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Drug Cocktail Interaction Study on the Effect of the Orally Administered Lavender Oil Preparation Silexan on Cytochrome P450 Enzymes in Healthy Volunteers

Oxana Doroshyenko, Dennis Rokitta, Gregor Zadoyan, Stephan Klement, Sandra Schläfke, Angelika Dienel, Thomas Gramatté, Hendrik Lück, and Uwe Fuhr

ITECRA GmbH & Co. KG, Cologne, Germany (O.D., G.Z., H.L., U.F.); Department of Pharmacology, Clinical Pharmacology Unit; University Hospital, University of Cologne, Cologne, Germany (D.R., G.Z., U.F.); Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany (S.K., S.S., A.D.); and Drug Development Consulting, Dresden, Germany (T.G.)

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ABSTRACT

This cocktail study evaluated the interaction potential of the oral lavender oil preparation silexan with major P450 (cytochrome P450) enzymes. Subjects and Methods: Sixteen healthy male or female Caucasians completed this double-blind, randomized, 2-fold cross-over study. Silexan (160 mg) or placebo were administered once daily for 11 days. Additionally, on day 11 of both study periods, 150 mg caffeine (CYP1A2), 125 mg tolbutamide (CYP2C9), 20 mg omeprazole (CYP2C19), 30 mg dextromethorphan-HBr (CYP2D6), and 2 mg midazolam (CYP3A4) were administered orally. Formal interaction was excluded if the 90% confidence interval (CI) for the silexan over placebo ratios for phenotyping metrics (primary: $AUC_{0–t}$) was within a 0.70–1.43 range. Results: According to the $AUC_{0–t}$ comparisons, silexan had no relevant effect on CYP1A2, 2C9, 2D6, and 3A4 activity. Secondary phenotyping metrics confirmed this result. Mean ratios for all omeprazole-derived metrics were close to unity. The 90% CI for the $AUC_{0–t}$ ratio of omeprazole but not for omeprazole/5-OH-omeprazole plasma ratio 3 hours post-dose or omeprazole/5-OH-omeprazole $AUC_{0–t}$ ratio (secondary CYP2C19 metrics) was above the predefined threshold of 1.43, probably caused by the inherent high variability of omeprazole pharmacokinetics. Silexan and the phenotyping drugs were well tolerated. Repeated silexan (160 mg/day) administration has no clinically relevant inhibitory or inducing effects on the CYP1A2, 2C9, 2C19, 2D6, and 3A4 enzymes in vivo.

Introduction

Assessing the potential of new drugs to change the activity of enzymes and/or transporters involved in pharmacokinetic processes and thus to cause respective drug-drug interactions is an integral part of clinical development also for herbal medicines. To this end, actual activity of many important drug-metabolizing enzymes in an individual may be quantified by phenotyping, i.e., by administration of an appropriate substrate for a given enzyme and subsequent determination of pharmacokinetic parameters reflecting activity of this enzyme (Fuhr et al., 2007). Phenotyping methods are extensively used for the qualitative and quantitative determination of factors influencing enzyme activity, including drug-drug interactions (Schellens et al., 1989; Adedoyin et al., 1998; Gorski et al., 2004; Zadoyan et al., 2012). Several selective substrates of important P450s may be administered concomitantly (cocktail) to simultaneously investigate effects of particular drugs toward the major drug-metabolizing enzymes (Frye et al., 1997; Tucker et al., 2001; Christensen et al., 2003; Sharma et al., 2004; Fuhr et al., 2007; Wohlfarth et al., 2012).

The oral lavender oil preparation silexan (the active substance of LASEA, W. Spitzner Arzneimittelfabrik GmbH, Ettlingen, Germany) 80 mg/day showed its efficacy as compared with placebo or to low-dose lorazepam 0.5 mg/day in patients with subthreshold and syndromal anxiety disorders (Kasper et al., 2010a,b; Woelk and Schläfke, 2010). Silexan has been approved in Germany for the treatment of restlessness with anxious mood [Summary of Product Characteristics (SmPC) for LASEA (NN, 2010)]. Pharmacokinetic studies performed with silexan demonstrate a rapid absorption and elimination of linalool with an apparent elimination half-life ($t_{1/2}$) of about 4 hours after a single dose and about 9 hours after 14 days of once daily administration (Kasper et al., 2010b).

Information from traditional use of *Lavandula angustifolia* (e.g., EMA community herbal monographs on *L. angustifolia* Miller, flos (European Medicines Agency, 2012a) and *aetheroleum* (European Medicines Agency, 2012b) and from limited in vitro tests conducted using human hepatocytes does not suggest that silexan would interact with cytochrome P450 enzymes (Dr. Willmar Schwabe GmbH & Co. KG, data on file). Because it is, however, questionable whether in vitro drug-drug interaction studies with herbal drugs are predictive for in vivo interactions, the present clinical study was conducted to

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ABBREVIATIONS: AE, adverse event; $AUC_{0–t}$, area under the plasma concentration-time curve between 0 and time of last quantifiable concentration; $AUC_{0–\infty}$, area under the plasma concentration-time curve extrapolated to infinity; C_{max}, maximal plasma concentration; CI, confidence interval; DMSO, dimethylsulfoxide; EI, electron ionization; linalool, 3,7-dimethylocta-1,6-dien-3-ol; linalyl acetate, 3,7-dimethylocta-1,6-dien-3-yl acetate; LLOQ, lower limit of quantification; P450, cytochrome P450.

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provide conclusive data on any potential effects of silexan on the activity of major P450s in humans. A cocktail approach was used to assess the interaction potential of silexan 160 mg toward CYP1A2, 2C9, 2C19, 2D6, and 3A4 enzymes.

Materials and Methods

Study Population and Study Design. The study protocol was approved by the Ethics Committee of the North Rhine Medical Association, Germany, and the study carried out in accordance with German laws, the Declaration of Helsinki, and other international guidelines. All study subjects provided written informed consent. Healthy male and female caucasians aged between 18 and 55 years were included in a single center, double-blind, randomized, placebo-controlled, two-period crossover design.

In the test period, 160 mg silexan (one soft gelatin capsule) was administered orally once daily on days 1–11. This preparation contains 160 mg of an essential oil produced from *L. angustifolia* flowers by steam distillation. It complies with the monograph Lavender oil (*Lavandulae aetheroleum*) of the *European Pharmacopeia* (Council of Europe, 2008) with respect to all quality parameters. According to these specifications, required contents are 20–45% and 25–46% for linalool and linalyl acetate, respectively. In the reference period, placebo capsules were administered instead. For each drug intake, the volunteers reported to the study ward.

In both study periods, administrations on day 11 were performed together with the five-probe phenotyping cocktail. The volunteers were hospitalized 12 hours before cocktail administration until 24 hours thereafter. Solid oral preparations of four cocktail drugs [150 mg caffeine (three tablets of Percoffedrinol N; Lindopharm GmbH, Germany), 125 mg tolbutamide (one quarter of a tablet, to be weighed; Actavis UK Limited, UK), 20 mg omeprazole (one tablet Omeprazol-ratiopharm NT 20 mg; ratiopharm GmbH, Germany), and 30 mg dextromethorphan-HBr (one capsule Hustenstiller ratiopharm; ratiopharm GmbH, Germany)] were administered orally together with silexan or placebo to evaluate the in vivo CYP1A2, 2C9, 2C19, and 2D6 activities, respectively. For the assessment of the total (liver and intestine) CYP3A4 activity, 2 mg midazolam [2 ml taken orally with 120 ml of water (Dormicum V injection solution 5 mg/5ml; Roche Pharma AG, Grenzach-Wyhlen, Germany)] were administered 1 minute thereafter.

Intake of food and beverages was standardized for the in-house phase. On day 11, the fasting period lasted from at least 9 hours before until 6 hours after dosing, and fluid intake regularization was applied from 1 hour before until 6 hours after dosing. During the ambulant periods (days 1–10 in both study periods and the washout phase of 21 days between cocktail administrations), nonalcoholic and noncaffeinated food and beverages without quinine or grapefruit could be consumed ad libitum. Alcohol and grapefruit juice were prohibited from 1 week prior to the study until the follow up examination performed 4–10 days after last dosing.

From 1 hour before administration of phenotyping cocktail until 4 hours post-dose, subjects remained in a recumbent position, which was continuously supervised by study personnel.

The study subjects were closely surveyed throughout the study for evidence of clinical or laboratory adverse events (AEs).

Blood Sampling. In both study periods, blood (10 ml per sample) for the determination of constituents of silexan was sampled approximately 10 minutes prior to the 5th, 10th, and 11th dosing to quantify exposure. The blood samples were collected into Sarstedt Monovette citrate tubes (Sarstedt AG and Co., Nümbrecht, Germany) and then centrifuged (2000*g*, room temperature, 10 minutes). The resulting plasma was transferred into two polypropylene tubes, immediately frozen, and stored in a freezer at –20° or below until assayed.

For determination of the phenotyping substances and calculation of the primary phenotyping metrics (Table 1), 9-ml blood samples were drawn approximately 10 minutes prior to dosing and 10, 20, 30, 45 minutes, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, and 24 hours post-dose. For determination of secondary phenotyping metrics (Table 2) with the purpose of further validation of the phenotyping results, additional blood sampling was carried out approximately 10 minutes prior to dosing and 3 and 6 hours post-dose. The blood samples were collected into Sarstedt Monovette lithium heparin tubes, immediately cooled in ice water, and then centrifuged (2000*g*, +4°C, 10 minutes). The supernatant

TABLE 1

Plasma pharmacokinetic parameters of the phenotyping drugs including primary phenotyping metrics

Phenotyping drug (P450)	Caffeine (1A2)		Tolbutamide (2C9)		Omeprazole (2C19)		Dextromethorphan (2D6)		Midazolam (3A4)	
Parameters[a]	N =16		N = 16		N = 16		N = 15[e]		N = 16	
Treatment	Placebo	Silexan	Placebo	Silexan	Placebo	Silexan	Placebo	Silexan	Placebo	Silexan
C_{max} [b]	22.81 (20.5–25.4)	21.20 (18.3–24.5)	59.0 (50.8–68.5)	59.3 (50.8–69.3)	524 (314–877)	561 (352–895)	3.42 (2.04–5.75)	3.61 (1.94-6.72)	37.0 (31.5-43.5)	34.4 (27.2–43.5)
t_{max} (h)	0.55 (0.18–1.50)	0.76 (0.17–3.02)	3.03 (1.50–5.00)	3.00 (0.75–6.00)	2.25 (0.50–4.00)	2.00 (0.50–6.00)	2.50 (0.50–4.00)	2.50 (1.00–5.00)	0.75 (0.50–1.00)	0.75 (0.33–2.50)
AUC_{0-t} [b,c]	**173 (135–221)**	**150 (114–198)**	**537 (458–631)**	**556 (477–648)**	**1018 (633–1636)**	**1164 (752–1800)**	**19.2 (9.8–37.7)**	**20.2 (9.40–43.3)**	**87.0 (72.3–104.6)**	**88.6 (71.3–110.0)**
$AUC_{0-\infty}$ [b]	192[d] (147–250)	160 (120–213)	589[d] (492–703)	654 (539–794)	1036 (646–1661)	1137[d] (721–1791)	28.2[f] (13.7–58.0)	33.1[g] (16.5–66.6)	95.0[h] (75.0–120.0)	94.9[d] (74.3–121.1)
$t_{1/2}$ (h)	4.75[d] (3.94–5.72)	4.30 (3.63–5.09)	7.23[d] (6.25–8.37)	8.06 (6.71–9.68)	0.834 (0.712–0.976)	0.812[d] (0.703–0.938)	6.50[f] (4.80–8.81)	6.61[g] (5.13–8.53)	4.33[h] (2.84–6.62)	3.53[d] (2.39–5.21)

AUC_{0-t}, area under the plasma concentration-time curve between 0 and time of last quantifiable concentration; $AUC_{0-\infty}$, area under the plasma concentration-time curve extrapolated to infinity; C_{max}, maximal plasma.

[a] AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, $t_{1/2}$: geometric mean (95% CI), t_{max}: median (range).

[b] Units for C_{max} of omeprazole, dextromethorphan, and midazolam are nM, those for caffeine and tolbutamide are μM; units for $AUC_{0-\infty}$ of omeprazole, midazolam, and dextromethorphan are h*nM, those for caffeine and tolbutamide are h*μM.

[c] The primary phenotyping metrics are printed in bold.

[d] N = 15.

[e] One subject excluded because identified as genetically poor metabolizer.

[f] N = 10.

[g] N = 12.

[h] N = 13.

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TABLE 2

Secondary phenotyping metrics

Secondary Phenotyping Metric[a]	CYP	Sample Size	Units	Geometric Mean (95% CI)	
				Placebo	Silexan
Molar paraxanthine/caffeine plasma concentration ratio 6 h post-dose	1A2	16	None	1.09 (0.76–1.56)	1.27 (0.93–1.73)
Tolbutamide plasma concentration 24 h post-dose	2C9	16	μM	6.16 (4.40–8.63)	6.81 (4.98–9.31)
Molar omeprazole/5-OH-omepazole plasma concentration ratio 3 h post-dose	2C19	16[b]	None	0.800 (0.583–1.10)	0.820 (0.561–1.20)
Molar omeprazole/5-OH-omepazole AUC_{0-t} ratio CYP2C19		16	None	0.795 (0.615–1.03)	0.884 (0.694–1.13)
Molar dextromethorphan/dextrorphan plasma concentration ratio 3 h post-dose	2D6	15[c]	None	0.263 (0.137–0.504)	0.297 (0.149–0.592)
Midazolam plasma concentration 6 h post-dose	3A4	16	nM	4.07 (3.19–5.20)	4.23 (3.25–5.52)

AUC_{0-t}, area under the plasma concentration-time curve between 0 and time of last quantifiable concentration.
[a] Units for concentrations of midazolam are nM, those for tolbutamide are μM.
[b] Concentration in one subject in the silexan period was below limit of quantification.
[c] One subject excluded because identified as genetically poor metabolizer.

plasma was transferred into four polypropylene tubes, immediately frozen, and stored deep-frozen at a temperature below –65°C.

At the eligibility assessment, blood was drawn for genotyping into Sarstedt Monovette EDTA tubes. Genotyping was performed to identify individuals with two important nonfunctional alleles of CYP2C19 (*2, *3) and/or CYP2D6 (*3, *4, *5, *6) (see http://www.cypalleles.ki.se/) with an approximate genotype frequency of 1 and 7%, respectively, in the Caucasian population. Such subjects were excluded from analyses for the respective metric because any interaction with regard to a specific enzyme cannot occur in its absence.

Analytical Methods. The quantification of phenotyping substrates (caffeine, tolbutamide, omeprazole, dextromethorphan, and midazolam) in plasma was performed using specific and sensitive liquid chromatography/tandem mass spectrometry methods as described earlier (Kasel et al., 2002; Jetter et al., 2004; Tomalik-Scharte et al., 2005; Frank et al., 2007; for specific details, see Zadoyan et al., 2012).

For the silexan constituents linalool (3,7-dimethylocta-1,6-dien-3-ol) and linalyl acetate (3,7-dimethylocta-1,6-dien-3-yl acetate), samples were analyzed by headspace gas chromatography-mass spectrometry with electron ionization mode using a headspace autosampler G1888 (Agilent Technologies, Santa Clara, CA), gas chromatograph 7890A (Agilent Technologies; from 80°C to 230°C with 10°C/min, helium, flow 1.5 ml/min), mass selective detector 5975C (Agilent Technologies), software MSDCHEM Station (Agilent Technologies), and a capillary column (J&W) DB WAXetr (60 m × 0.32 mm, 0.5 μm; Agilent Technologies). Dimethylsulfoxide (DMSO) was used as solvent for test and calibration solutions. Extraction and purification of reference substances was done in-house by Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany, achieving 97.6% purity of linalool and 97.7% purity for linalyl acetate. Quantification of both substances was performed by linear regression with the primary reference standard linalool (ion 93.1 *m/z*) and internal standard. Lower limit of quantification (LLOQ) for linalool and linalyl acetate was 2 ng/ml, working range was 2–2000 ng/ml. Interday precision for linalool was between 0.1 and 7.0% and for linalyl acetate between 0.4 and 12.0%, with interday accuracy between –6.0 and 1.6%, and –7.2 and 5.6%, respectively.

Data Analysis. Pharmacokinetic parameters of phenotyping drugs were determined from actual blood sampling times (relative to drug administration) for post-dose samples, and assayed drug plasma concentrations at these times using standard noncompartmental methods (WinNonlin Professional, version 5.2; Pharsight Corporation, Palo Alto, CA).

Plasma concentrations and all phenotyping metrics were assumed to arise from a log-normal distribution (multiplicative model).

For all probe substances of the cocktail, the main phenotyping metric was the area under the plasma concentration-time curve between administration and time of last quantifiable concentration (AUC_{0-t}) of the parent compound (Tables 1 and 2). The treatments were compared using standard average bioequivalence procedures (Steinijans et al., 1996) for the respective phenotyping metrics (Tables 1 and 2) obtained following silexan (test) and placebo (reference) treatments. There was no adjustment for multiple comparisons because all assessments were considered as separate tests for the respective enzyme. Linear correlation was used to assess the relationship between primary and secondary phenotyping parameters.

Sample Size Determination. Intraindividual CVs were assumed not to exceed 25% for any phenotyping metric (Fuhr et al., 2007). Lack of interaction was assumed if the 90% CI for estimated ratio $\mu_{test}/\mu_{reference}$ did not exceed a tolerance zone of 0.70–1.43 for phenotyping metrics. For $0.95 \le$ true ratio $\mu_{test}/\mu_{reference} \le 1.05$, $N = 14$ would allow rejection of each null hypothesis "interaction present" with $\alpha = 0.05$ (two-sided) and a power of at least 90%. Two additional subjects were included to account for eventual drop-outs as a safety margin, resulting in a sample size of $N = 16$.

Results

Demographic Data. In total, 17 white Caucasian subjects (8 males, 9 females) participated in this study. The respective means and ranges for age and body mass index were 37 (21–52) years and 23.5 (19.7–26.9) kg/m^2. All subjects were nonsmokers at the time of the study, three subjects (17.6%) had smoked in the past. Six of the female subjects used oral contraceptives prior to and during the study. All subjects were healthy as confirmed by an extensive prestudy examination. After completion of the first study period, one subject withdrew due to AEs. Sixteen subjects completed the study and were included in the analysis.

Concentrations of Linalool and Linalyl Acetate in Plasma. Following administration of silexan, concentrations of linalyl acetate were unquantifiably low (<2 ng/ml) in all samples, whereas the presence of linalool (concentrations ranged from 2.2 to 9.0 ng/ml, LLOQ 2 ng/ml) confirmed compliance in all cases. Mean values for the linalool concentrations were 2.02 ng/ml, 3.30 ng/ml, and 2.95 ng/ml prior to the 5th, 10th, and 11th dose of silexan, respectively, indicating that steady state has been reached on the phenotyping day.

Identification of Genotypes Coding for Absent Protein Expression. One study subject was identified as a poor metabolizer for *CYP2D6* and was excluded from the analysis of interaction for this enzyme. With respect to the *CYP2C19* genotype, no carriers of two alleles coding for nonfunctional enzyme were identified in the study population.

Pharmacokinetic Parameters for Phenotyping Substrates. Concentration-time profiles of phenotyping drugs are shown in Fig. 1. Phenotyping metrics of the P450 substrates calculated following administration of the test and reference treatments are presented in Tables 1 and 2. A summary of the statistical analysis, i.e., point estimates and 90% CIs, on the effect of silexan on phenotyping metrics is provided in Table 3.

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Mean C_{max}, $AUC_{0–t}$, and and area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0–\infty}$) (reflecting the extent of drug absorption and exposure) as well as median t_{max} (indicating the rate of drug absorption) and mean $t_{1/2}$ (reflecting drug elimination) values of the probe substances were in most cases very similar after both treatments (Tables 1 and 2), with a few apparent exceptions. Median t_{max} of a CYP1A2 probe drug caffeine occurred later after silexan (0.76 hour) than after placebo (0.55 hour) administration. Mean $AUC_{0–t}$ of the CYP2C19 probe substrate omeprazole was slightly increased after treatment with silexan (1164 hours*nM) compared with the value observed after placebo treatment (1018 hours*nM). For the CYP3A4 probe substrate midazolam, mean $t_{1/2}$ was shorter after silexan administration (3.53 hours) compared with placebo administration (4.33 hours).

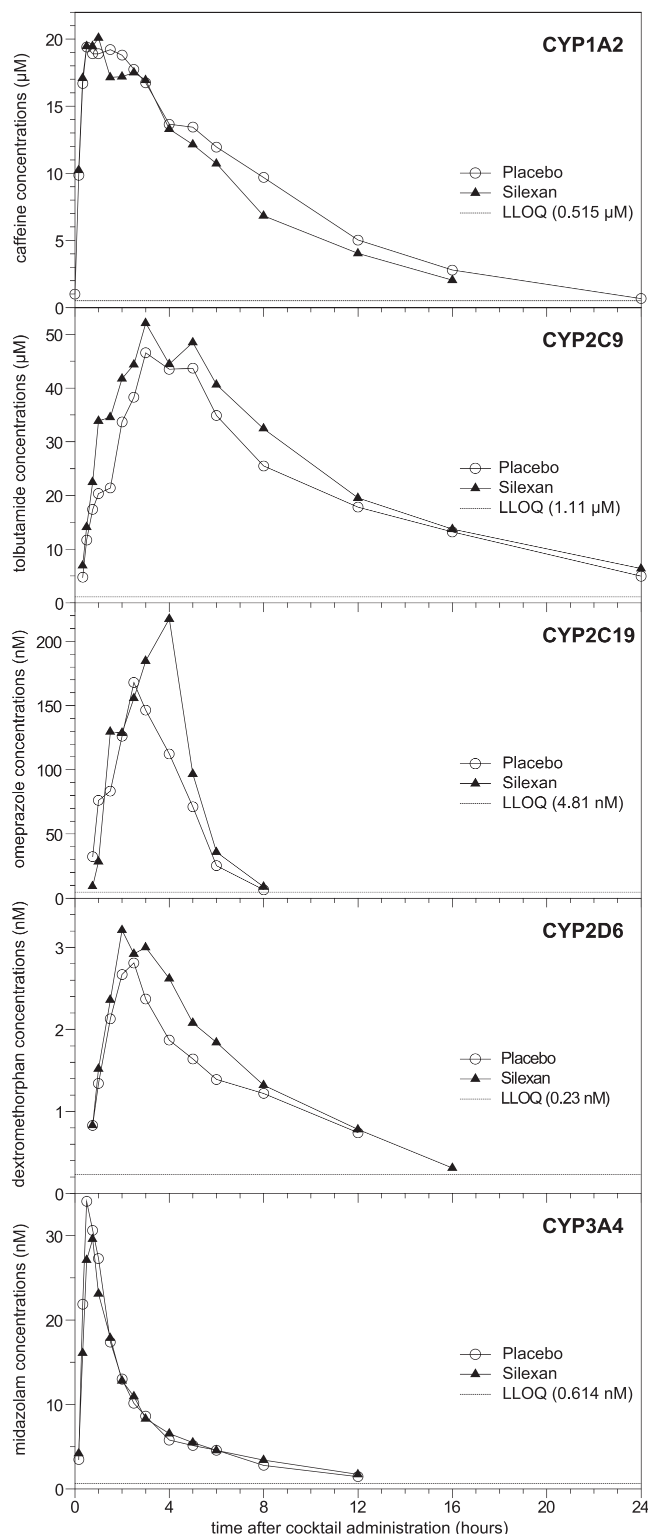

Fig. 1. Median plasma concentration-time profiles of the phenotyping drugs in silexan and placebo periods. Values below respective LLOQ are not shown.

Phenotyping Metrics and Effect of Silexan on the Activity of P450 Enzymes. For CYP1A2, 2C9, 2D6, and 3A4 metrics, the 90% CIs for the ratios (silexan/placebo) of the primary and secondary phenotyping metrics were well within the predefined acceptance range of 0.70–1.43 (Table 3). Thus, a pharmacokinetic interaction between silexan and drugs which are substrates of these enzymes could be excluded.

The upper bound of the 90% CI for the $AUC_{0–t}$ ratio of the CYP2C19 probe substrate omeprazole was above the threshold of 1.43, while the respective values for secondary phenotyping metrics (molar omeprazole/5-OH-omepazole plasma concentration ratio 3 hours post-dose and molar omeprazole/5-OH-omepazole $AUC_{0–t}$ ratio) were within the acceptance range. Point estimates for the ratios silexan/placebo of all CYP2C19 metrics used were close to unity (Table 3). Marked heterogeneity of measurements with respect to the $AUC_{0–t}$, $AUC_{0–\infty}$, and C_{max} values for all omeprazole metrics used was observed. The exceeding of the acceptance range for the main phenotyping metric of omeprazole thus is probably caused by the inherent high variability of omeprazole pharmacokinetics. Therefore, a clinically relevant pharmacokinetic interaction between silexan and CYP2C19 substrates is not expected.

In general, secondary phenotyping metrics provided similar results as the primary ones with regard to a potential interaction. Intraindividual variability of secondary phenotyping metrics depended on the type of metric and on phenotyping drug and could be lower or higher than for the primary metric (Table 3). Correlations between the main and the secondary phenotyping metrics, calculated to provide further information for the use of simplified phenotyping strategies, were significant ($P < 0.05$) in all cases (Table 4).

Safety and Tolerability. Eleven AEs were observed in 5/16 (31.3%) subjects and 30 AEs in 15/17 (88.2%) subjects during and until 7 days after last placebo or silexan administration.

Mild eructation occurred shortly after drug intake and was the most frequently reported AE, which was experienced by 10 (58.8%) subjects (in five subjects as a single event) after treatment with silexan and by no subject after placebo.

With respect to the double-blind treatment, the causal relationship with silexan was considered as probable for five AEs (five cases of eructation, silexan), as possible for eight AEs [eructation (five cases, silexan), diarrhea (one case, silexan), nausea (one case each, silexan and placebo)], and as unlikely for 27 AEs.

With respect to the phenotyping cocktail, the causal relationship was considered as possible for three AEs (nausea, dizziness, and vomiting) in two subjects and as unlikely for three AEs (cold, increased hematocrit, and increased erythrocytes count) in two subjects.

No severe or serious AEs occurred during the study. One subject dropped out due to moderate AEs (nausea before intake of study drug and vomiting after administration of the phenotyping cocktail) in the study period with silexan treatment.

Mean vital signs, ECG, and laboratory parameters showed no clinically relevant changes during the study.

Thus, repeated administration of silexan (160 mg/day) alone or together with the probe substrates were well tolerated by healthy subjects in this study.

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TABLE 3

Assessment of the effect of silexan on phenotyping metrics given as ratios for silexan over placebo periods

CYP	Parameter[a]	Sample Size	Ratio	90% CI	CV_{intra}%
1A2	**AUC_{0-t} of caffeine in plasma**	16	**0.869**	**0.786–0.961**	**16.2**
	Molar paraxanthine / caffeine plasma concentration ratio 6 h post-dose	16	1.16	0.956–1.414	32.2
2C9	**AUC_{0-t} of tolbutamide in plasma**	16	**1.035**	**0.973–1.101**	**10.0**
	Tolbutamide plasma concentration 24 h post-dose	16	1.106	0.970–1.261	21.3
2C19	**AUC_{0-t} of omeprazole in plasma**	16	**1.143**	**0.810–1.614**	**59.9**
	Molar omeprazole / 5-OH-omepazole plasma concentration ratio 3 h post-dose	15[b]	1.044	0.842–1.294	34.3
	Molar omeprazole / 5-OH-omepazole AUC_{0-t} ratio	16	1.111	0.998–1.237	17.4
2D6	**AUC_{0-t} of dextromethorphan in plasma**	15[c]	**1.046**	**0.838–1.305**	**36.7**
	Molar dextromethorphan / dextrorphan plasma concentration ratio 3 h post-dose	15[c]	1.131	0.962–1.328	25.2
3A4	**AUC_{0-t} of midazolam in plasma**	16	**1.018**	**0.894–1.159**	**21.1**
	Midazolam plasma concentration 6 h post-dose	16	1.040	0.847–1.277	33.8

AUC_{0-t}, area under the plasma concentration-time curve between 0 and time of last quantifiable concentration; CI, confidence interval; CV_{intra}, intrasubject coefficient of variation.

[a] The main phenotyping metrics are printed in bold.

[b] Concentration in one subject in the silexan period was below limit of quantification.

[c] One subject excluded because identified as genetically poor metabolizer.

Discussion

The objective of the study was to assess the in vivo interaction potential of silexan with respect to the activities of five major P450 enzymes using the cocktail approach. The study using state-of-the-art methodology showed that repeated administration of silexan has no clinically relevant inhibitory or inducing effects toward the CYP1A2, 2C9, 2C19, 2D6 and 3A4 enzymes.

A higher silexan dose (160 mg/day) as compared with the standard dose of 80 mg/day was chosen in the study to maximize exposure in the present drug interaction study (European Medicines Agency, 2012c; Food and Drug Administration, 2012).

Plasma concentration measurements of the silexan constituents (linalool and linalyl acetate) confirm the compliance of study subjects. Based on linalool concentration data, it could be concluded that the interaction potential of silexan was assessed under steady-state conditions. Plasma concentrations of linalyl acetate below LLOQ can be explained by the pharmacokinetic properties of the compound (rapid elimination) and the time point of sampling.

The current pharmacokinetic approach allowed the effects of silexan toward major P450 enzymes to be examined systematically, simultaneously, and under standardized conditions. For scientific reasons and in line with existing guidelines on drug interactions (European Medicines Agency, 2012c; Food and Drug Administration, 2012), a crossover design of the study was chosen as the within-subject variability was expected to be smaller than the between-subject variability. Based on the a priori estimation of the sample size required, the number of study participants turned out to be sufficient for all enzymes except CYP2C19, although intraindividual variability for several phenotyping metrics was higher than expected (Table 3).

The probe drugs were selected in accordance with existing guidelines (Food and Drug Administration, 2012; European Medicines Agency, 2012c) and scientific literature (Gorski et al., 2004; Frank et al., 2007; Fuhr et al., 2007; Wohlfarth et al., 2012). All of these drugs are established probe substrates meeting the important criteria for cocktail drugs: selectivity toward the respective P450s (i.e., the probe drug is cleared predominantly by a single P450 enzyme), absence of interference with the metabolism and clearance of other drugs in the cocktail, safety and good tolerability, availability and validity of bioanalytical assays, and appropriateness of phenotyping metrics. Selectivity of these substrates for respective P450s is supported by a number of investigations; following single doses, they do not affect the in vivo activity of any other of the enzymes to a relevant extent and no mutual interactions by their coadministration has been reported (Endres et al., 1996; Frye et al., 1997; Streetman et al., 2000; Wang et al., 2001; Zhu et al., 2001; Fuhr et al., 2007; Turpault et al., 2009). There is, however, a caveat for CYP2C19 phenotyping. The EMA Guideline on the Investigation of Drug Interactions (European Medicines Agency, 2012c) considers omeprazole as not sufficiently validated as a phenotyping drug, but accepts its use as a "standard of

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TABLE 4

Correlation of secondary phenotyping metrics to the primary metric AUC_{0-t} of the parent substance

CYP	Secondary Metric	Silexan Treatment		Placebo Treatment	
		n	r[d]	*n*	r[d]
1A2	Molar paraxanthine / caffeine plasma concentration ratio 6 h post-dose[a]	16	0.531*	16	0.698**
2C9	Tolbutamide plasma concentration 24 h post-dose	16	0.842**	16	0.928**
2C19	Molar omeprazole/5-OH-omepazole plasma concentration ratio 3 h post-dose	15[b]	0.697**	16	0.539*
	Molar omeprazole/5-OH-omepazole AUC_{0-t} ratio	16	0.905**	16	0.771**
2D6	Molar dextromethorphan/dextrorphan plasma concentration ratio 3 h post-dose	15[c]	0.971**	15[c]	0.983**
3A4	Midazolam plasma concentration 6 h post-dose	16	0.933**	16	0.855**

[a] For calculation of this correlation, the inverse value (i.e., caffeine/paraxanthine ratio) was used.

[b] Concentration in one subject in the silexan period was below LLOQ.

[c] One subject excluded because identified as genetically poor metabolizer.

[d] Pearson's correlation coefficient.

$*P = 0.05$; $**P = 0.01$.

convenience" in the absence of better choices, because mephenytoin is no longer available (Klaassen et al., 2008).

The main phenotyping metric for respective enzyme was the AUC_{0-t} of the parent drug, a reliable metric reflecting the activity of the particular enzyme, although it requires multiple blood collections and is time-consuming. Irrespective of the phenotyping drug, assessment of full concentration-time profile is also recommended by the EMA Guideline on the Investigation of Drug Interactions (European Medicines Agency, 2012c), most probably because pharmacokinetic interactions other than those caused by modification of enzyme activity could occur, such as delayed absorption, which may erroneously be attributed to changes in enzyme activity, if only a sample would be available. Still, phenotyping metrics based on single-point plasma concentration and molar metabolic ratios (Tables 1 and 2) are appealing, and suitability of such (secondary) phenotyping metrics had been previously assessed in cocktail interaction studies (Jetter et al., 2004; Frank et al., 2007; Fuhr et al., 2007; Zadoyan et al., 2012) and were also assessed in the present study. Intraindividual variability of secondary phenotyping metrics (Table 3) obviously depended on the type of metric and on gastrointestinal absorption of the phenotyping drug. For single point measurements of the parent drug only (tolbutamide, midazolam), it was higher than that for AUC_{0-t}. For single-point metabolic ratios, variability was lower for drugs with poor and irregular bioavailability (omeprazole, dextromethorphan), but higher for caffeine, which is rapidly and completely absorbed and does not undergo first-pass metabolism. The molar AUC ratio, combining an assessment based on many samples (thus leveling out inaccuracies) and on standardization with regard to absorption differences, had the lowest variability of the CYP2C19 metrics tested. Both similar results with regard to the interaction tested and the close correlations between most of the phenotyping metrics (Table 4) support the use of such phenotyping metrics, with preference on metrics with lower variability, explained by avoiding confounders.

A clinically relevant effect of a perpetrator drug on the activity of a given enzyme is difficult to define and depends on the victim drug. For the purpose of this study, formally interaction was excluded if the 90% CI for the ratios active treatment over placebo was within a 0.7–1.43 range. These boundaries have often been used to assess drug-drug interactions (Steinijans et al., 1996; Rani and Pargal, 2004; Tomalik-Scharte et al., 2005; Fuhr et al., 2007).

The upper bound of the 90% CI for the AUC_{0-t} ratio of omeprazole (a CYP2C19 probe substrate) was above the threshold of 1.43 with the mean AUC_{0-t} ratio close to unity. Assuming this to reflect a real effect of silexan on CYP2C19 activity, an inhibitory action of silexan on CYP2C19 cannot be excluded formally. For the assessment of relevance, however, it has to be considered that: 1) as shown previously by genotype/phenotype relationships, omeprazole clearance is primarily mediated by the CYP2C19 activity, and the enzyme activity itself is highly variable (Andersson et al., 1993; Chang et al., 1995; Roh et al., 1996); 2) the inability to formally exclude an interaction toward CYP2C19 was observed only for the most variable (albeit primary) of the three phenotyping metrics applied; 3) as described above, the main phenotyping metric (AUC of parent compound) is not fully validated (Fuhr et al., 2007; European Medicines Agency, 2012c); 4) other factors may contribute for a high inter- and intraindividual variability of the omeprazole-derived CYP2C19 metrics. Specifically, omeprazole is acid labile and, therefore, is administered as acid-fast preparations dissolving in the small intestine [Howden et al., 1984; Andersson et al., 1991; SmPC for omeprazol-ratiopharm NT, 20 mg hard capsule (NN, 2008); SmPC for omeprazole, 20 mg capsules (NN, 2012)], and the highly variable gastric emptying is expected to contribute to variability in pharmacokinetic parameters. An intraindividual comparison of the ratio for omeprazole revealed that in 6 of 16 volunteers the ratio AUC-silexan to AUC-placebo was $\geq$1.25, but in five volunteers it was below one. The group of the six volunteers with ratios $\geq$1.25 showed the lowest median of their AUC values after placebo treatment and individual ratios $\geq$1.25 were observed only for low omeprazole AUCs under placebo. Ratios $\geq$1.25 are primarily caused by particularly low AUCs after placebo and not by high AUCs after silexan. The terminal elimination half-life of omeprazole was not affected by silexan. For these reasons, most probably the high variability and not an inhibitory effect of silexan on CYP2C19 is the explanation for the observations.

Repeated administration of silexan 160 mg/day was well tolerated when given alone and together with the phenotyping cocktail. Most AEs observed in this trial (eructation, nausea) were expected (SmPC for LASEA; NN, 2010), their nature and intensity were in line with previously reported data to silexan safety and tolerability profile (Kasper et al., 2010a; Kasper et al., 2010b; Woelk and Schläfke, 2010). Also, the phenotyping cocktail used in the study is considered to be safe and well-tolerated taking into account that the phenotyping substances have been widely used for various therapeutic applications in patients, their doses in the present study were reduced as far as possible compared with the therapeutic doses by the application of highly sensitive analytical methods, only two single doses of each drug were administered for phenotyping, and available reports of cocktail interactions studies confirm their appropriate safety and good tolerability (Frye et al., 1997; Streetman et al., 2000; Wang et al., 2001; Zhu et al., 2001; Tomalik-Scharte et al., 2005; Turpault et al., 2009; Zadoyan et al., 2012).

The use of a crossover design with well controlled conditions, including supervised intake of study drug, the clinically relevant dose of the drug to be tested, proof of exposure, and standardized food and fluid intake for assessment of enzyme activity may be considered as state of the art and is stipulated by the respective guidelines (Food and Drug Administration, 2012; European Medicines Agency, 2012c).

The data also suggest that the use of simplified phenotyping metrics should take absorption properties of the phenotyping drugs into account.

In conclusion, the study reported here provides information to the in vivo interaction potential of silexan at therapeutic doses toward major P450s as well as further data to respective phenotyping metrics. Repeated oral administration of the standardized lavender oil preparation silexan at the dose 160 mg/day does not cause clinically relevant inhibitory or inducing effects on the CYP1A2, 2C9, 2C19, 2D6, and 3A4 enzymes.

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Authorship Contributions

Participated in research design: Klement, Dienel, Fuhr.

Conducted experiments: Lück, Fuhr.

Performed data analysis: Doroshyenko, Rokitta, Zadoyan, Schläfke, Lück.

Wrote or contributed to the writing of the manuscript: Doroshyenko, Rokitta, Zadoyan, Klement, Gramatté, Lück, Fuhr.

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Address correspondence to: Dr. Uwe Fuhr, Institut für Pharmakologie, Klinische Pharmakologie, Universität zu Köln, Gleueler Straße 24, 50931 Köln, Germany. E-mail: uwe.fuhr@uk-koeln.de

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