Escitalopram Is a Weak Inhibitor of the CYP2D6-Catalyzed *O*-Demethylation of (+)-Tramadol but Does Not Reduce the Hypoalgesic Effect in Experimental Pain

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Tramadol is *O*-demethylated to the active metabolite (+)-*O*-desmethyltramadol ((+)-M1) via CYP2D6, an enzyme that is weakly inhibited by escitalopram. We investigated the possibility of a pharmacokinetic (PK) and pharmacodynamic (PD) effect of escitalopram on tramadol metabolism. Fifteen healthy subjects completed this randomized, double-blind, three-phase, crossover trial. Combinations of escitalopram 20 mg/day or placebo together with tramadol 150 mg or placebo were used. Blood samples for pharmacokinetics were drawn at 0–24 h after medication. The analgesic effect of (+)-M was assessed by the cold pressor test (CPT) (area under effect curve, 1–12 h after medication ($AUEC_{1-12}$)). The median area under plasma concentration–time curve extrapolated to infinity ($AUC_{0-\infty}$) of (+)-M1 was 2.75 µmol/l·h after placebo pretreatment compared with 1.95 µmol/l·h after escitalopram ($P = 0.0027$). The mean $AUEC_{1-12}$ of CPT were 4,140 and 4,388 cm·s after placebo and escitalopram, respectively ($P = 0.71$). Although escitalopram is a weak inhibitor of CYP2D6, it does not impair the analgesic effect of tramadol.

Escitalopram is the active (S)-enantiomer of citalopram and the most selective serotonin reuptake inhibitor.[1] A recent meta-analysis comparing the efficacy and acceptability of 12 new-generation antidepressants found that escitalopram and sertraline had the best profiles with respect to efficacy and acceptability.[2] Therefore, an increase in the number of prescriptions for escitalopram is anticipated. Escitalopram is metabolized in the liver by CYP2C19, CYP2D6, and CYP3A4.[3,4] *In vitro* studies have shown that escitalopram is a weak CYP2D6 inhibitor—in human liver microsomes, the mean 50% inhibitory concentration of the CYP2D6-catalyzed *O*-demethylation of dextromethorphan to dextrorphan was 73 µmol/l for escitalopram, whereas it was 2.6 µmol/l for the well-established potent inhibitor paroxetine.[4] This was very similar to previous results for citalopram.[5,6] Racemic citalopram is a weak inhibitor of CYP2D6 *in vivo*,[7–9] but published *in vivo* data on the CYP2D6 inhibitory effect of escitalopram are very sparse. In fact, they are limited to one study examining the effect of escitalopram on metoprolol.[10] In a group of healthy subjects, the mean maximum plasma concentration (C_{max}) and the mean area under plasma concentration–time curve (AUC) of a single 100-mg dose of metoprolol were statistically significantly increased when the subjects were treated at steady state with escitalopram 20 mg/day.[10]

Depression and pain are conditions that often coexist.[11] In a study among the Australian veteran population, it was shown that, on average, 7.7% of veterans who used antidepressants used the analgesic drug tramadol, dispensed in an episodic pattern.[12] Tramadol is metabolized in the liver to the µ-opioid receptor agonist metabolite (+)-*O*-desmethyltramadol ((+)-M1) by CYP2D6.[13] (+)-M1 is crucial for the analgesic effect of tramadol in both experimental and postoperative pain.[14,15] We have previously shown that paroxetine markedly diminished, but did not abolish, the analgesic effect of tramadol through inhibition of the CYP2D6-catalyzed +M1 formation in experimental pain.[16]

In this study, we investigated a putative interaction of escitalopram on the pharmacokinetics and pharmacodynamics

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of tramadol. Our hypothesis was that escitalopram is a weak inhibitor of CYP2D6; the formation of (+)-M1 and the derived analgesic effect should be only weakly or moderately diminished. The analgesic effect was measured by the cold pressor test (CPT), an experimental pain model that has been shown to be sensitive to μ-opioid receptor agonists.[17,18] The critical flicker fusion threshold (CFFT)—a direct measure of the capacity for processing cognitive information—was used to assess the psychomotor effect.[19] Static and dynamic pupillometry, which have been found to be useful pharmacodynamic (PD) parameters for the effectiveness of tramadol, were implemented in the study.[20,21]

RESULTS

Safety and tolerability

During pretreatment, 31 adverse events were reported with escitalopram (treatment c) and 22 adverse events with placebo (treatments a and b). Tiredness, nausea, and headache were the most frequently reported adverse events during treatment for both escitalopram and the equivalent placebo. Tramadol treatment gave rise to reports of 81 adverse events; 48 such events were reported after pretreatment with escitalopram (treatment c), and 33 were reported after pretreatment with placebo (treatment b). The most frequently reported adverse events were the same during both active and placebo treatment, namely, tiredness, nausea, and dizziness. The incidence of adverse events during tramadol treatment was not statistically different between treatments b and c (χ^2-test: $\chi^2_{observed} = 2.78$; $\chi^2_{critical} = 3.84$; degrees of freedom = 1; $P = 0.10$). None of the adverse events was severe or unexpected, and all disappeared shortly after discontinuation of treatment.

Pharmacokinetics

The mean ± SE of escitalopram plasma concentrations on study days 8, 9, and 10 were 77.9 ± 9.9, 71.6 ± 8.9, and 76.2 ± 10.7 μmol/l, respectively; a conversion factor of 1 ng/ml escitalopram = 3.08 nmol/l was used. Because there were no statistically significant differences in the three trough plasma concentrations, it was concluded that a steady state of escitalopram treatment had been achieved. All subjects were compliant with escitalopram treatment.

The formation of the pharmacologically and therapeutically important (+)-M1 was statistically significantly decreased by ~20% after pretreatment with escitalopram as compared with placebo (see **Table 2**). The time courses of the median plasma concentrations of (+)/(−)-tramadol and (+)/(−)-M1

Table 1 Median and (range) pharmacokinetic values for (+)- and (−)-tramadol and the metabolites (+)- and (−)-M1, after a single oral dose of 150 mg tramadol, with statistical inference of the ratio between pretreatment with 20 mg/day escitalopram and placebo ($n = 15$)

	Placebo	Escitalopram	Statistical inference[a]	*P* value
(+)-Tramadol				
C_{max}, μmol/l[b]	0.74 (0.42–0.74)	0.97 (0.62–1.61)	1.36 (1.17–1.59)	0.0008
t_{max}, h	4.0 (2.0–4.1)	3.1 (2.0–4.1)	0.05 (−0.54 to 1.05)[c]	0.49
$AUC_{0-\infty}$, μmol/l·h	8.1 (3.4–13.8)	10.7 (6.4–21.0)	1.36 (1.17–1.57)	0.0004
$t_{½}$, h	5.8 (3.9–7.8)	5.8 (4.8–8.4)	1.07 (0.96–1.19)	0.19
CL/F, l/h	18.6 (10.8–44.2)	14.0 (7.1–23.6)	0.73 (0.64–0.85)	0.0004
(−)-Tramadol				
C_{max}, μmol/l[b]	0.65 (0.35–1.18)	0.86 (0.53–1.53)	1.36 (1.16–1.59)	0.0011
t_{max}, h	3.1 (2.0–4.1)	3.1 (2.0–4.1)	0.04 (−0.54 to 1.00)[c]	0.62
$AUC_{0-\infty}$, μmol/l·h	6.5 (2.6–11.3)	9.01 (5.06–18.98)	1.34 (1.18–1.53)	0.0003
$t_{½}$, h	5.3 (3.6–6.9)	5.4 (3.4–8.1)	1.05 (0.95–1.16)	0.35
CL/F, l/h	23.0 (13.2–57.4)	16.6 (7.9–29.7)	0.75 (0.65–0.85)	0.0003
(+)-M1				
C_{max}, μmol/l[d]	0.19 (0.7–0.28)	0.12 (0.05–0.21)	0.68 (0.56–0.84)	0.0014
t_{max}, h	4.0 (20–8.0)	4.0 (2.1–8.0)	−0.06 (−1.03–0.93)[c]	0.53
$AUC_{0-\infty}$, μmol/l·h	2.75 (1.36–3.80)	1.95 (1.24–2.62)	0.78 (0.67–0.90)	0.0027
$t_{½}$, h	6.9 (4.4–10.2)	7.1 (4.9–14.1)	1.12 (0.98–1.29)	0.91
(−)-M1				
C_{max}, μmol/l[d]	0.19 (0.12–0.29)	0.15 (0.10–0.22)	0.77 (0.676–0.88)	0.0011
t_{max}, h	4.0 (2.0–6.0)	4.0 (2.0–6.0)	0.01 (−0.98 to 0.95)[c]	0.87
$AUC_{0-\infty}$, μmol/l·h	2.27 (1.57–3.29)	2.00 (1.42–2.73)	0.81 (0.72–0.91)	0.0020
$t_{½}$, h	6.8 (3.9–9.9)	5.8 (4.7–8.8)	0.98 (0.88–1.09)	0.47

$AUC_{0-\infty}$, area under plasma concentration–time curve from time zero to infinity; CL/F, oral clearance; C_{max}, maximum observed plasma concentration; $t_{½}$, apparent elimination half-life in plasma; t_{max}, time to C_{max}.

[a]Geometric mean ratio (escitalopram/placebo) (95% confidence interval) and *P* value. [b]Conversion factor: 1 ng/ml (±)-tramadol = 3.34 nmol/l. [c]Hodges–Lehmann estimates of median difference (95% confidence interval) and *P* value. [d]Conversion factor: 1 ng/ml (±)-M1 = 3.50 nmol/l.

are depicted in Figure 1. The estimated pharmacokinetic (PK) parameters of the enantiomers of tramadol and M1 following treatments b and c are summarized in Table 1, together with statistical inference of the ratios between treatments b and c. The metabolism of both enantiomers of tramadol was impaired by escitalopram: the C_{max} and$AUC_{0-\infty}$ of both parent compounds were statistically significantly increased, and the C_{max} and $AUC_{0-\infty}$ of the respective metabolites of the enantiomers were accordingly reduced. Pretreatment with escitalopram decreased the oral clearance of both (+)- and (−)-tramadol by ~25% as compared with placebo. There were no statistically significant differences of time to C_{max} (t_{max}) and half-life ($t_{½}$) in either of the enantiomers of the parent drug or their metabolites.

Pharmacodynamics

One subject did not perform the PD tests at 12 h after medication on two occasions. Accordingly, in all three treatment regimens, the areas under effect curve (AUECs) for this subject were calculated for 1–8 h after medication.

The mean and SE of baseline values for all PD parameters are presented in Table 2, along with statistical inferences for escitalopram vs. placebo. The mean $AUEC_{1-12} \pm$ SE for the three treatment phases a, b, and c are listed in Table 3, with statistical inferences of the differences of $AUEC_{1-12}$ between placebo (treatment a) and tramadol (treatment b) and between tramadol without escitalopram (treatment b) and tramadol with escitalopram (treatment c). Figure 2 shows the time course of the median effect values of the four main PD parameters (area under the pain

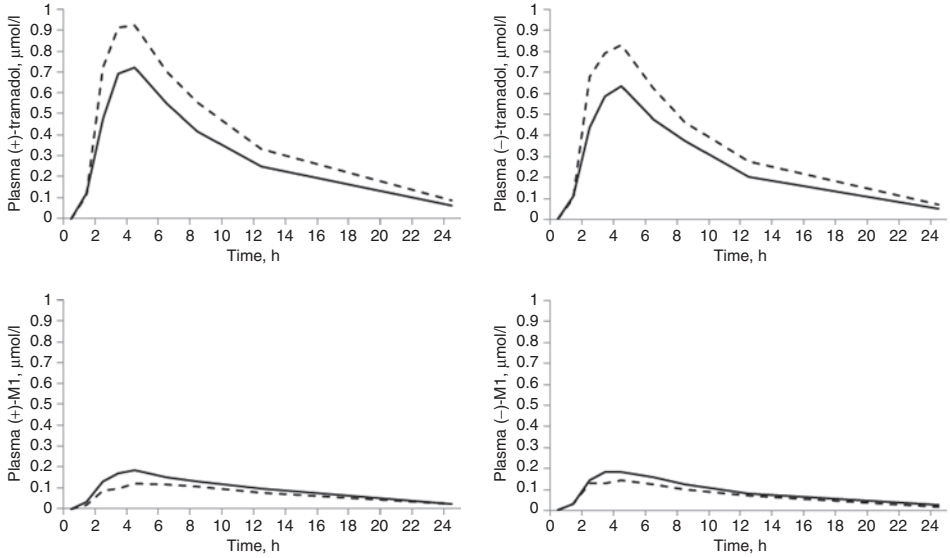

Figure 1 Median plasma concentrations of (+)-tramadol, (−)-tramadol, (+)-M1, and (−)-M1 vs. time during treatment b: placebo – tramadol (solid lines) and treatment c: escitalopram – tramadol (broken lines) ($n = 15$).

Table 2 Mean baseline values ± SE for treatment: (a) placebo–placebo, (b) placebo–tramadol, and (c) escitalopram–tramadol and statistical inference estimated as differences in baseline values after pretreatment with escitalopram vs. placebo (data pooled from treatments a and b) with 95% confidence interval (CI) and *P* value

	Baseline values			Statistical inference		
	Treatment			Escitalopram vs. placebo		
Parameter	a	b	c	Difference	95% CI	*P* value
Pain-AUC, cm·s	451 ± 60	467 ± 66	494 ± 59	35	−42–111	0.38
Pain/discomfort, cm	5.9 ± 0.5	6.4 ± 0.5	6.6 ± 0.4	0.4	−0.1–1.0	0.12
CFFT, Hz	25.2 ± 0.9	25.5 ± 0.7	25.6 ± 0.8	0.2	−0.6–1.0	0.57
MAX, mm	6.2 ± 0.2	6.2 ± 0.2	6.7 ± 0.1	0.5	0.2–0.7	0.000
MIN, mm	3.6 ± 0.1	3.6 ± 0.1	4.1 ± 0.1	0.5	0.3–0.6	0.000
Lat, s	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	−0.01	−0.02–0.01	0.37
MCV, mm/s	−6.5 ± 0.2	−6.3 ± 0.2	−6.4 ± 0.2	0.1	−0.3–0.5	0.77
CV, mm/s	−2.8 ± 0.1	−2.8 ± 0.1	−2.9 ± 0.1	−0.1	−0.2–0.1	0.34
DV, mm/s	1.3 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	0.1	−0.0–0.3	0.098
Rel. ampl.	0.42 ± 0.01	0.42 ± 0.01	0.39 ± 0.01	−0.02	−0.04 to −0.00	0.014

CFFT, critical flicker fusion threshold; CV, constriction velocity; DV, dilation velocity; Lat, latency; MAX, maximum pupil diameter; MCV, maximum constriction velocity; MIN, minimum pupil diameter; pain-AUC, area under the pain intensity–time curve; Rel. ampl., relative amplitude.

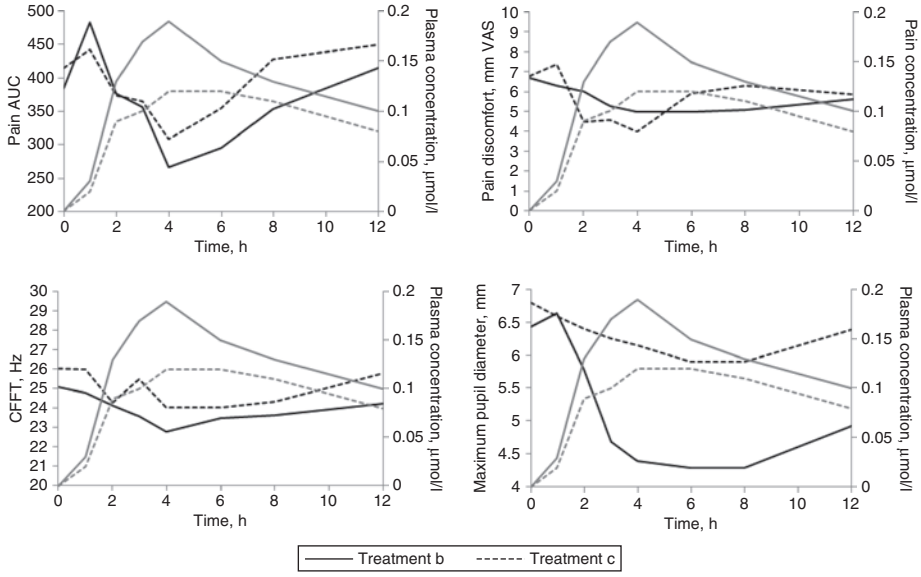

Figure 2 Median values of four different pharmacodynamic (PD) tests vs. time and the corresponding plasma concentrations of (+)-M1 (lines in gray) at b: placebo – tramadol and treatment c: escitalopram – tramadol ($n = 15$; except at $t = 12$, where $n = 14$ for the PD parameters). AUC, area under plasma concentration–time curve; CFFT, critical flicker fusion threshold; VAS, visual analog scale.

Table 3 Mean area under effect curve (AUEC) ± SE for treatment: (a) placebo–placebo, (b) placebo–tramadol, and (c) escitalopram–tramadol and statistical inference estimated as difference in AUEC with 95% confidence interval (CI) and *P* value for treatment a vs. b and treatment c vs. b

	$AUEC_{1-12}$			Statistical inference					
	Treatment			Treatment a vs. b			Treatment c vs. b		
Parameter	a	b	c	Difference	95% CI	*P* value	Difference	95% CI	*P* value
Pain-AUC, cm·s	5,112 ± 535	4,140 ± 518	4,388 ± 540	1,059	550–1,567	0.000	96	−414 to 606	0.71
Pain/discomfort, cm	68.4 ± 6.2	54.9 ± 5.0	58.4 ± 5.6	15.8	10.5–21.1	0.000	2.8	−2.3 to 7.9	0.28
CFFT, Hz	273 ± 13	259 ± 12	265 ± 12	15.9	9.8–22.0	0.000	6.0	−0.1 to 12.1	0.055
MAX, mm	66.9 ± 3.1	52.9 ± 3.0	64.2 ± 3.2	13.9	9.2–18.5	0.000	8.2	3.1–13.3	0.002
MIN, mm	39.2 ± 1.9	32.8 ± 1.7	39.1 ± 2.0	6.3	3.7–8.9	0.000	2.5	−0.6 to 5.5	0.11
Lat, s	2.0 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	−0.3	−0.4 to −0.2	0.000	−0.1	−0.3 to −0.0	0.036
MCV, mm/s	−67.3 ± 2.2	−55.9 ± 2.7	−64.0 ± 2.4	−11.5	−13.8 to −9.2	0.000	−8.1	−10.4 to −5.9	0.000
CV, mm/s	−31.1 ± 1.3	−23.7 ± 1.5	−28.5 ± 1.3	−7.4	−9.2 to −5.6	0.000	−4.8	−6.6 to −3.0	0.000
DV, mm/s	14.0 ± 1.0	11.3 ± 0.9	14.5 ± 0.9	2.6	1.4–3.8	0.000	2.8	1.5–4.0	0.000
Rel. ampl.	4.4 ± 0.1	4.0 ± 0.2	4.2 ± 0.1	0.4	0.3–0.6	0.000	0.2	0.1–0.3	0.010

Differences are corrected for baseline values ($n = 15$).

CFFT, critical flicker fusion threshold; CV, constriction velocity; DV, dilation velocity; Lat, latency; MAX, maximum pupil diameter; MCV, maximum constriction velocity; MIN, minimum pupil diameter; pain-AUC, area under the pain intensity–time curve; Rel. ampl., relative amplitude.

intensity–time curve (pain-AUC), pain/discomfort, CFFT, and maximum pupil diameter) together with median plasma concentrations of (+)-M1 in treatment b and treatment c.

Comparison of baseline values revealed that escitalopram had no effect on either the pain-AUC or the pain/discomfort of the CPT; the respective mean differences between baseline values after placebo and escitalopram treatments were 35 cm·s (95% confidence interval (CI): −42 to 111 cm·s; $P = 0.38$) and 0.4 cm (95% CI: −0.1 to 1.0 cm; $P = 0.12$; see **Table 2**). Tramadol statistically significantly reduced both parameters of CPT as compared with placebo: differences in $AUEC_{1-12}$ between treatments a and c were 1,059 cm·s (95% CI; 555–1,567 cm·s; $P < 0.001$) and 15.8 cm (95% CI: 10.5–21.1; $P < 0.001$) for pain-AUC and pain/discomfort, respectively (see **Table 3**). The reduction in pain scores achieved by tramadol was not affected by pretreatment with escitalopram: pain-AUC $AUEC_{1-12}$ = 4,140 cm·s after treatment b vs. 4,388 cm·s after treatment c ($P = 0.71$); and pain/discomfort $AUEC_{1-12}$ = 54.9 cm after treatment b vs. 58.4 cm·s after treatment c ($P = 0.28$). Inspection of the effect vs. time curves (**Figure 2**) indicated that the pain-AUC was slightly attenuated at 3–12 h after medication; however, this did not result in

a statistically significant difference in the $AUEC_{1-12}$. For both pain-AUC and pain/discomfort (see **Figure 2**),the maximum effect of tramadol was seen at 4 h after medication.

CFFT, the psychomotor-effect model, was not affected by escitalopram as compared with placebo (see **Table 2**), whereas tramadol significantly reduced the CFFT (see **Table 3**), indicating a reduction in the capacity for processing cognitive information, similar to sedation. Compared with placebo, pretreatment with escitalopram tended to minimize this effect; however, this was not statistically significant (differences between treatments c and b were 6.0 Hz; 95% CI: −0.1 to 12.1; $P = 0.055$). The maximum effect of tramadol on the CFFT was seen at 4 h after medication (**Figure 2**).

The static pupillometry was statistically significantly affected by escitalopram treatment as compared with placebo: escitalopram increased maximum pupil diameter at baseline by 0.5 mm (95% CI: 0.2–0.7 mm; $P < 0.001$). The temporal parameters of dynamic pupillometry—latency, constriction velocity, and dilation velocity—were not affected by escitalopram treatment, whereas the minimum pupil diameter was statistically significantly increased ($P < 0.001$) and the relative amplitude was statistically significantly reduced by 0.02 (95% CI: −0.4 to −0.00; $P = 0.014$). Tramadol treatment had a statistically significant effect on all pupillometry parameters as compared with placebo, and escitalopram opposed this effect in all parameters, except minimum pupil diameter; e.g., tramadol reduced $AUEC_{1-12}$ of maximum pupil diameter by 13.9 mm·h (95% CI: 9.2–18.5 mm·h; $P < 0.001$) as compared with placebo. When escitalopram was given prior to tramadol, the reduction in $AUEC_{max}$ was 8.2 mm·h (95% CI: 3.1–13.3 mm·h; $P = 0.002$) (see **Table 3**). The maximum effect of tramadol on pupil diameter occurred 4–6 h after administration of medication.

The mean maximum differences from baseline pupil diameter were −0.37, −1.82, and −1.10 mm for treatments a, b, and c, respectively. The difference between tramadol (b) and placebo (a) was statistically significant ($P < 0.001$), as was the difference between tramadol with (c) and without (b) escitalopram pretreatment ($P = 0.006$).

DISCUSSION

In this study, a statistically significant effect of escitalopram on the CYP2D6-mediated metabolism of tramadol to the (+)-M1 was shown. The estimated median AUC of (+)-M1 was decreased ~20% when subjects were pretreated with escitalopram at a dosage of 20 mg/day at steady state. According to the US Food and Drug Administration draft guidance for drug–drug interaction studies to the pharmaceutical industry,[22] escitalopram should be classified as a weak CYP2D6 inhibitor. This is in good agreement with the *in vitro* and *in vivo* data identified in the literature search and websites.[4,10,23] Despite the decreases in plasma concentrations of (+)-M1, the antinociceptive effect of tramadol was not significantly affected by pretreatment with escitalopram in both parameters of the applied experimental pain model.

Tramadol decreased CFFT, indicating a sedative effect. This effect was borderline significantly diminished by escitalopram pretreatment. Further, at a clinically relevant dose, escitalopram did not demonstrate a sedative effect. This finding is complementary to other studies of escitalopram psychomotor function, in which no effect was found in various other psychomotor tests.[24,25] The alteration in CFFT after pretreatment with escitalopram as compared with placebo is thus most likely due to a reduction in exposure to (+)-M1; the alteration in CFFT is not a direct effect of escitalopram.

Tramadol caused a reduction in pupil size (miosis) of a magnitude consistent with the findings of previous studies of tramadol in healthy subjects.[20,26] Furthermore, the baseline pupillometry data showed that escitalopram statistically significantly increased the maximum pupil diameter (mydriasis). In a previous study of the effect of escitalopram on pupil diameter, it was not possible to detect an effect after single or repeated doses of 10 mg escitalopram.[3] This discrepancy might be due simply to dose differences. Escitalopram reduced the relative reflex amplitude in this study, which is in accordance with the previous study's findings.[3] The minimum pupil diameter is highly correlated with the initial pupil diameter,[27] and the observed reduction in minimum pupil diameter after escitalopram treatment is considered a related finding, not an independent one. When the drugs are administered in combination, the combined result of the two opposing effects of escitalopram and tramadol on pupil diameter is difficult to predict. The miotic effect of tramadol was reduced after pretreatment with escitalopram, indicating that the pupil size is a result of a balance between the miotic effect of tramadol and the mydriatic effect of escitalopram.

In this study, we used extra gelatin capsules for the tramadol. The t_{max} of tramadol and metabolites were thus long as compared with previous findings.[20,28] However, the other PK parameters of tramadol found in this study are in good agreement with the results from two former studies of tramadol metabolism in CYP2D6 extensive metabolizers,[20,28] and the overall conclusions from the study are considered to be unaffected by the formulation of tramadol used.

The CPT has been proven to be a sensitive measure of the effects of μ-receptor agonists.[17,18] However, the analgesic effect of tramadol is based on a multimodal synergistic mechanism; in addition to the μ-receptor agonistic effect of (+)-M1, (+)-tramadol is a serotonin reuptake inhibitor, and (−)-tramadol acts as a noradrenaline reuptake inhibitor.[29] Escitalopram is a potent selective serotonin reuptake inhibitor, and it might therefore contribute to the analgesic effect of (+)-tramadol. Combining these drugs might be beneficial to the overall analgesic treatment effect and should be explored, either in experimental pain studies applying tests that are sensitive to the monoamine-mediated analgesic effect or in future clinical trials addressing the clinical implications of the present findings.

In conclusion, we found that escitalopram:

- Is a weak inhibitor of CYP2D6
- Reduces the formation of the (+)-M1 (μ-receptor agonist) metabolite of tramadol by ~20%
- Does not impair the analgesic effect of tramadol
- Does not affect psychomotor function but did diminish the sedative effect of tramadol
- Causes mydriasis and thus reduces the miotic effect of tramadol

The present results do not support advising against concomitant treatment with escitalopram and tramadol.

METHODS

Subjects. Sixteen healthy subjects (3 men and 13 women) were included, after they had given written informed consent. One woman withdrew her consent before receiving any trial medication. Fifteen subjects completed the clinical trial. Subjects were recruited from a panel of CYP2C19- and CYP2D6-phenotyped subjects at the Institute of Public Health, Clinical Pharmacology, University of Southern Denmark, in Odense, Denmark. The subjects were phenotyped as CYP2C19 and CYP2D6 extensive metabolizers using omeprazole and tramadol, respectively, as the probe drugs. The plasma metabolic ratios (MR_{ome}) of omeprazole vs. 5-OH-omeprazole were calculated, and a cutoff point of 6 (antimode) was used.[30] The mean MR_{ome} was 0.95 (range 0.15–2.73). The urine MR of the tramadol metabolites (−)-M1 vs. (+)-M1 (MR_2) in 0–8-h urine samples were calculated; the antimode = 2 was used.[31] The mean MR_2 was 0.72 (range 0.50–1.51). All subjects were assessed via physical examination, review of medical history, and appropriate laboratory testing and were found to have normal cardiovascular, renal, and hepatic functions and to be free of alcohol and drug abuse. The mean age of the subjects was 25 years (range 21–30 years), and the mean body mass index was 23 (range 19–28). Subjects were not allowed to use any analgesics or to consume alcohol within 24 h before treatment and during each treatment period. They were also asked to restrict their daily consumption of caffeine-containing beverages to a maximum of six cups a day.

Study design. This was a randomized, double-blind, placebo-controlled, three-phase, crossover interaction study. The study procedures at each phase were identical and were conducted as follows. the individual subject was instructed to take the trial medication (escitalopram or placebo) at home each morning at 8:00 AM on study days 1–8. All subjects were provided with a diary and instructed to note the exact time of medication intake and any adverse events occurring during treatment at home. On study day 9, they consumed their usual breakfast at 7:00 AM before appearing at the clinical trial unit at the Institute of Public Health at 7:30 AM. The test procedures were recapitulated as subsequently described, and an intravenous catheter was placed in a forearm vein of the subjects. At 8:00 AM, subjects received the last dose of escitalopram or placebo, together with a single oral dose of tramadol or placebo.

The three phases consisted of the following combinations of active drugs and equivalent placebo: treatment a, placebo for escitalopram and placebo for tramadol; treatment b, placebo for escitalopram and tramadol; and treatment c, escitalopram and tramadol. The drugs used were escitalopram, 3 days of 10 mg/day, followed by 6 days of 20 mg/day or placebo (all manufactured and provided by H. Lundbeck, Valby, Denmark), or tramadol, 3 × 50 mg as a single dose (Nobligan; Grünenthal Denmark, Copenhagen, Denmark). The tramadol capsules were put inside gelatin capsules in order to make them appear identical to the placebo capsules. The placebo for tramadol was manufactured by the Hospital Pharmacy Fyn, Odense University Hospital, Odense, Denmark. All trial medications were packed by the Hospital Pharmacy Fyn. The treatment order was randomized in blocks of four. The participants themselves randomly and blindly chose individual packages of trial medication. The treatment phases were separated by washout periods of at least 15 days.

This study was registered in the European Clinical Trial Database (EudraCT no. 2007-004470-10). The protocol was approved by the Danish Medicines Agency (J no. 2612-3633), the Danish Data Protecting Agency (J no. 2007-41-1565), and the Regional Committee on Biomedical Research Ethics of Vejle and Funen Counties (project ID S-20070113). The study was conducted in accordance with good clinical practice and monitored by the good clinical practice unit of Odense University Hospital, Odense, Denmark. The trial was registered in the US National Institutes of Health registry at http://www.clinicaltrials.gov (NCT00692263).

PK tests. Blood samples for the PK analysis of tramadol and metabolites were drawn from the intravenous catheter before and at 1, 2, 3, 4, 6, 8, 12, and 24 h after medication. At each sample time, 2 × 10 ml EDTA-stabilized blood was drawn. Blood was centrifuged at 2,400 *g* for 10 min, and plasma was kept at −20 °C until drug analysis.

Additional 2 × 10-ml EDTA blood samples were drawn prior to medication at treatment days 8, 9, and 10, and plasma concentrations of escitalopram were measured for evaluation of compliance and the achievement of a steady state of escitalopram treatment.

Plasma tramadol and metabolites analysis: Solid-phase extraction was used to extract (+)- and (−)-tramadol and (+)- and (−)-M1 from plasma. Quantification was carried out with a high-performance liquid chromatography method with fluorescence detection.[32] The chiral column was replaced with Daicel Chiralpak IA 250 × 4.6 mm^2 (Chiral Technologies Europe, Illkirch, France). The lower limit of quantification for tramadol and M1 was unchanged at 5 nmol/l.

Plasma escitalopram and metabolites analysis: After extraction from plasma with a solid-phase extraction method, plasma escitalopram was assessed by high-performance liquid chromatography with fluorescence detection.[3]

PK data analysis: The AUC extrapolated to infinity ($AUC_{0-\infty}$) of (+)- and (−)-tramadol and (+)- and (−)-M1 was calculated using the linear trapezoidal method. The values of C_{max} and the t_{max} of (+)- and (−)-tramadol and (+)- and (−)-M1 were read directly from the data. The terminal elimination $t_{½}$ of (+)- and (−)-tramadol and (+)- and (−)-M1 was calculated as $t_{½} = \ln 2/\lambda$, where λ is the terminal slope of the time vs. log concentrations as calculated by linear regression. The total body clearance (CL/F) of (+)- and (−)-tramadol were calculated as CL/F = dose/$AUC_{0-\infty}$. The PK parameters were calculated by noncompartmental methods using the WinNonlin Professional software package, version 5.1 (Pharsight, Mountain View, CA).

PD tests. At the time of enrollment, the participants were familiarized with the PD tests described below, and in order to minimize adaptation phenomena, the tests were repeated when the participants arrived on each study day. The PD tests were carried out in the order in which they are described in the following, before (baseline) and at 1, 2, 3, 4, 6, 8, and 12 h after medication.

CFFT: CFTT was assessed using the Leeds Psychomotor Tester (ZAK, Simbach am Inn, Germany). The subjects were seated 1 m in front of four red light–emitting diodes and instructed to respond, by pushing a button, when they perceived a change in the emitted light from a flicker to a fusion, or vice versa. Subjects wore adjustable stenopeic eyeglasses, opaque except for a 1.5-mm opening. The test was repeated three times with increasing flicker frequencies and three times with decreasing flicker frequencies. The overall mean of the ascending and the descending values was used as the CFFT value.[33]

CPT: Each participant immersed the left hand up to the wrist in ice-chilled water (1.0 ± 0.3 °C) that was continuously stirred by an air pump. The hand was kept in the water for 2 min or until the pain was considered intolerable. During the test, the participant periodically rated the pain intensity using an electronic visual analog scale. The pain-AUC was used as the pain score. Immediately after the CPT, participants rated their overall discomfort during the test by use of a visual analog scale (pain/discomfort).[34]

Pupillometry: Static and dynamic pupillometry were performed using the NeurOptics Pupillometer-PLR (NeurOptics, San Clemente, CA). The PLR is a handheld, monocular, infrared optical scanner. A digital camera, integrated infrared illumination, a visible flash-stimulus source, and a microprocessor are integrated in the pupillometer.

Data were acquired at a rate of 38.6 frames per second. A measurement sequence consisted of a 5-s targeting phase followed by a 3.2-s acquisition phase, including a single-flash visual stimulus of 0.8 s. During measurement, a live image of the pupil is displayed on a color liquid crystal display monitor. Measurements could be reviewed via the liquid crystal display and accepted or rejected immediately after capture. After measurement, data analysis was automatically initiated,

and the results of the test were shown on the liquid crystal display together with the pupil trajectory profile as a function of time and pupil size.

The results of a measurement consisted of the following parameters: static pupillometry, maximum pupil diameter (mm), and dynamic pupillometry; and, following the single-flash stimuli: minimum pupil diameter (mm), latency (s), constriction velocity (mm/s), maximum constriction velocity (mm/s), and dilation velocity (mm/s). Relative amplitude was calculated as (maximum pupil diameter − minimum pupil diameter)/maximum pupil diameter.

Pupillometry was carried out in a room with a fixed light intensity of 25 cd/m^2 as measured by the Testo 545 light Level Lux Meter (Testo, Hampshire, UK). In order to avoid accommodation during measurement, subjects were instructed to focus on a mark placed ~2 m away. After a 2-min period of dark adaptation, two measurements were recorded 2 min apart.

PD data analysis: AUEC at 1–12 h after treatment ($AUEC_{1-12}$) was calculated for each PD parameter through linear interpolation using the noncompartmental model 220 for drug effect in the WinNonlin Professional software package. Baseline values at $t = 0$ were not included in the AUEC calculation but were used as a fixed factor in the linear mixed-effect model to correct for any effect of escitalopram on the PD tests.

Adverse events. Adverse events occurring during unsupervised treatment were recorded in a diary by the subject. During study day 9, the subjects were monitored closely, and the trial manager recorded any adverse events on the case report form.

Statistical methods. The sample-size calculation was based on the primary outcome: $AUC_{0-\infty}$ of (+)-M1, with the assumption of a log-normal distribution and an interindividual coefficient of variance of 34%.[20] It was calculated that 16 subjects were needed to complete the study if a difference of 27% in $AUC_{0-\infty}$ of (+)-M1 between treatments b and c should be detected with a two-sided α of 0.05 and a power $(1 - \beta)$ of 80%.

The secondary PK end points were C_{max} and $t_{½}$ of (+)-M. One and secondary PD end points were $AUEC_{1-12}$ of CFFT, pain-$AUC_{0-2\ min}$, pain/discomfort, and maximum pupil diameter. The remaining PK and PD parameters were used for explorative purposes.

The PK data are presented as medians and ranges, unless otherwise indicated. Before statistical analysis, all data except t_{max} were transformed by the natural logarithm to create a Gaussian distribution. Parameters were transformed back to original scale when the effects were described (Microsoft Office Excel 2007; Microsoft Denmark, Hellerup, Denmark).

Statistical inferences of escitalopram on tramadol PK parameters were analyzed by paired *t*-test and are presented as geometric mean ratios with exact 95% CIs and associated *P* values (GraphPad QuickCalcs, http://graphpad.com/quickcalcs/index.cfm; GraphPad Software, San Diego, CA). Inference tests on t_{max} were analyzed by Hodges–Lehmann estimates of median differences with exact 95% CIs (StatXact-3; Cytel Software, Cambridge, MA).

PD data are presented as means and SEs. A linear mixed-effect model tested statistical inferences of escitalopram treatment on PD parameters with subject as random effect. Fixed effects were treatment and baseline values.[35,36] The residuals were graphically tested and found to be independent of each other and distributed identically to the normal distribution (STATA/SE 9.2 for Windows; StataCorp, College Station, TX).

The distribution of adverse-event frequencies was tested using χ^2-test (STATA/SE 9.2 for Windows; StataCorp, College Station, TX).

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CONFLICT OF INTEREST

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