I).

In phase I of the investigation, the patients received one placebo tablet administered on the morning for 15 days. On the 15th day, immediately after the last placebo dose, the patients were equipped with a 24-h ambulatory BP monitor (SpaceLabs 90207, Redmond, Wash.), programmed to read BP and heart rate every 15 min from 0800 hours to 2200 hours and every 30 min from 2200 hours to 0800 hours. Mean daytime (0800–2200 hours) and night time (2200–0800 hours) BP and heart rate were calculated. In phase II, the patients received one coat core 20-mg tablet of racemic nisoldipine (Syscor AP, Zeneca). On the 15th day of treatment, the patients were admitted to the hospital for serial blood collections after a 12-h fast and received the daily dose of nisoldipine in the morning. The breakfast was served 2 h after the drug administration. Blood samples (approximate 5-ml volumes in syringes heparinised with Liquemine 500 IU, Roche) were obtained over the 24-h dose interval at times zero, 0.5, 1, 2, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 20 and 24 h, centrifuged for 20 min at 1800 *g*, and plasmas were stored at –20°C protected from light.

All patients admitted to the study were submitted to the lidocaine test. During the hospitalisation phase they received 1 mg kg^{-1} lidocaine hydrochloride (2% Xylocaine, Astra) by the intravascular route, i.e. a slow infusion over a period of 1–2 min. Blood samples (approximate volumes of 5 ml in heparinised syringes) were obtained immediately before infusion and 15 min and 30 min after the end of infusion. Plasma was obtained by centrifugation at 1800 *g* for 20 min and stored at –20°C until the time for analysis. Electrocardiographic monitoring was performed during lidocaine administration, although there are no reports of the onset of convulsions or of the induction of cardiac arrhythmia during this process [29].

Blood samples for the determination of noradrenaline concentration in plasma were obtained during three different phases: at the end of the wash-out period of 15 days, at the end of placebo administration, and at the end of nisoldipine administration (15 days). Collections were made with the patient in the supine position and in a state of relaxation 30 min after catheter insertion. Blood samples (approximate volumes of 5 ml) were transferred to tubes immersed in ice containing 50 µl of a solution of EGTA (ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid) and glutathione (4.75 g EGTA and 3 g glutathione dissolved in 50 ml water with pH adjusted to 6–7). The blood samples were

Table 1 Characteristics of the hypertensive patients investigated. Data are reported as means (min–max). *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *δ-GT* δ-glutamyltransferase

	Non-diabetic	Diabetic
n	8	9
Sex (male/female)	(1/7)	(2/7)
Age (years)	47.6 (29.0–64.0)	53.2 (41.0–63.0)
Weight (kg)	76.5 (62.0–89.0)	74.4 (46.0–118.0)
Height (cm)	162.4 (156.0–180.0)	157.3 (147.0–167.0)
Combined drugs	Aminophylline	Glibenclamide, metformine, aminophylline, chlorpropamide, ASA
Glucose (mg dl^{-1})	92.5 (68.8–108.0)	181.5* (130.0–311.0)
Serum creatinine (mg dl^{-1})	0.9 (0.7–1.2)	0.8 (0.5–0.9)
$Cl_{Creatinine}$ (ml min^{-1}/1.73m^2)	85.6 (63.0–129.0)	95.9 (74.6–116.0)
Urea (mg dl^{-1})	35.6 (22.0–47.0)	33.3 (18.0–49.6)
AST (U l^{-1})	20.21 (14.0–28.2)	18.1 (10.0–22.8)
ALT (U l^{-1})	19.9 (12.8–28.2)	27.7 (22.0–36.0)
δ-GT (U l^{-1})	26.6 (16.8–34.0)	33.0 (18.0–51.6)
Na (mEq l^{-1})	144.1 (142.0–148.0)	141.8 (134.0–147.0)
K (mEq l^{-1})	4.4 (3.8–5.2)	4.4 (4.0–4.7)
[a]Office systolic BP (mmHg)	162 (142–168)	156 (152–163)
[a]Office diastolic BP (mmHg)	89 (84–95)	90 (87–96)

[a]Office systolic BP and diastolic BP were read at the end of the wash-out period
*$P < 0.05$, unpaired *t*-test

centrifuged at 4°C (2400 *g* for 5 min) and plasma aliquots were stored at –70°C until analysis.

Methods

Analytical procedure for measurement of nisoldipine enantiomers in plasma

The plasma levels of nisoldipine enantiomers were determined by chiral high-performance liquid chromatography combined with gas-chromatographic/mass spectrometry (GC-MS) as described by Marques et al. [30]. Aliquots (2 ml) of plasma samples were basified and extracted over a period of 1 h (horizontal shaker, 220 ± 10 cycles min^{-1}) with 8 ml toluene and the residues were reconstituted in the mobile phase and used for high-performance liquid chromatography (HPLC). The enantiomers were resolved on a Chiralcel OD-H column using hexane:ethanol (97.5:2.5, v/v) and the (+)- and (–)-fractions were collected separately with the diode array detector switched off. After evaporation of the organic solvent, each fraction was resuspended in toluene and added to a solution of the internal standard (nitrendipine). For the quantification of the isolated enantiomers of nisoldipine, a GC-MS with an Ultra 1 Hewlett-Packard column was used with the detector operated in the single-ion monitoring mode with electron-impact ionisation (*m/z* 371.35 and 270.20 for nisoldipine and *m/z* 360.00 for the internal standard, nitrendipine). The limit of quantification was 0.05 ng ml^{-1} for each enantiomer and the linear range was 0.05–50.0 ng ml^{-1} for each enantiomer. The coefficients of variation for both within-day and between-day assays were below 15%. The mean concentrations obtained for both nisoldipine enantiomers were within the 15% limit of the real values. All analytical procedures were carried out under yellow light since nisoldipine is photosensitive.

Analytical procedure for measurement of lidocaine and its metabolite MEGX in plasma

Plasma samples (1 ml) were extracted with basic medium containing 4 ml hexane:dichloromethane (82:18, v/v) after saturation of the aqueous phase with 500 mg sodium chloride (45 min in a horizontal shaker, 220 ± 10 cycles min^{-1}). The residues were taken up again in the mobile phase and 20-µl aliquots were analysed using HPLC. Lidocaine and the MEGX metabolite were separated on a Lichrospher 60 RP-Select B column (Merck, Darmstadt, Germany) with the mobile phase consisting of a mixture of 25 mM phosphate buffer, pH 4.5, and acetonitrile (82:18, v/v) with an elution rate of 1 ml min^{-1}. The system functioned with the ultraviolet detector operating at 205 nm.

Analytical procedure for the measurement of noradrenaline in plasma

Plasma noradrenaline was analysed using the method of Forster and Macdonald [31]. Extracts of 500 µl plasma samples were analysed by HPLC using a system with an electrochemical detector with a Ag/AgCl electrode operating with a +0.70-V potential. Catecholamines were separated on an end-capped Purospher RP-18 column (Merck, Darmstadt, Germany). The calibration curve was constructed for the 1–10 pmol noradrenaline/ml plasma interval.

Pharmacokinetic analysis

The enantioselective kinetic disposition of nisoldipine was evaluated in a steady state for the 0- to 24-h dose interval [1, 4, 5]. Maximum plasma concentration (C_{max}) and time to reach C_{max} (t_{max}) were calculated directly from the experimental data. The area under the curve concentration versus time in the steady state was calculated during the dose interval (AUC_{ss}^{0-24}) using the trapezoidal method. This parameter was used for the calculation of apparent clearance ($Cl/f = dose/AUC_{ss}^{0-24}$).

Statistical analysis

The statistical tests were applied with the aid of the GraphPad Instat software in order to obtain the median, mean, 95% confidence interval and standard error of the mean (SEM). The Wilcoxon test was used to evaluate the pharmacodynamic data and the ratios of the (+)/(–)-nisoldipine enantiomers, and the Mann-Whitney test was used for the evaluation of the effect on type-2 DM on the enantioselective pharmacokinetics of nisoldipine, with the level of significance set at $P < 0.05$.

Nonparametric Spearman correlation was used to evaluate the correlation between the individual data of the ratios of plasma lidocaine/MEGX concentration and the apparent clearance of nisoldipine enantiomers obtained for diabetic and non-diabetic hypertensive patients.

Results

The plasma concentration profiles of the nisoldipine enantiomers are shown in Fig. 2 and the pharmacokinetic parameters are summarised in Table 2. Concentration and consequently the AUC for plasma concentration versus time was higher for the (+)-nisoldipine enantiomer than for the (–)-nisoldipine antipode for diabetic (51.47 ng ml^{-1} h vs 9.35 ng ml^{-1} h) and non-diabetic (26.50 ng ml^{-1} h vs 3.84 ng ml^{-1} h) hypertensive patients. The AUC (+)/AUC(–) ratios ranged from 5.71 to 6.97, respectively, for the diabetic and non-diabetic groups, with no significant differences between groups. Figure 3 shows that the (+)/(–) enantiomer ratios were not constant during the 24-h dose interval. The ratio increased up to the time when maximum plasma concentration was observed (t_{max}),

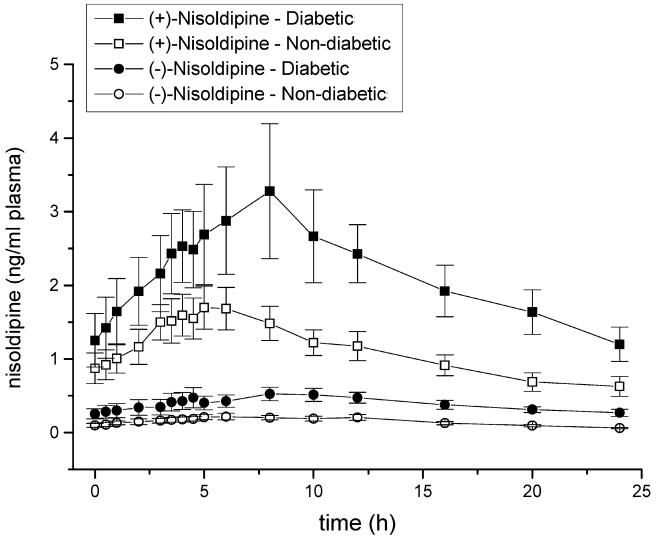

Fig. 2 Plasma concentrations of nisoldipine enantiomers versus time for diabetic and non-diabetic hypertensive patients treated with rac-nisoldipine (20 mg/24 h) as coat core tablets for 15 days. Mean ± SEM

Table 2 Kinetic disposition of (+) and (–)-nisoldipine in hypertensive patients with or without type-2 diabetes mellitus. Data are reported as mean (95% CI) and median. t_{max} time to reach C_{max}, C_{max} maximum plasma concentration, AUC plasma concentration versus time area under the curve, Cl/f apparent total clearance

Parameter	Non-diabetic ($n=8$) (+)-Nisoldipine	Non-diabetic ($n=8$) (–)-Nisoldipine	Diabetic ($n=9$) (+)-Nisoldipine	Diabetic ($n=9$) (–)-Nisoldipine
t_{max} (h)	5.37 (3.72–7.03) 5.50	6.75 (4.27–9.23) 5.50	6.61 (4.23–8.99) 6.00	8.89 (6.69–11.09) 10.00
C_{max} (ng·ml^{-1})	1.99 (1.32–2.66) 1.89	0.27[a] (0.17–0.37) 0.27	3.89 (1.72–6.05) 3.00	0.68[a*] (0.39–0.98) 0.52
AUC (ng·ml^{-1}·h)	26.50 (17.47–35.52) 22.21	3.84[a] (2.81–4.86) 3.53	51.47* (28.95–74.01) 44.01	9.35[a*] (5.85–12.84) 7.88
Cl/f (l·h^{-1}·kg^{-1})	5.91 (4.31–7.50) 6.07	38.85[a] (26.75–50.96) 33.41	3.64* (1.94–5.35) 3.45	18.70[a*] (11.69–25.70) 18.35
AUC (+)/(–)	6.97 (5.71–8.22) 7.17		5.71 (3.61–7.81) 4.73	

*$P<0.05$ Mann-Whitney test, non-diabetic versus diabetic
[a]$P<0.05$ Wilcoxon test, (–)-nisoldipine versus (+)-nisoldipine

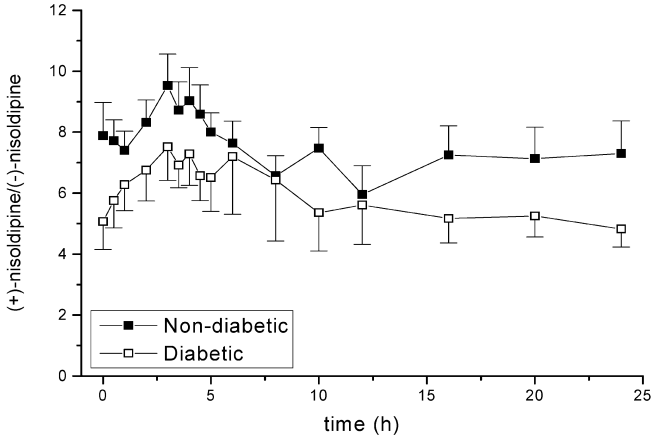

Fig. 3 Plasma concentration ratios of (+) and (–)-nisoldipine during the dose interval of 24 h in diabetic ($n=9$) and non-diabetic ($n=8$) hypertensive patients treated with rac-nisoldipine (20 mg/24 h) as coat core tablets for 15 days. Mean ± SEM

suggesting preferential metabolism of the (–)-nisoldipine enantiomer, and decreased thereafter to reach values close to those observed immediately before dihydropyridine administration (Fig. 3).

The AUC for plasma (+)-nisoldipine concentration versus time, expressed as the mean, ranged from 26.50 ng ml^{-1} h (non-diabetic) to 51.47 ng ml^{-1} h (diabetic) ($P<0.05$), characterising the influence of type-2 DM on the stereoselective pharmacokinetics of nisoldipine. The same observation was made for the (–)-nisoldipine enantiomer, with the AUC parameter increasing from 3.84 ng ml^{-1} h to 9.35 ng ml^{-1} h, respectively, for the non-diabetic and diabetic groups (Table 2). The increased plasma concentrations of both nisoldipine enantiomers in the diabetic group is the consequence of a lower apparent clearance for the (+)-nisoldipine (5.91 l h^{-1} vs 3.64 l h^{-1}) and (–)-nisoldipine (38.85 l h^{-1} vs 18.70 l h^{-1}) enantiomers in these patients.

The lidocaine test carried out as an in vivo marker of activities of CYP3A4 (and CYP1A2) resulted in higher ratios of plasma lidocaine/MEGX concentrations (15 min and 30 min after i.v. administration of lidocaine) for hypertensive patients with type-2 DM (11.07 vs 18.58) (Table 3).

Significant correlations ($P<0.05$) were found between the ratios of plasma lidocaine/MEGX concentrations and the apparent clearances of nisoldipine enantiomers obtained for both groups (Fig. 4).

The data presented in Table 4 demonstrate that plasma noradrenaline concentrations were increased during nisoldipine administration.

Table 3 Lidocaine and monoethylglycinaxilidide (MEGX) plasma concentrations in hypertensive patients with or without type-2 diabetes mellitus. Data are reported as means (95% CI) and median

	Non-diabetic ($n=8$)	Diabetic ($n=9$)
Lidocaine 15 min (μg·ml^{-1})	1.88 (1.29–2.47) 1.74	2.27 (1.85–2.69) 2.29
MEGX 15 min (μg·ml^{-1})	66.54 (39.58–93.50) 62.55	42.53 (26.05–59.01) 40.0
Lidocaine/MEGX 15 min	33.16 (13.60–52.72) 28.25	61.32* (34.77–87.87) 56.30
Lidocaine 30 min (μg·ml^{-1})	0.78 (0.52–1.03) 0.75	0.84 (0.53–1.14) 0.73
MEGX 30 min (μg·ml^{-1})	75.96 (49.83–102.09) 71.95	48.86 (30.62–67.09) 42.90
Lidocaine/MEGX 30 min	11.07 (7.22–14.93) 10.8	18.58* (12.26–24.91) 20.60

*$P<0.05$, Mann-Whitney test

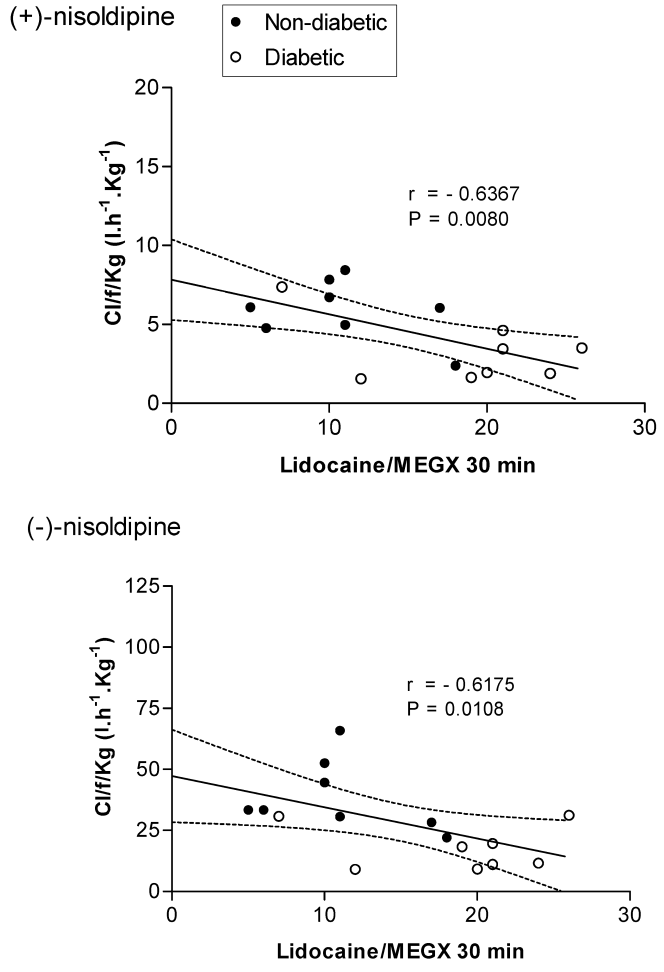

Fig. 4 Correlation between the individual data of the ratios of plasma lidocaine/MEGX concentration and the apparent clearance of nisoldipine enantiomers obtained for diabetic ($n=9$) and non-diabetic ($n=8$) hypertensive patients

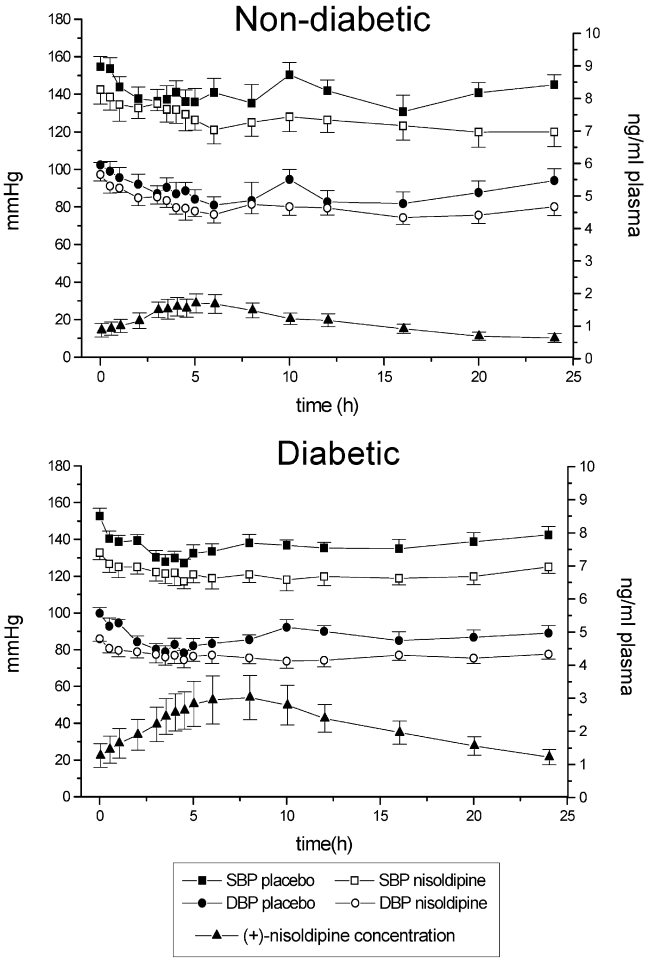

Fig. 5 Mean systolic (SBP) and diastolic (DBP) blood pressures and plasma concentrations of (+)-nisoldipine in diabetic ($n=9$) and non-diabetic ($n=8$) hypertensive patients treated with rac-nisoldipine (20 mg/24 h) as coat core tablets for 15 days. Mean ± SEM

Table 4 Plasma concentrations of noradrenaline. Data are reported as means (95%CI) and median

	Noradrenaline (pmol ml^{-1} plasma)	
	Placebo	Nisoldipine
Non-diabetic ($n=8$)	2.72 (1.45–4.00) 2.50	4.80[a](3.01–6.59) 5.300
Diabetic ($n=9$)	1.68 (0.96–2.40) 1.30	2.86[a, b](2.06–3.64) 2.40

[a] $P<0.05$ Wilcoxon test, placebo versus nisoldipine
[b] $P<0.05$ Mann-Whitney test, non-diabetic versus diabetic

BP data plotted together with plasma concentrations of the (+)-nisoldipine eutomer during the 24-h dose interval are presented in Fig. 5. Nisoldipine (coat core tablets) significantly reduced (Wilcoxon test, $P<0.05$; Fig. 5) 24-h mean systolic BP (122 ± 12 mmHg vs 133 ± 11 mmHg and 124 ± 17 mmHg vs 137 ± 13 mmHg) and 24-h mean diastolic BP (76 ± 8 mmHg vs 82 ± 8 mmHg and 76 ± 10 mmHg vs 86 ± 10 mmHg), respectively, in diabetic and non-diabetic patients relative to placebo.

Discussion

DM is associated with suppression or induction of the expression of different isoforms of CYP [24]. There are no data in the literature about the influence of DM on the stereoselective pharmacokinetics of nisoldipine, a dihydropyridine essentially eliminated by CYP3A4-dependent metabolism.

The stereoselective pharmacokinetics of nisoldipine was evaluated in a steady state after administration of racemic nisoldipine in the form of coat core tablets every 24 h for 15 days. The higher concentrations of (+)-nisoldipine relative to the (–) enantiomer found in this study were similar to data reported in other settings. Frost et al. [8] reported that the plasma concentrations of (+)-nisoldipine were 6.3 times higher than those observed for the (–) enantiomer when a single dose of an oral solution of the pseudoracemate was administered to healthy volunteers. Heinig et al. [6] reported AUC(+)/AUC(–) ratios of 13 for healthy volunteers treated with

a single dose of racemic nisoldipine by the peroral route (controlled release formulation) and of 5 for hypertensive patients treated with racemic nisoldipine in a multiple dose regimen (controlled release formulation).

The plasma accumulation of the (+)-nisoldipine enantiomer is a consequence of the lower apparent clearance (Table 2). There are no data in the literature about the distribution volume and apparent clearance values for the nisoldipine enantiomers. The apparent distribution volume (Vd/f) of nisoldipine as an enantiomeric mixture is high, with reported values of the order of 1.6–7.1 l kg^{-1} [11], but no data are available about the individual enantiomers. The apparent clearance (Cl/f) of nisoldipine as an enantiomeric mixture is high, with the values reported in the literature ranging from 6.7 l min^{-1} to 20.0 l min^{-1} when the racemic drug is administered to healthy volunteers or to hypertensive patients in the form of coat core tablets [11]. The data presented in Table 2 demonstrated that the apparent clearance of (–)-nisoldipine was significantly higher than that of (+)-nisoldipine for all patients investigated.

A lower clearance of both nisoldipine enantiomers in the patients with diabetes suggests that the enzyme(s) mediating its metabolism may be less active in this group. Lidocaine *N*-deethylation is a nonspecific phenotyping reaction for CYP3A4 activity (Wang et al.) [16]. The significant correlations between individual lidocaine/MEGX ratios versus (+) and (–)-nisoldipine clearances indicate that the substrates are oxidised by the same or similar CYP isoforms, i.e. mainly CYP3A4. The data in Table 3 show that the plasma lidocaine/MEGX ratios evaluated 15 min and 30 min after lidocaine administration were significantly higher for diabetic than non-diabetic patients, suggesting a lower CYP3A4 activity in patients with type-2 DM. This result is in accordance with the reduction in apparent clearance of both nisoldipine enantiomers and the role of CYP3A4 in the metabolism of nisoldipine.

Vascular smooth muscle is the main target site for the clinical effect of the calcium antagonist dihydropyridine. [10]. Increased plasma noradrenaline concentrations have been reported for vasodilating drugs as a counter-regulatory behaviour [8]. The data presented in Table 4 show that nisoldipine increases noradrenaline concentrations in plasma of hypertensive patients with or without type-2 DM. The data also demonstrate that the plasma concentrations of noradrenaline 30 min after nisoldipine administration were lower in diabetic than in non-diabetic patients (Table 4).

As a consequence of peripheral arterial vasodilation, nisoldipine reduces systemic resistance and BP [10]. This was seen in both groups of patients (Fig. 5). Despite the significant differences in the plasma concentrations of both nisoldipine enantiomers, no significant differences in BP reduction were observed between diabetic and non-diabetic patients. Other studies using pharmacokinetic–pharmacodynamic models could be developed in order to explain these data. The data also show that nisoldipine reduced BP throughout the 24-h dose interval and that the maximum reduction in systolic and diastolic BP occurred approximately at the same time as the maximum plasma concentration of the (+)-nisoldipine eutomer.

Zannad [10] reported that nisoldipine can increase heart rate by reflex activation of the sympathetic nervous system. However, when the controlled-release coat core formulation is administered, no or very small changes in heart rate are expected because of the low peak plasma concentrations (C_{max}). The data obtained here permit us to infer that nisoldipine (coat core tablets) does not change the heart rate of diabetic (79.6 bpm vs 82.3 bpm) or non-diabetic (80.7 bpm vs 85.3 bpm) patients relative to placebo.

In conclusion, type-2 DM alters the kinetic disposition of both nisoldipine enantiomers through the inhibition of CYP3A4, although it does not modify the clinical effect produced by the reduction of systolic and diastolic BP.

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