Clin Drug Investig (2015) 35:601–612
DOI 10.1007/s40261-015-0318-2

ORIGINAL RESEARCH ARTICLE

Evaluation of Cytochrome P450 (CYP) 3A4-Based Interactions of Levomilnacipran with Ketoconazole, Carbamazepine or Alprazolam in Healthy Subjects

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Published online: 28 August 2015

Abstract

Background and Objectives Levomilnacipran is a serotonin and norepinephrine reuptake inhibitor with balanced potency for the reuptake inhibition of norepinephrine and serotonin, approved in the USA for the treatment of major depressive disorder (MDD) in adults. We conducted studies in healthy human subjects to investigate pharmacokinetic interactions when levomilnacipran extended-release (ER) is administered in combination with an inhibitor (ketoconazole), an inducer (carbamazepine), or a substrate (alprazolam) of cytochrome P450 (CYP) 3A4.

Methods Randomised, open-label studies were conducted in healthy volunteers ($n = 34$ ketoconazole, $n = 34$ carbamazepine, $n = 30$ alprazolam) and pharmacokinetic parameters were determined when levomilnacipran was administered alone or together with the relevant study drug.

Results Co-administration of ketoconazole with levomilnacipran ER increased levomilnacipran maximum concentration (C_{max}) by 39 % [90 % confidence interval (CI) 31–47 %] and area under the concentration–time curve (AUC) by 57 % (90 % CI 47–67 %), whereas carbamazepine reduced the C_{max} and AUC of levomilnacipran by 26 % (90 % CI 22–30 %) and 29 % (90 % CI 26–32 %), respectively. Levomilnacipran at steady state had no significant effect on the pharmacokinetics of a single 1 mg dose of alprazolam extended release (XR); neither did single-dose alprazolam XR affect the steady-state pharmacokinetics of levomilnacipran. No new safety concerns were noted in these studies.

Conclusions Based on these results, the levomilnacipran ER dose should not exceed 80 mg once daily when used with ketoconazole, compared to 120 mg once daily in the absence of ketoconazole. No dose adjustment for levomilnacipran is suggested when levomilnacipran ER is co-administered with carbamazepine or other CYP3A4 inducers. Co-administration with levomilnacipran of drugs metabolised by CYP3A4, such as alprazolam, requires no dose adjustment due to pharmacokinetic considerations.

Electronic supplementary material The online version of this article (doi:10.1007/s40261-015-0318-2) contains supplementary material, which is available to authorized users.

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Key Points

Levomilnacipran exposure was increased by approximately 50 % when it was co-administered with ketoconazole, a strong CYP3A4 inhibitor; a maximum dose of 80 mg of levomilnacipran extended release (ER) is therefore recommended when the drug is used with ketoconazole or other strong CYP3A4 inhibitors, compared to 120 mg once daily in the absence of strong CYP3A4 inhibitors.

The pharmacokinetics of levomilnacipran were not clinically meaningfully affected by co-administration with carbamazepine, an inducer of CYP3A4; thus, dose adjustments are not required.

Maximal-dose levomilnacipran at steady state did not affect the pharmacokinetics of single-dose alprazolam extended release (XR), a substrate of CYP3A4; neither did single-dose alprazolam XR affect the steady-state pharmacokinetics of levomilnacipran ER. Thus, dose adjustment is generally not needed based on pharmacokinetic considerations for drugs metabolised by CYP3A4 enzymes when they are co-administered with levomilnacipran ER.

1 Introduction

Levomilnacipran (1S, 2R-milnacipran; Fetzima®, Forest Laboratories, LLC, St Louis, MO, USA) is a potent and selective serotonin and norepinephrine reuptake inhibitor (SNRI) approved by the US Food and Drug Administration (FDA) for the treatment of major depressive disorder (MDD) in adults. Levomilnacipran has balanced potency for inhibiting the norepinephrine transporter and the serotonin transporter. Conversely, the SNRIs duloxetine and venlafaxine have greater potency for serotonin reuptake inhibition than norepinephrine reuptake inhibition [1, 2]. An extended-release (ER) formulation was developed to facilitate once-daily dosing.

The efficacy and safety of levomilnacipran ER in the treatment of MDD have been evaluated in a large phase II study [3] and four phase III studies [4–7]. Four of these studies showed a statistically significant improvement for levomilnacipran ER on the Montgomery–Åsberg Depression Rating Scale total score compared with placebo [3–6]. A 48-week, open-label, follow-up study [8] examined the longer-term safety and tolerability of levomilnacipran in patients who had completed one of three earlier trials [4, 6, 7]. The results supported the safety and tolerability profile observed during the earlier studies. Post hoc pooled analyses of the five studies indicated that levomilnacipran ER was effective across a wide range of patients with MDD [9] and that functional impairment was significantly improved compared with placebo, regardless of the baseline level of functional impairment [10].

Pharmacokinetic studies of the ER formulation showed that levomilnacipran has a median time to reach maximum drug plasma concentration (t_{max}) of 6–8 h and an apparent terminal elimination half-life ($t_{½}$) of 12 h [11, 12]. The pharmacokinetics of levomilnacipran are dose proportional following a single dose of 25–120 mg and multiple doses of 25–300 mg/day. Food does not have a significant effect on the bioavailability of levomilnacipran ER capsules [12].

Levomilnacipran is eliminated primarily by renal excretion; about 58 % of an orally administered dose is excreted unchanged in the urine. The remainder is eliminated by multiple metabolic pathways including glucuronidation, desethylation and *p*-hydroxylation. Desethylation to form *N*-desethyl levomilnacipran (also called F17400) is a major metabolic pathway accounting for approximately 18 % of total clearance; none of the other metabolic pathways contributes more than 5 % of total clearance in humans. In vitro studies have shown that, while multiple cytochrome P450 (CYP) enzymes are capable of catalysing this desethylation, CYP3A4 is the primary catalyst [13]. Therefore, there is the potential that levomilnacipran pharmacokinetics may be influenced by CYP3A4 modulators. None of the identified metabolites are pharmacologically active [11].

In vitro studies using validated methods showed that levomilnacipran did not induce CYP 1A2, 2C9, 2C19 or 3A4/5 when tested using freshly isolated human hepatocytes [14]. Nor did it directly inhibit CYP1A2, 2A6, 2C8, 2C9, 2C19, 2D6 or 2E1 in a pooled human liver microsomal system; only CYP3A4/5 was inhibited, with a half-maximal inhibitory concentration (IC_{50}) of >30 μM [15]. As this is about 21-fold higher than the steady-state maximum plasma concentration of 1.4 μM achieved with 120 mg/day [11], the highest recommended therapeutic dose, the potential for levomilnacipran to inhibit CYP3A4/5 is low. Similarly, *N*-desethyl levomilnacipran did not directly inhibit CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 or 3A4/5 at clinically relevant concentrations [16]. No time-dependent or metabolism-dependent inhibition was observed for levomilnacipran or the metabolite at concentrations exceeding those that are clinically relevant. In addition, based on in vitro evaluations, drug transporters of *P*-glycoprotein—BCRP, OATP1B1, OATP1B3, OAT1, OAT3 or OCT2—have no significant interaction with levomilnacipran at therapeutically relevant concentrations [11]. Thus, the in vitro studies suggested that there is low

potential for levomilnacipran to inhibit the metabolism of substrates of CYP3A4.

In order to further evaluate the in vitro findings, three in vivo pharmacokinetic studies were conducted in healthy human subjects to investigate pharmacokinetic interactions when levomilnacipran ER is administered in combination with an inhibitor (ketoconazole), an inducer (carbamazepine) or a substrate (alprazolam) of CYP3A4.

2 Methods

2.1 Study Designs

All three studies were designed in accordance with the FDA guidelines for drug-interaction studies [17]. No concomitant medications were permitted during the studies. Since food has no significant effect on the pharmacokinetics of levomilnacipran, study drugs were administered with a meal to potentially improve tolerability. As food affects the bioavailability of alprazolam, subjects were required to take the drug alone or co-administered with levomilnacipran under fasted conditions. In all three studies, blood samples were collected at appropriate predefined intervals for analysis of the relevant drug concentrations.

2.1.1 Study 1 (Levomilnacipran ER Plus Ketoconazole)

This was a single-centre, randomised, open-label, two-period crossover study of the effects of steady-state ketoconazole on the pharmacokinetics of a single dose of levomilnacipran ER in healthy subjects. Subjects were randomised to one of two treatments (levomilnacipran alone or combined levomilnacipran and ketoconazole) in two sequences as described in Online Resource 1.

2.1.2 Study 2 (Levomilnacipran ER Plus Carbamazepine Extended Release [XR])

This was a single-centre, open-label, fixed-sequence, multiple-dose, four-period study in which subjects received Treatments A (levomilnacipran alone); B (carbamazepine alone); C (combined levomilnacipran and carbamazepine); and D (carbamazepine down-taper) in a fixed order with a washout period between Treatments A and B (Online Resource 2).

2.1.3 Study 3 (Levomilnacipran ER Plus Single-Dose Alprazolam Extended Release [XR])

This was single-centre, randomised, open-label, 2×2 crossover study to assess the effect of levomilnacipran ER at steady state on the pharmacokinetics of alprazolam in healthy subjects. Participants were randomised to one of two treatments (alprazolam alone or combined alprazolam and levomilnacipran) in two sequences as described in Online Resource 3.

2.2 Inclusion and exclusion criteria

The studies were conducted in healthy, non-smoking adults aged 18–45 years with a body mass index (BMI) of between 18 and 30 kg/m^2, and resting pulse rate of 50–100 beats per minute (bpm). Female participants were required to have a negative pregnancy test at screening and on Day −1, and agreed to use a non-hormonal double-barrier method of contraception during the study period.

Exclusion criteria for all three studies included: known hypersensitivity to study treatments, selective serotonin reuptake inhibitors (SSRIs) or other noradrenergic drugs; clinically significant electrocardiogram (ECG) or laboratory abnormalities; history of alcohol or substance abuse within the past 5 years; positive test for cocaine, methadone, barbiturates, amphetamines, benzodiazepines, alcohol, cannabinoids, opiates, phencyclidine or cotinine; consumption of caffeine or grapefruit-containing products within 48 h or alcohol consumption 72 h before Day 1; concomitant medications (within 14 days) or hormonal drugs (within 30 days) before study; pregnancy or breast-feeding; suicide risk or history of narrow-angle glaucoma. Study 2 had an additional exclusion criterion of Asian ancestry or allele positive for HLA-B* 1502.

Subjects underwent physical examination and clinical laboratory testing prior to receiving study drug(s). All participants provided informed consent at screening. Study protocols were approved and studies overseen by the designated independent Institutional Review Board: Independent Investigational Review Board, Plantation, Florida, USA (Studies 1 and 3) and the PRACS Institute Ltd Institutional Review Board (Study 2). The studies were conducted in compliance with the principles of good clinical practice.

2.3 Bioanalytical Methods

Blood sampling was performed as shown in the Electronic Supplementary Material (Online Resources 1, 2 and 3). Validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods were used to measure plasma concentrations of drugs and their metabolites (see Online Resource 4).

2.4 Pharmacokinetic Analyses

Pharmacokinetic parameters were derived from plasma concentrations by non-compartmental analysis using WinNonlin® (Pharsight, Cary, NC) version 5.2.1 (Study 1) or version 6.1 (Studies 2 and 3).

In Study 1 (levomilnacipran and ketoconazole), the pharmacokinetic population comprised all subjects who had evaluable pharmacokinetic parameters in both treatment periods. For levomilnacipran, the following were determined: area under the plasma concentration versus time curve (AUC) from time zero to time t (AUC_{0-t}) and from time zero to infinity ($AUC_{0-\infty}$) (using the linear trapezoidal rule), maximum concentration (C_{max}), t_{max}, $t_{½}$, apparent total clearance of drug from plasma after oral administration (CL/F) and apparent volume of distribution at steady state ($V_{SS/F}$). For N-desethyl levomilnacipran, C_{max}, t_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and $t_{½}$ were determined. Log-transformed values for the C_{max} and AUC of levomilnacipran and N-desethyl levomilnacipran for levomilnacipran administered with ketoconazole or alone were compared by means of an analysis-of-variance (ANOVA) model using SAS version 9.1.3 under the UNIX operating system. A general linear model was fitted with sequence, subject within sequence, treatment and period as factors.

In Study 2 (levomilnacipran and carbamazepine XR), the pharmacokinetic population comprised all subjects who had evaluable pharmacokinetic parameters for both treatments in each comparison. The following pharmacokinetic parameters were assessed for levomilnacipran, N-desethyl levomilnacipran, carbamazepine and carbamazepine-10,11-epoxide: AUC during the dosing interval, τ, at steady state ($AUC_{0-\tau,SS}$), C_{max} at steady state ($C_{max,SS}$), t_{max} at steady state ($t_{max,SS}$), average plasma drug concentration at steady state ($C_{av,SS}$), and minimum plasma drug concentrations at steady state ($C_{min,SS}$). The $t_{½}$ of levomilnacipran and N-desethyl levomilnacipran were also determined. Log-transformed values for $C_{max,SS}$ and $AUC_{0-\tau,SS}$ of levomilnacipran, N-desethyl levomilnacipran, carbamazepine and carbamazepine-10,11-epoxide for drug in combination or alone were compared by means of ANOVA models using SAS version 9.2 under the Windows operating system. General linear models were fitted with treatment and subject as factors.

In Study 3 (levomilnacipran and alprazolam), the pharmacokinetic population was defined as all subjects who completed the study and had evaluable pharmacokinetic parameters. For levomilnacipran, $AUC_{0-\tau}$, $C_{max,SS}$, $C_{av,SS}$, $C_{min,SS}$ and $t_{max,SS}$, and for alprazolam, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ (using the linear trapezoidal rule), t_{max}, $t_{½}$, CL/F and V_z/F (apparent volume of distribution during terminal phase after non-intravenous administration) were determined. The pharmacokinetic parameters C_{max} and AUC (after single or multiple dose, as applicable) were compared between treatments (drug in combination vs. drug alone) using ANOVA. A general linear model with sequence, subject within sequence, treatment and period as factors was fitted to log-transformed values of C_{max} and AUC.

For all three studies, two-sided 90 % confidence intervals (CIs) were constructed for the ratio of least squares (LS) geometric means of C_{max} and the relevant AUC parameters for the relevant drugs administered in combination versus drug alone. The 90 % CI for the LS geometric means ratios of C_{max} and AUC parameters needed to be within 80–125 % in order to conclude that each drug had no effect on the pharmacokinetic parameters of the other drug [18]. T_{max} for the relevant drug alone or in combination with the other was compared using the Wilcoxon signed-rank test.

2.5 Safety

Safety was assessed by means of adverse event (AE) monitoring, clinical laboratory evaluations, vital sign assessments, ECGs, physical examinations and Columbia–Suicide Severity Rating Scale (C-SSRS) results. An AE was "any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product" [19]. AEs were recorded as they were reported spontaneously by the subjects, and subjects were asked about their well-being each time vital signs were taken. AEs were classified as mild, moderate or severe by investigators, based on the following definitions: a mild AE was an annoyance to the subject but did not further hinder baseline functioning; a moderate AE caused the subject some discomfort or interference with normal activities but was not hazardous to health; a severe AE caused the subject to experience severe discomfort or severely limited or prevented normal activities and represented a definite hazard to health.

Participants who took one or more doses of any of the study drugs were included in the safety population. Any AE occurring subsequent to the first dose of study drug was counted as a treatment-emergent AE (TEAE). Incidence tables were compiled for AEs; for other safety parameters, descriptive statistics were calculated.

3 Results

3.1 Study 1 (Levomilnacipran ER and Ketoconazole)

3.1.1 Pharmacokinetics

Thirty-four healthy subjects were enrolled and all completed Study 1. Mean age ± standard deviation (SD) was 38.2 ± 5.9 years; mean BMI 26.1 ± 2.3 kg/m^2; 17 participants (50 %) were female; 88.2 % were white and 11.8 % black.

Administration of levomilnacipran ER with ketoconazole resulted in a 57 % increase in both AUC_{0-t} and $AUC_{0-\infty}$ and a 39 % increase in C_{max} of levomilnacipran compared with administration of levomilnacipran ER alone (all statistically

significant as indicated by the 90 % CIs for the geometric LS means ratios) (Fig. 1; Table 1). Median t_{max} was delayed for 2 h ($p < 0.0001$), and clearance reduced from 22 to 14 L/h. $T_{½}$ was similar for the two treatment arms (Table 1).

For *N*-desethyl levomilnacipran, AUC_{0-t} and $AUC_{0-\infty}$ were also elevated by approximately 22 and 20 % respectively, following co-administration with steady-state ketoconazole. C_{max} was reduced by 14 % (statistically significant). Median t_{max} was 12 h for both treatments; however, the range of t_{max} was wider, and the mean t_{max} was longer (17.46 vs. 10.94 h), with co-administration ($p < 0.0001$). Mean $t_{½}$ was similar for both treatment arms (Table 1).

3.1.2 Safety Analyses

More TEAEs were reported with co-administration than with levomilnacipran ER alone. The most frequently reported TEAE was headache, reported by 10 subjects with co-administration and none with levomilnacipran ER alone (Table 2). No serious AEs (SAEs) were reported; all TEAEs were considered by the investigator to be mild in intensity.

A small mean increase in creatinine was observed in participants in Sequence I (12.48 µmol/L), which was

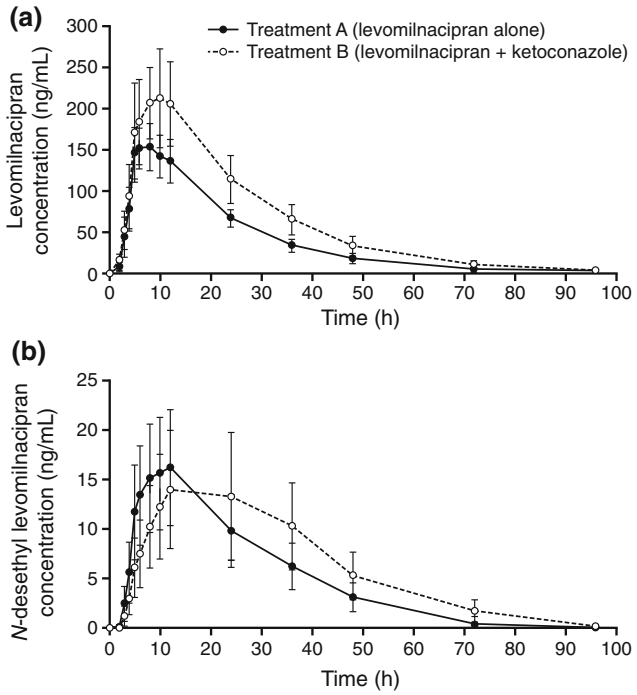

Fig. 1 Plasma concentrations of **a** levomilnacipran and **b** *N*-desethyl-levomilnacipran versus time following a single-dose administration of 80 mg levomilnacipran ER alone (Treatment A) and in combination with 400 mg ketoconazole at steady state (Treatment B). Pharmacokinetic analysis population ($N = 33$). Values shown are mean ± standard deviation. *ER* extended release

Table 1 Pharmacokinetic parameters of levomilnacipran and *N*-desethyl levomilnacipran following single-dose levomilnacipran ER 80 mg alone and with co-administration of ketoconazole 400 mg OD at steady state

Pharmacokinetic parameters	LVM ER alone ($n = 33$)	LVM ER + ketoconazole ($n = 33$)	Ratio of geometric means, % [90 % CI] or difference (p value)
Levomilnacipran			
$AUC_{(0-t)}$ (ng·h/mL)	3684 ± 552	5911 ± 1350	156.8 [147.17–167.09]
$AUC_{(0-\infty)}$ (ng·h/mL)	3730 ± 565	5976 ± 1371	156.6 [146.93–166.89]
C_{max} (ng/mL)	163.0 ± 27.8	231.0 ± 60.1	138.7 [130.72–147.07]
t_{max} (h)	7.0 ± 2.1	8.8 ± 2.5	2.0[b] ($p < 0.0001$)[c]
	6.0 (5.0–12.0)[a]	8.0 (5.0–12.0)[a]	
$t_{½}$ (h)	12.2 ± 2.5	13.1 ± 2.4	–
CL/*F* (L/h)	21.9 ± 3.3	14.3 ± 4.7	–
V_{SS}/F (L)	469.6 ± 74.8	339.0 ± 85.3	–
N-desethyl levomilnacipran			
$AUC_{(0-t)}$ (ng·h/mL)	447.2 ± 167.3	566.5 ± 247.7	122.2 [112.9–132.3]
$AUC_{(0-\infty)}$ (ng·h/mL)	492.8 ± 167.1	606.9 ± 243.8	120.0 [111.2–129.5]
C_{max} (ng/mL)	16.6 ± 5.9	14.7 ± 6.6	85.8 [79.96–91.98]
t_{max} (h)	10.9 ± 1.9	17.5 ± 6.1	– ($p < 0.0001$)[c]
	12.0 (5.0–12.0)[a]	12.0 (12.0–24.0)[a]	
$t_{½}$ (h)	14.3 ± 2.9	15.6 ± 3.7	–

Values are mean ± SD unless otherwise stated

$AUC_{0-\infty}$ area under the plasma concentration versus time curve from time zero to infinity, *AUC_{0-t}* area under the plasma concentration versus time curve from time zero to time *t*, *C_{max}* maximum plasma drug concentration, *CI* confidence interval, *CL/F* apparent total clearance of drug from plasma after oral administration, *LVM ER* levomilnacipran extended release, *OD* once daily, *SD* standard deviation, *t_{max}* time of maximum plasma drug concentration, *$t_{½}$* terminal elimination half-life, *V_{SS}/F* apparent volume of distribution at steady state

[a] Median (minimum–maximum)

[b] Difference in median t_{max}

[c] Based on Wilcoxon signed-rank test

Table 2 Incidence of TEAEs affecting ≥5 % of subjects receiving levomilnacipran ER 80 mg alone or levomilnacipran ER 80 mg plus ketoconazole 400 mg OD at steady state

Preferred term[a]	LVM ER alone *N* (%) ($n = 34$)	LVM ER + ketoconazole *N* (%) ($n = 34$)
Number of TEAEs	12	35
Subjects with at least one TEAE[b]	11 (32.4)	18 (52.9)
Headache	0	10 (29.4)
Nausea	2 (5.9)	5 (14.7)
Somnolence	7 (20.6)	4 (11.8)
Hypertension	2 (5.9)	3 (8.8)
Dizziness	0	2 (5.9)
Vomiting	0	2 (5.9)

LVM ER levomilnacipran extended release, *OD* once daily, *TEAE* treatment-emergent adverse event

[a] MedDRA dictionary version 13.0 was used in the coding of all adverse events

[b] Subjects who took any study drug (counted only once)

larger than in Sequence II (1.56 μmol/L). Pulse rate increased by mean (±SD) 9.4 (±10.3) bpm in the safety population, with changes similar between treatments. No other clinically meaningful findings regarding vital signs or ECG parameters were observed; no suicidal ideation or behaviour was reported.

3.2 Study 2 (Levomilnacipran ER Plus Carbamazepine XR)

3.2.1 Pharmacokinetics

Thirty-four healthy subjects were enrolled; eight discontinued prematurely (four due to TEAEs, three due to protocol violations, one withdrew consent). Mean age ± SD was 26.3 ± 6.6 years; mean BMI was 25.1 ± 2.7 kg/m^2; 11 participants (32.2 %) were female; 79.4 % were white and 20.6 % black.

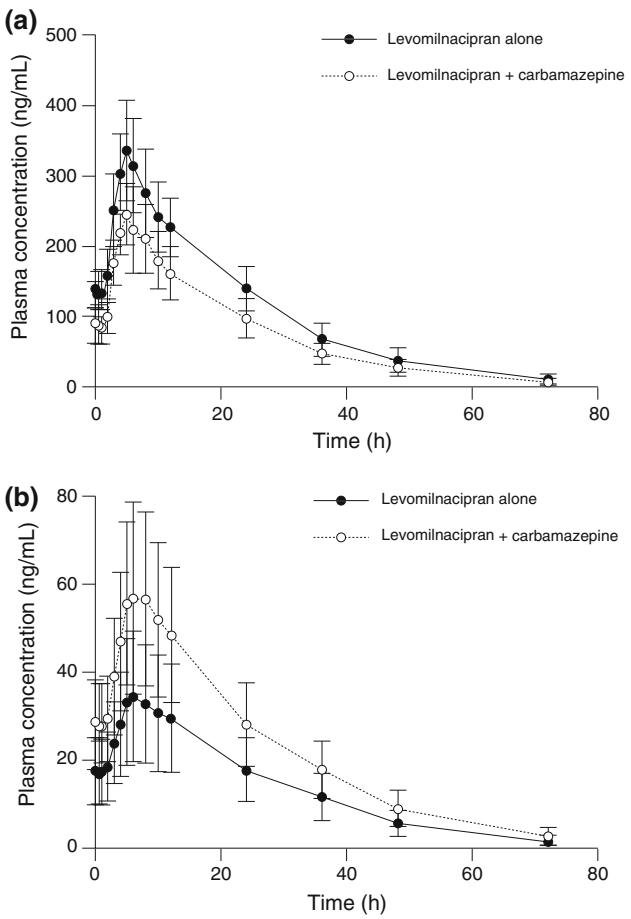

Fig. 2 Plasma concentrations of **a** levomilnacipran and **b** *N*-desethyl levomilnacipran at steady state (levomilnacipran ER 120 mg OD) versus time in the absence and presence of 200 mg BID carbamazepine XR. Pharmacokinetic analysis population ($N = 27$). Values shown are mean ± standard deviation. *BID* twice daily, *ER* extended release, *OD* once daily, *XR* extended release

Table 3 Pharmacokinetic parameters for the levomilnacipran ER:-carbamazepine XR interaction study: levomilnacipran and *N*-desethyl levomilnacipran at steady state following levomilnacipran ER 120 mg OD alone and with co-administration of carbamazepine XR 200 mg BID at steady state

	LVM ER alone ($n = 27$)	LVM ER + CBZ XR ($n = 27$)	Ratio of geometric means, % [90 % CI]
Levomilnacipran pharmacokinetics			
$AUC_{(0-\tau)}$ (ng·h/mL)	5196 ± 950	3713 ± 755	71.1 [68.0–74.4]
$C_{max,SS}$ (ng/mL)	340.9 ± 68.5	249.7 ± 43.9	73.6 [69.8–77.7]
$t_{max,SS}$ (h)[a]	5.0 (3.0–6.0)	5.0 (3.0–8.0)	–
$t_{½}$ (h)	13.2 ± 2.9	12.9 ± 3.0	–
$C_{av,SS}$ (ng/mL)	216.5 ± 39.6	154.7 ± 31.4	–
$C_{min,SS}$ (ng/mL)	140.7 ± 31.4	98.4 ± 28.2	–
N-desethyl levomilnacipran			
$AUC_{(0-\tau)}$ (ng·h/mL)	625.0 ± 253.3	1026.4 ± 332.7	169.5 [159.1–180.6]
$C_{max,SS}$ (ng/mL)	35.8 ± 14.9	59.9 ± 19.1	172.9 [162.8–183.6]
$t_{max,SS}$ (h)[a]	6.0 (5.0–12.0)[a]	6.0 (5.0–12.0)[a]	–
$t_{½}$ (h)	14.1 ± 2.9	13.8 ± 2.8	–
$C_{av,SS}$ (ng/mL)	26.0 ± 10.6	42.8 ± 13.9	–
$C_{min,SS}$ (ng/mL)	17.9 ± 7.3	28.3 ± 9.5	–

Values are mean ± SD unless otherwise stated

$AUC_{0-\tau}$ area under the plasma concentration versus time curve during a dosing interval τ at steady state, *BID* twice daily, *$C_{max,SS}$* maximum plasma drug concentration at steady state, *$C_{min,SS}$* minimum plasma drug concentration at steady state, *$C_{av,SS}$* average plasma drug concentration at steady state, *CBZ XR* carbamazepine extended release, *CI* confidence interval, LVM ER levomilnacipran extended release, *OD* once daily, *SD* standard deviation, *$t_{½}$* terminal elimination half-life, *$t_{max,SS}$* time of maximum plasma drug concentration at steady state

[a] Median (minimum–maximum)

Administration of levomilnacipran ER with carbamazepine XR resulted in a 29 and 26 % decrease in levomilnacipran $AUC_{0-\tau}$ and $C_{max,SS}$, respectively, compared with levomilnacipran ER alone (statistically significant) (Fig. 2; Table 3). The $AUC_{0-\tau}$ and $C_{max,SS}$ for *N*-desethyl levomilnacipran were elevated by 70 and 73 %, respectively, for combined treatment compared with levomilnacipran ER alone (statistically significant) (Table 3).

Carbamazepine $AUC_{0-\tau}$ and $C_{max,SS}$ were decreased by 2 and 4 %, respectively, when carbamazepine XR and levomilnacipran ER were co-administered (not statistically significant) (Fig. 3; Table 4). $AUC_{0-\tau}$ and $C_{max,SS}$ of

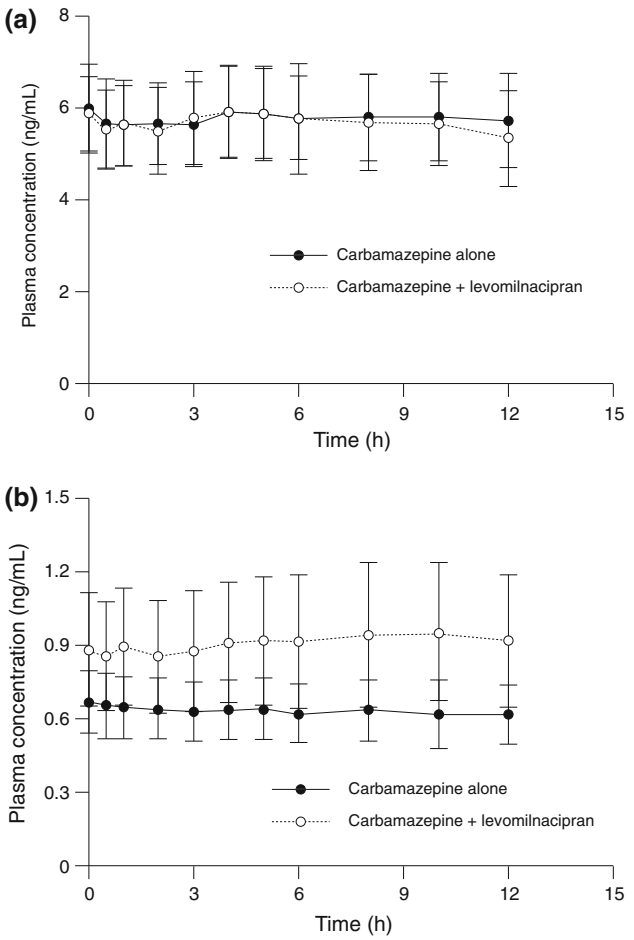

Fig. 3 Plasma concentrations of **a** carbamazepine and **b** carbamazepine-10,11-epoxide at steady state (carbamazepine XR 200 mg BID) in the absence and presence of levomilnacipran at steady state (levomilnacipran ER 120 mg OD). Pharmacokinetic analysis population ($N = 27$). Values shown are mean ± standard deviation. *BID* twice daily, *ER* extended release, *OD* once daily, *XR* extended release

carbamazepine-10,11-epoxide were increased by 42 and 36 %, respectively (statistically significant).

3.2.2 Safety Analyses

The number of subjects experiencing TEAEs was similar between treatment periods, although there were more TEAEs in the combined treatment period (Treatment C) than levomilnacipran (Treatment A) or carbamazepine treatment (Treatment B) alone (Table 5). All TEAEs were mild or moderate in intensity.

One subject in each treatment group discontinued due to AEs (four in total). This included one 39-year-old male who experienced an SAE [atrial fibrillation (AF)] on Day 11, considered moderate in intensity and related to levomilnacipran administration. The subject recovered from the AF with rapid ventricular response and aberrancy

Table 4 Pharmacokinetic parameters for the levomilnacipran ER:carbamazepine XR interaction study: carbamazepine and its metabolite at steady state following carbamazepine XR 200 mg BID alone and with co-administration of levomilnacipran ER 120 mg OD at steady state

	CBZ XR alone ($n = 27$)	LVM ER + CBZ XR ($n = 27$)	Ratio of geometric means, % [90 % CI]
Carbamazepine pharmacokinetics			
$AUC_{(0-\tau)}$ (μg·h/mL)	69.3 ± 10.5	68.3 ± 11.2	98.3 [91.5–105.5]
$C_{max,SS}$ (μg/mL)	6.5 ± 1.0	6.3 ± 1.0	96.1 [89.6–103.2]
$t_{max,SS}$ (h)	5.0 (0.0–12.0)[a]	4.0 (0.0–10.0)[a]	–
$C_{av,SS}$ (μg/mL)	5.8 ± 0.9	5.7 ± 0.9	–
$C_{min,SS}$ (μg/mL)	5.7 ± 1.0	5.4 ± 1.0	–
Carbamazepine-10,11-epoxide			
$AUC_{(0-\tau)}$ (μg·h/mL)	7.6 ± 0.1	11.0 ± 3.0	141.6 [126.5–158.4]
$C_{max,SS}$ (μg/mL)	0.7 ± 0.1	1.0 ± 0.3	136.1 [121.7–153.8]
$t_{max,SS}$ (h)	1.0 (0.0–10.0)	6.0 (0.0–12.0)[a]	–
$C_{av,SS}$ (μg/mL)	0.6 ± 0.1	0.9 ± 0.3	–
$C_{min,SS}$ (μg/mL)	0.6 ± 0.1	0.9 ± 0.3	–

Values are mean ± SD unless otherwise stated

$AUC_{0-\tau}$ area under the plasma concentration versus time curve during a dosing interval τ at steady state, *BID* twice daily, *$C_{max,SS}$* maximum plasma drug concentration at steady state, *$C_{min,SS}$* minimum plasma drug concentration at steady state, *$C_{av,SS}$* average plasma drug concentration at steady state, *CBZ XR* carbamazepine extended release, *CI* confidence interval, *LVM ER* levomilnacipran extended release, *OD* once daily, *SD* standard deviation, *$t_{max,SS}$* time of maximum plasma drug concentration at steady state

[a] Median (minimum–maximum)

the next day, after withdrawal from the study. Other withdrawals were due to rash (two subjects) and urinary tract obstruction (one subject).

One subject experienced a decrease in weight ≥7 %; no other clinically significant changes in vital signs, clinical laboratory or ECG parameters were observed. No suicidal ideation or behaviour was reported. The combined administration was generally well tolerated, although with a higher frequency of TEAEs than with either drug alone.

3.3 Study 3 (Levomilnacipran ER Plus Single-Dose Alprazolam XR)

3.3.1 Pharmacokinetics

Thirty subjects were enrolled and all completed the study. Mean age ± SD was 36.5 ± 7.4 years; mean BMI ± SD was 26.21 ± 3.01 kg/m^2; 12 participants (40 %) were female; 93.3 % were white and 6.7 % black.

Combined administration did not markedly alter the AUC and C_{max} of alprazolam (Fig. 4; Table 6). Median t_{max} for alprazolam was similar when administered alone or with levomilnacipran (9 vs. 8 h; $p = 0.3756$).

Levomilnacipran $AUC_{0-\tau}$, $C_{max,SS}$ and $C_{min,SS}$ were not meaningfully affected by co-administration with single-

Table 5 Incidence of TEAEs affecting ≥5 % of subjects receiving levomilnacipran ER 120 mg alone, levomilnacipran plus carbamazepine XR 200 mg BID, or carbamazepine 100 or 200 mg BID or OD

Preferred term[a]	LVM ER alone (Treatment A) *N* (%) (*n* = 34)	CBZ XR (Treatment B) *N* (%) (*n* = 29)	LVM ER + CBZ XR (Treatment C) *N* (%) (*n* = 28)	CBZ XR (Treatment D) *N* (%) (*n* = 27)
Number of TEAEs	43	31	54	13
Subjects with at least one TEAE[b]	14 (41.2)	11 (37.9)	14 (50.0)	5 (18.5)
Headache	5 (14.7)	5 (17.2)	6 (21.4)	3 (11.3)
Nausea	3 (8.8)	1 (3.4)	5 (17.9)	–
Dizziness	2 (5.9)	1 (3.4)	3 (10.7)	1 (3.7)
Palpitations	2 (5.9)	–	2 (7.1)	–
Fatigue	2 (5.9)	–	2 (7.1)	–
Testicular pain	1 (5.9)	–	3 (10.7)	–
Oropharyngeal pain	–	2 (6.9)	2 (7.1)	–
Chest pain	2 (5.9)	–	–	1 (3.7)
Musculoskeletal pain	–	–	2 (7.1)	–
Abnormal dreams	–	1 (3.4)	2 (7.1)	–
Rhinorrhoea	–	–	2 (7.1)	–

Treatment A: Levomilnacipran ER 20 mg for 1 day, followed by levomilnacipran ER 40 mg once daily for 3 days, followed by levomilnacipran ER 80 mg once daily for 3 days, followed by levomilnacipran ER 120 mg once daily for 4 days

Treatment B: Carbamazepine XR 100 mg twice daily for 4 days, followed by carbamazepine XR 200 mg twice daily for 17 days

Treatment C: Carbamazepine XR 200 mg twice daily for the entire period and levomilnacipran ER 20 mg for 1 day, followed by levomilnacipran ER 40 mg once daily for 3 days, followed by levomilnacipran ER 80 mg once daily for 3 days, followed by levomilnacipran ER 120 mg once daily for 4 days

Treatment D: Carbamazepine XR 200 mg and carbamazepine XR 100 mg for 1 day, carbamazepine XR 100 mg twice daily for 2 days, followed by carbamazepine XR 100 mg once a day for 1 day

BID twice daily, *CBZ XR* carbamazepine extended release, *LVM ER* levomilnacipran extended release, *OD* once daily, *TEAE* treatment-emergent adverse event

[a] MedDRA dictionary version 13.0 was used in the coding of all adverse events

[b] Subjects who took any study drug (counted only once)

dose alprazolam (Fig. 5; Table 7). Median $t_{max,SS}$ for levomilnacipran was similar (5 h) in the absence or presence of single-dose alprazolam, but in some subjects individual $t_{max,SS}$ was delayed in the presence of alprazolam ($p = 0.0055$) (Table 7).

3.3.2 Safety Analyses

There were no premature discontinuations, deaths or SAEs. No suicidal ideation or behaviour was reported. TEAEs reported in two or more patients during the trial are shown in Table 8. The most common TEAE in both treatment groups was somnolence, a known side-effect of alprazolam [20].

Treatment-emergent AEs were reported more frequently with combined administration than with alprazolam XR alone. However, Treatment A was only

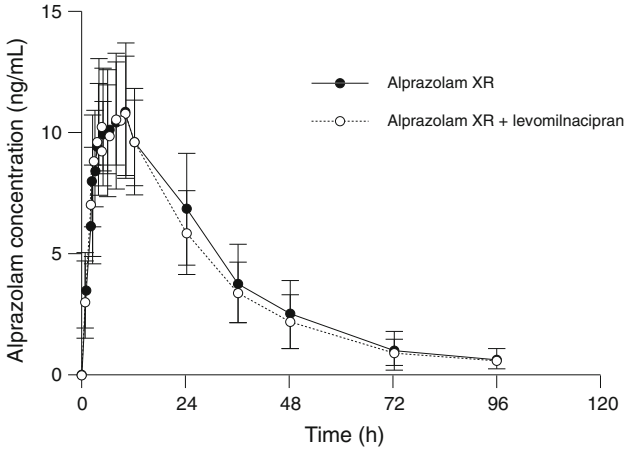

Fig. 4 Plasma concentrations of alprazolam after administration of single-dose alprazolam XR (1 mg) in the absence and presence of levomilnacipran at steady state (levomilnacipran ER 120 mg OD). Pharmacokinetic analysis population ($N = 30$). Values shown are mean ± standard deviation. *ER* extended release, *OD* once daily, *XR* extended release

Table 6 Pharmacokinetic parameter values for the interaction study between single-dose alprazolam XR 1 mg and levomilnacipran ER 120 mg at steady state: Effect of levomilnacipran ER on alprazolam XR

Alprazolam pharmacokinetics	APZ XR alone Mean[a] ± SD (*n* = 30)	APZ XR + LVM ER Mean[a] ± SD (*n* = 30)	Ratio of LS geometric means (combination:drug alone), % [90 % CI] or *p* value (Wilcoxon)
C_{max} (ng/mL)	11.71 ± 2.60	12.12 ± 2.64	103.6 [97.3–110.4]
AUC_{0-t} (ng·h/mL)	362.6 ± 116.7	337.1 ± 89.4	94.3 [88.9–100.0]
$AUC_{0-\infty}$ (ng·h/mL)	378.0 ± 128.3	351.4 ± 92.4	94.7 [89.3–100.5]
t_{max} (h)[b]	9.0 (3.0–12.0)	8.0 (4.0–12.0)	$p = 0.376$
$t_{½}$ (h)	15.9 ± 4.7	15.4 ± 4.2	–
CL/*F* (L/h)	2.9 ± 1.0	3.1 ± 0.9	–
V_z/F (L)	63.2 ± 16.1	64.7 ± 12.8	–

Values are mean ± SD unless otherwise stated

APZ XR alprazolam extended release, $AUC_{0-\infty}$ area under the plasma concentration versus time curve from time zero to infinity, AUC_{0-t} area under the plasma concentration versus time curve from time zero to time *t*, *CI* confidence interval, *CL/F* apparent total clearance of drug from plasma after oral administration, C_{max} maximum plasma drug concentration, $C_{max,SS}$ maximum plasma drug concentration at steady state, *LS* least squares, *LVM ER* levomilnacipran extended release, *SD* standard deviation, $t_{½}$ terminal elimination half-life, t_{max} time of maximum plasma drug concentration, *Vz/F* apparent volume of distribution during terminal phase after non-intravenous administration

[a] Arithmetic mean

[b] Median (minimum–maximum)

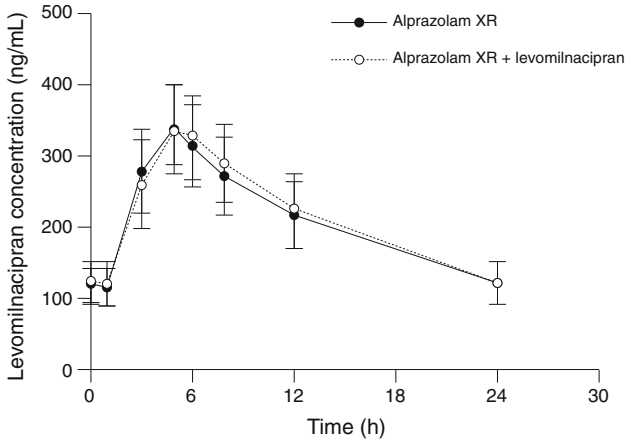

Fig. 5 Plasma concentrations of levomilnacipran at steady state (levomilnacipran ER 120 mg OD) versus time, alone or with a single dose (1 mg) of alprazolam XR. Pharmacokinetic analysis population ($N = 30$). Values shown are mean ± standard deviation. *ER* extended release, *OD* once daily, *XR* extended release

1 day in duration while Treatment B was 14 days in duration. All AEs were considered mild, except for one incidence of moderate urinary retention in the combined group.

There were no clinically significant changes in clinical laboratory results, vital signs or ECG parameters except for mean ventricular heart rate, which increased by (mean ± SD) 18.9 ± 10.0 bpm from screening to end of study. Systolic and diastolic blood pressure increased by 3.4 ± 10.4 and 1.0 ± 7.6 mm Hg, respectively. Only two subjects had potentially clinically significant changes in vital signs at any point during the study. One had a high pulse rate of 122 bpm 6 h after dosing with levomilnacipran alone, but 92 bpm (close to predose value) 11 h after dosing. A second subject had a low pulse of 48 bpm at 4 h after dosing with single-dose alprazolam alone, but 64 bpm upon repeat.

Table 8 Incidence of common ($n \geq 2$ subjects in any group) treatment-emergent adverse events in the safety population

Preferred term	Treatment A *n* (%) ($N = 30$)	Treatment B *n* (%) ($N = 30$)	All subjects *n* (%) ($N = 30$)
At least 1 TEAE	21 (70)	26 (86.7)	28 (93.3)
Somnolence	20 (66.7)	18 (60.0)	26 (86.7)
Headache	0	8 (26.7)	8 (26.7)
Constipation	1 (3.3)	3 (10.0)	4 (13.3)
Urinary retention	0	4 (13.3)	4 (13.3)
Tachycardia	0	3 (10.0)	3 (10.0)
Sinus tachycardia	1 (3.3)	2 (6.7)	2 (6.7)
Testicular pain	0	3 (10.0)	3 (10.0)
Nausea	0	3 (10.0)	3 (10.0)
Dry mouth	0	2 (6.7)	2 (6.7)
Dizziness	0	2 (6.7)	2 (6.7)
Insomnia	0	2 (6.7)	2 (6.7)
Ejaculation disorder	0	2 (6.7)	2 (6.7)
Oropharyngeal pain	0	2 (6.7)	2 (6.7)

Treatment A (treatment duration 1 day): Single oral dose of alprazolam XR 1 mg tablet under fasted conditions

Treatment B (treatment duration 14 days): Alprazolam XR 1 mg tablet co-administered with LVM (LVM ER capsules 20 mg for 1 day, 40 mg once daily for 3 days, 80 mg once daily for 3 days, 120 mg once daily for 4 days [fourth dose of 120 mg LVM administered under fasted conditions], co-administration of LVM ER 120 mg plus alprazolam XR 1 mg for 1 day under fasted conditions, followed by LVM ER 120 mg once daily for 2 days)

LVM ER levomilnacipran extended release, *TEAE* treatment-emergent adverse event, *XR* extended release

Table 7 Pharmacokinetic parameter values for the interaction study between single-dose alprazolam XR 1 mg and levomilnacipran ER 120 mg at steady state: Effect of alprazolam XR on levomilnacipran ER

Levomilnacipran pharmacokinetics	LVM ER alone Mean[a] ± SD ($n = 30$)	LVM ER + APZ XR Mean[a] ± SD ($n = 30$)	Ratio of LS geometric means (combination:drug alone), % [90 % CI] or *p* value (Wilcoxon)
$AUC_{0-\tau}$ (ng·h/mL)	5045 ± 953	5124 ± 873	101.8 [98.9–104.8]
$C_{max,SS}$ (ng·h/mL)	344.6 ± 55.8	346.8 ± 63.5	100.4 [96.9–104.0]
$C_{av,SS}$ (ng/mL)	210.2 ± 39.7	213.5 ± 36.4	–
$C_{min,SS}$ (ng/mL)	122.5 ± 29.3	119.7 ± 29.5	97.8 [93.8–101.9]
$t_{max,SS}$ (h)[b]	5.0 (5.0–6.0)	5.0 (5.0–8.0)	$p = 0.0055$

Values are mean ± SD unless otherwise stated

APZ XR alprazolam extended release, $AUC_{0-\tau}$ area under the plasma concentration versus time curve during a dosing interval τ at steady state, *CI* confidence interval, $C_{max,SS}$ maximum plasma drug concentration at steady state, $C_{min,SS}$ minimum plasma drug concentration at steady state, *LS* least squares, *LVM ER* levomilnacipran extended release, *SD* standard deviation, t_{max} time of maximum plasma drug concentration, $t_{max,SS}$ time of maximum plasma drug concentration at steady state

[a] Arithmetic mean

[b] Median (minimum–maximum)

4 Discussion

The studies reported here investigated the effects of a known strong CYP3A4 inhibitor (ketoconazole) and a strong CYP3A4 inducer (carbamazepine) on levomilnacipran pharmacokinetics because earlier in vitro results had shown that CYP3A4 is the predominant CYP isozyme involved in the metabolism of levomilnacipran (Forest Laboratories, Data on file, Xenotech Study no XT104114), and therefore the one most susceptible to drug-drug interactions. Additionally, the effect of levomilnacipran on the pharmacokinetics of alprazolam, a CYP3A4 substrate, in healthy adults was investigated. Alprazolam was chosen as the CYP3A4 substrate because it is likely to be co-prescribed with levomilnacipran ER in patients with psychiatric disorders.

Ketoconazole co-administered with levomilnacipran ER in healthy adults had a statistically significant impact on the pharmacokinetics of levomilnacipran, increasing the AUC_{0-t} and $AUC_{0-\infty}$ of levomilnacipran by 57 %, and those of *N*-desethyl levomilnacipran by about 20 %. C_{max} of levomilnacipran and *N*-desethyl levomilnacipran increased by 39 and 14 %, respectively, when co-administered with ketoconazole.

N-desethylation, mediated mainly by CYP3A4, contributes to about 18 % of the total clearance of levomilnacipran. The increase in AUC of levomilnacipran resulting from co-administration with ketoconazole is therefore slightly greater than expected if it were assumed that CYP3A4 is solely responsible for the conversion of levomilnacipran to *N*-desethyl levomilnacipran and that this pathway was completely blocked. Also, the AUC of *N*-desethyl levomilnacipran was increased by co-administration with ketoconazole, rather than decreased as would be expected, suggesting that besides the inhibition of the CYP3A4 metabolic pathway, other mechanisms may also be involved in this interaction. For example, the delayed levomilnacipran t_{max} in the presence of ketoconazole suggests that ketoconazole may affect absorption of levomilnacipran. Ketoconazole has been reported to affect transporters [21, 22] and renal function [23]; thus, alterations of these functions by ketoconazole may also affect the disposition of both levomilnacipran and *N*-desethyl levomilnacipran in the body because renal excretion is the main elimination pathway for both levomilnacipran and the metabolite [11]. Furthermore, as indicated by in vitro CYP phenotyping results [13], CYP3A4 is likely not the sole enzyme involved in the formation of *N*-desethyl levomilnacipran, and ketoconazole may have additional, unquantified effects on these alternate enzymes. In a study in hepatically impaired subjects, the increase in levomilnacipran AUC in the severely impaired participants relative to healthy control subjects was only approximately 30 %, consistently indicating that hepatic metabolism plays a minor role in levomilnacipran elimination [12].

Overall, concomitant administration of levomilnacipran ER with ketoconazole was well tolerated and there were no clinically meaningful effects of co-administration on safety parameters. The increased number of headaches reported with co-administration was likely due to an increase of levomilnacipran exposure to levels that would be expected with a single-dose administration of a 120 mg dose. In clinical practice, levomilnacipran doses of 120 mg are administered following up-titration with a starting dose of 20 mg; thus, patients would not be exposed to high-plasma levomilnacipran exposures without prior administration of lower doses.

Based on these results (57 % increase in levomilnacipran AUC), and considering the overall safety profile of levomilnacipran ER and available capsule strengths (20, 40, 80, and 120 mg), it is recommended that the dose of levomilnacipran ER should not exceed 80 mg once daily when used with strong CYP3A4 inhibitors such as ketoconazole [11].

Carbamazepine also appeared to have a statistically significant effect on the metabolism of levomilnacipran via CYP3A4. $C_{max,SS}$ and $AUC_{0-\tau}$ for levomilnacipran were lower by 26 and 29 %, respectively, and $C_{max,SS}$ and $AUC_{0-\tau}$ for *N*-desethyl levomilnacipran were higher by around 70 % under co-administration, compared with levomilnacipran ER alone. The decreases in levomilnacipran plasma exposure are not considered clinically meaningful, as levomilnacipran has demonstrated efficacy and safety over a wide dose range of 40–120 mg/day [4], and dosing increases are based on individual efficacy and tolerability.

The plasma exposure of carbamazepine was unchanged when administered with levomilnacipran ER. However, $AUC_{0-\tau}$ and $C_{max,SS}$ of carbamazepine-10,11-epoxide were increased by 36 and 42 %, respectively. These increases are unlikely to result from any interaction with levomilnacipran, which has been shown in in vitro studies to not inhibit CYP3A4 at clinically relevant levels reported above. It is possible that steady state might not have been reached for carbamazepine 10,11-epoxide, given that the carbamazepine-mediated induction half-life for the formation of the metabolite may be up to 1180 h [24]. Concomitant administration of levomilnacipran ER with carbamazepine was well tolerated and there were no clinically meaningful effects of co-administration on safety parameters.

Consistent with the lack of pharmacokinetic effect of levomilnacipran on carbamazepine, the pharmacokinetics of alprazolam, a CYP3A4 substrate, were not altered

following multiple-dose administration of levomilnacipran at the highest recommended therapeutic dose of 120 mg/day. These in vivo observations are in agreement with the in vitro findings that levomilnacipran is a poor inhibitor of CYP3A4 as well as other CYP enzymes evaluated and does not induce CYP3A4 [15, 16]. Therefore, it can also be expected that levomilnacipran is unlikely to interfere with drugs metabolised by other CYP enzymes. Concomitant alprazolam plus levomilnacipran demonstrated no additional safety and tolerability issues compared with each of the drugs administered separately.

The safety profile was generally consistent with what would be expected based on the mechanisms of action (reuptake inhibition of serotonin and norepinephrine). There was one SAE of atrial fibrillation; however, this was not reported as an AE in any of the clinical trials [3–7].

5 Conclusions

Co-administration of levomilnacipran with ketoconazole, a strong CYP3A4 inhibitor, increased levomilnacipran exposure by up to 57 %, with the possible involvement of multiple mechanisms; it is conservatively recommended that the dose of levomilnacipran ER should not exceed 80 mg once daily when used with ketoconazole or other strong CYP3A4 inhibitors (maximum recommended levomilnacipran ER dose is 120 mg once daily).

Co-administration of levomilnacipran with carbamazepine, an inducer of CYP3A4, did not cause clinically meaningful changes in the pharmacokinetics of levomilnacipran.

Based on the results of these drug–drug interaction studies, modulators of other CYP isozymes are not expected to affect the pharmacokinetics of levomilnacipran to a clinically significant degree, because in vitro studies have shown that biotransformation of levomilnacipran via *N*-desethylation is facilitated primarily by CYP3A4.

Furthermore, as in vitro studies demonstrated only a minor inhibition of CYP3A4 and lack of inhibition of other major CYP enzymes at levels of levomilnacipran much higher than therapeutic concentrations, the lack of meaningful pharmacokinetic changes in alprazolam pharmacokinetics when co-administered with levomilnacipran suggests that no dose adjustment for pharmacokinetic reasons would generally be recommended for drugs metabolised by CYP enzymes when they are co-administered with levomilnacipran ER.

Acknowledgments We thank Hao Chen (Forest Research Institute, Inc., NY) and Valerie Brunner (Pierre Fabre Médicament, Toulouse, France) for their work on the in vitro studies that preceded this work.

Compliance with Ethical Standards

Funding These studies were supported by funding from Forest Research Institute, Inc., an affiliate of Actavis, Inc. (New York, NY), and Pierre Fabre Médicament, Boulogne, France. Both companies were involved in the study design, collection (via contracted clinical investigator sites), analysis and interpretation of data, and the decision to present these results.
Writing assistance, editorial, and logistical support for this manuscript was provided by Grace Townshend and Daria Renshaw of Watermeadow Medical, UK, and funded by Forest Research Institute, Inc.

Conflicts of interest LC, RB and AP are all employees of Forest Research Institute, Inc., an affiliate of Actavis, Inc.
LC, RB and AP hold stock/stock options in Actavis, Inc.
WMG, NG, JW and PG were employees of Forest Research Institute, Inc., at the time of these studies. WMG held stock/stock options in Forest Research Institute, Inc. while an employee, but no longer holds them.

Ethical approval All procedures in the three studies were in accordance with the 1964 Helsinki Declaration (and its amendments), and the Ethical Committee or institutional review board that approved the study.

Informed consent Written informed consent was obtained from all subjects.

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