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PHARMACOGENETICS

Impact of CYP2C19 phenotypes on escitalopram metabolism and an evaluation of pupillometry as a serotonergic biomarker

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Abstract
Purpose To investigate the impact of cytochrome P450 2C19 (CYP2C19) phenotypes on escitalopram metabolism and to evaluate pupillometry as a serotonergic biomarker.
Methods This was a double-blind, crossover design study with single and multiple doses of 10 mg escitalopram and placebo in panels of CYP2C19 extensive (EM) and poor metabolisers (PM). Pupillometry was measured by a NeurOptics Pupillometer-PLR.
Results Five PM and eight EM completed the study. The CYP2C19 phenotype significantly affected the metabolism of escitalopram. The area under the time–plasma concentration curve ($AUC_{0\text{-}24}$) was 1.8-fold higher in PM than in EM after both single and multiple doses. Escitalopram treatment did not affect the maximum pupil size, but it did statistically significantly decrease the relative amplitude of the pupil light reflex compared to the placebo; this effect was equal in both phenotype groups.
Conclusions The CYP2C19 polymorphism affects escitalopram metabolism, but the difference does not justify dose adjustment. The puzzling results from pupillometry can be due to interplay between a central and a local serotonergic effect. Based on these results, pupillometry can not be recommended as a serotonergic biomarker.

Keywords Biomarker · CYP2C19 · Escitalopram · Pharmacodynamics · Pharmacokinetics · Pupillometry

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Introduction

Escitalopram is the S-enantiomer of the selective serotonin reuptake inhibitor (SSRI) citalopram, and it is used for the treatment of depression, a variety of other affective disorders, a number of anxiety disorders and obsessive compulsive disorder (OCD) [1]. Citalopram is a racemate consisting of equal proportions of an R- and an S-enantiomer, and it has been demonstrated that the S-enantiomer is a far more potent inhibitor of the presynaptic serotonin transporter (SERT) than the R-enantiomer and, consequently, that the therapeutic effect of citalopram probably originates almost exclusively from S-citalopram [2]. The R-enantiomer inhibits the effect of S-citalopram through allosteric modulation of the SERT [3]. The antidepressant mechanism of escitalopram has not yet been fully elucidated, but the prevailing hypothesis is that it is mediated by an inhibition of SERT, leading to an increased level of serotonin (5-HT) in the synaptic cleft [4].

Previous studies have shown that racemic citalopram is mainly metabolised by cytochrome P450 2C19 (CYP2C19)

[5]. Up to 5% of Blacks and Caucasians are phenotyped as poor metabolisers (PM), whereas up to 20% of Asians are PM [6]. There are at least nine different variants of the *CYP2C19* gene associated with no enzyme activity [7, 8] .

Racemic citalopram is enantioselectively metabolised via CYP2C19, and the S-enantiomer is more rapidly metabolised than the R-enantiomer, as was demonstrated in a panel study of healthy volunteers [9] and in a retrospective study of therapeutic drug monitoring samples from psychiatric patients [10]. To the best of our knowledge, no formal panel study addressing the role of CYP2C19 in the metabolism of escitalopram has been performed. Consequently, the main aim of this study was to investigate the impact of CYP2C19 polymorphism, as assessed by phenotype, on escitalopram and demethylescitalopram pharmacokinetics (PK) in panels of CYP2C19 extensive metabolisers (EM) and CYP2C19 PM.

The pupil diameter is known to be a useful biomarker in studies of opioid-like drugs and is capable of distinguishing between CYP2D6 EM and PM after a single dose of tramadol [11]. In a recent double-blind, crossover study of healthy subjects, citalopram and sertraline induced a marked acute and steady increase of pupil diameter, but the mechanism is unclear [12].

Based on the results of pupillometry as a biomarker of opioid effect and CYP2D6 activity, we decided to implement and evaluate pupillometry as a potential biomarker of the serotonergic effect of escitalopram, as a broad range of plasma concentrations of escitalopram was expected in the study population. Thus, the secondary purpose of this study was to evaluate static and dynamic pupillometry as a possible biomarker for the serotonergic effect of escitalopram. Our hypothesis was that the anticipated mydriatic effect of escitalopram—all other things equal—would be higher in CYP2C19 PM than in CYP2C19 EM due to the higher plasma concentrations this drug.

Methods and materials

Study design and study procedures

The study was conducted as a randomised, double-blind, placebo-controlled, two-phase, crossover, phenotype panel trial with single and multiple doses (1 dose/day for 8 days) of 10 mg escitalopram (H. Lundbeck A/S, Copenhagen, Denmark) and equivalent placebo (H. Lundbeck A/S). Subjects were classified into groups according to their CYP2C19 phenotype, as determined by their omeprazole metabolic capacity. *CYP2C19* genotypes were determined after the subjects were entered into the study.

The treatment consisted of two phases: one with escitalopram and one with placebo. A total of 20 packages of trial medication were packed by Hospital Pharmacy Fyn, Odense University Hospital. Randomisation of treatment order was done in ten blocks of two subjects. The individual package of trial medication was randomly chosen by the subjects. On the first study day, subjects came to the Department of Clinical Pharmacology at 7:30 a.m. They were instructed beforehand to eat their usual breakfast at 7:00 a.m. and were not to eat or drink anything except water until they received a free meal at 12:00 noon. A venous canula was placed in the forearm of the subjects for blood sampling. At 8:00 a.m., the drug was taken with approximately 100 ml of tap water. Blood samples and pupillometry were undertaken at pre-arranged times, as outlined below. Subjects stayed at the trial unit until 4:00 p.m., then the venous canula was removed, and they were free to leave the trial unit. Subjects returned shortly before 8:30 p.m. and 8:00 a.m. the next morning for pupillometry and blood sampling. Participants were instructed to continue their intake of the trial medication at 8:00 a.m. for the following five days. On day 8, subjects returned to the trial unit and the same procedure as that carried out on the first day was repeated. Following the multiple dose regimen, blood sampling and pupillometry were performed on an outpatient basis, every morning for 6 days. The two phases were separated by at least 15 days of wash-out, and a follow-up visit was arranged at least 15 days after the last dose.

Information on adverse events was recorded by the simple question: “How are you?” when the subjects turned up for blood sampling and pupillometry. The subjects were also instructed to report any adverse event that occurred during the 5 days of self-administered medication.

This study was registered in the European Clinical Trial Database (EudraCT no.: 2006-001976-19). The protocol was approved by the Danish Medicines Agency (J. no: 2612-3153), the Danish Data Protection Agency (J. no. 2006-41-6658) and the Regional Committee on Biomedical Research Ethics of Vejle and Funen Counties (Project ID: VF-20060045). The study was conducted in accordance with Good Clinical Practice (GCP) and monitored by the GCP-unit, Odense University Hospital, Odense, Denmark. The trial was registered in the U.S. National Institute of Health register (www.clinicaltrials.gov) as trial NCT00397059.

Subjects

Subjects were selected from 306 healthy volunteers phenotyped for CYP2C19 using omeprazole as a probe drug [13] who had been recruited mainly among students at the University of Southern Denmark, Odense, Denmark and University College Lillebaelt, Denmark. All subjects (101 men and 205 women, aged 19–45 years) were Caucasian. More than 95% belonged to the Nordic population, whereas the remaining 5% or so came from southern Europe or the Middle East.

Nine subjects were phenotyped as PM, and all were invited to participate in the study. Two declined—one due to family reasons and one due to a previous experience with adverse effects during treatment with escitalopram—and a third PM was excluded as she had started treatment with escitalopram after being phenotyped.

Six PM (one man and five women) and eight EM (one man and seven women) gave informed written consent. One female PM withdrew her consent before receiving any trial medication due to a change in residence. In total, 13 subjects completed the trial. All subjects had normal cardiovascular, renal and hepatic functions as assessed by a physical examination, review of the medical history and appropriate laboratory testing. Subjects were interviewed on their use of drugs, herbal medicine and alcohol; none of the subjects had any history of drug or alcohol abuse. The eight subjects in the EM group had a median age of 25 years (range 21–32 years), a median weight of 67 kg (range 51–88 kg), and a median height of 169.5 cm (range 165–173 cm). The five PM had a median age of 24 years (range 21–27 years), a median weight of 59 kg (55–74 kg) and a median height of 169 cm (range 165–177 cm). There were no differences in mean age or body mass index between the phenotype groups (P=0.52 and P=0.25, respectively). All subjects were genotyped as *CYP2D6* EM.

Phenotyping The CYP2C19 phenotype was determined with omeprazole (AstraZeneca, Albertslund, Denmark) as probe drug [14, 15]. The metabolic ratio (MR) of omeprazole to hydroxyomeprazole was used to assess the individual capacity of CYP2C19. The EM were defined as having a MR<6 and the PM as having a MR≥6 [14, 15].

In the total group, the 297 EM had a median MR of 0.9 (range 0.07–5.1), and the nine PM had a median MR of 13.6 (range 6.3–25.6). In the study reported here, the eight EM had a median MR of 0.9 (range 0.3–1.7), and the five PM had median MR of 13.6 (range 6.4–16.3). There was no statistical significant difference in the median MR of the subjects participating in the clinical study and that of the entire population, either in the EM group or the PM group (P=0.59 and P=0.84, respectively).

Genotyping After inclusion to the trial, each subject was genotyped for *CYP2C19 *2, *3,* and**4*. The *CYP2C19*2* allele was identified using a commercially available 5′-exonuclease-dependent assay that includes proprietary amplification primers and two allele-specific fluorescence-labeled probes (Applied Biosystems, Foster City, CA). Determination of *CYP2C19*3* and *CYP2C19*4* were based on PCR analysis) and restriction enzyme treatment of amplified fragments [16, 17].

The *CYP2C19* genotype distribution in accordance with the phenotypes were: EM: *CYP2C19*1/*1* (n=7), *CYP2C19*1/*2* (n=1); PM: *CYP2C19*1/*2* (n=1), *CYP2C19*2/*2* (n=3)and *CYP2C19*2/*4* (n=1).

Blood samples Blood samples for the PK analysis were drawn prior to and at 1, 2, 3, 4, 6, 8, 12 and 24 h after the administration of the single and multiple dose medication. Following the multiple dose treatment, blood sampling was repeated every 24 h (at 8:00 a.m.) until 144 h after last medication. Samples (2×10 ml) of K-EDTA blood were drawn at each sampling time; these were centrifuged for 10 min at 2400 *g* and the plasma separated and kept at −20°C until drug analysis.

Drug analysis

Escitalopram and demethylescitalopram were extracted from plasma using Bond Elut–C18 solid-phase extraction (SPE; 100 mg, 3 mL) cartridges (Varian, Palo Alto, CA). The SPE cartridge was preconditioned by 2 mL methanol, 1 mL 1 mol/L hydrochloric acid and 1 mL Milli-Q water. A 1 mL aliquot of plasma and 10 μL 0.1 mmol/L alprenolol (internal standard) was whirlimixed for 5 s and applied to the SPE cartridge. The sample was allowed to run slowly through the column by the use of gravity and a minimum use of vacuum. This was followed by a washing procedure consisting of 1 mL Milli-Q water, 1 mL 50% methanol and 1 mL acetonitril. The columns were allowed to dry 1 min at full vacuum. The compounds were eluted using 1 mL of a freshly prepared 2% formic acid in methanol. The eluent was evaporated to dryness at 50°C under a gentle stream of nitrogen. The compounds were redissolved in 200 μL of eluent A (see below) and transferred to 300-μL conical high-performance liquid chromatography (HPLC) micro-vials. The vials were centrifuged at 5000 *g* for 2 min, and a 125-μL sample was transferred to a new microvial. A 75-μL aliquot was injected onto the analytical system that consisted of a LaChrom HPLC system equipped with a fluorescence detector (λ_{ex}=250 nm; λ_{em}=325 nm) (Merck-Hitachi, Darmstadt, Germany). The separation was performed on a 150×4.6 mm Synergi Polar-RP column (particle size 4 μm) (Phenomenex, Torrance, CA) using a two-phase gradient elution. The eluents consisted of 10 mmol/L KH_2PO_4 (pH 4):acetonitril (67:33) (eluent A) and 10 mmol/L KH_2PO_4 (pH 4):acetonitrile (40:60) (eluent B). The gradient profile was 0–12 min: 0% B; 12.1–14 min: 0–100% B, 14.1–20 min: 100% B; 20.1–22 min: 100–0% B; 22.1–35 min: 0% B. The flow rate was 1 mL/min.

The linearity of the method was investigated for escitalopram and demethylescitalopram in a range of 0–200 nmol/L. The correlation coefficient for each compound was >0.996. The mean recovery of the extraction procedure was 86% for escitalopram and 88% for demethylescitalo-

pram. The intraday variability was < 6% for escitalopram and < 5% for demethylescitalopram. The interday variability (n=5 days) was investigated for three concentration levels (7.5, 20 and 38 nmol/L) and was < 1% for escitalopram and < 1.3 % for demethylescitalopram. The accuracy for escitalopram ranged from 100.5 to 101.2% and from 99.6 to 100.9% for demethylescitalopram. The lower limit of detection (LOD) was 3 nmol/L and the lower limit of quantification (LOQ) was 4 nmol/L for both escitalopram and demethylescitalopram. The following conversion factors were used: 1 ng/ml escitalopram=3.08 nmol/L; 1 ng/ml demethylescitalopram=3.22 nmol/L.

Pupillometry Static and dynamic pupillometry was performed using the hand-held infra-red NeurOptics Pupillometer-PLR (NeurOptics, San Clemente, CA). The following parameters were measured: maximum pupil diameter (mm) (MAX) and minimum pupil diameter (mm) (MIN). Relative amplitude (REL AMPL) was calculated as (MAX − MIN)/MAX. A schematic drawing of the pupil trajectory profile displayed as a function of time and pupil size is depicted in Fig. 1.

Pupillometry was recorded prior to medication and at 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 12, and 24 h after medication intake on days 1 and 8. After multiple doses, the measurements were repeated at 24-h intervals until 144 h after the last dose had been taken.

Pupillometry was carried out in a room without windows, and the light intensity was kept at 5 cd m^{-2}(Testo 545 Light Level Lux Meter; Testo, Hampshire, UK). After a 2-min period of dark adaptation, the subject was instructed to focus on a mark placed approximately 4 m away in order to avoid accommodation during the measurement. Based on the results from the pupillometer evaluation, we decided to record two measurements with an interval of 2 min. The maximum limit of acceptable difference between the two measurements of maximum pupil diameters was set at 0.7 mm; if the limit was exceeded, a third measurement was performed. The mean values of the two measurements were used in subsequent analyses.

Pharmacokinetic data analysis The PK parameters for escitalopram and demethylescitalopram were calculated by standard non-compartmental methods using the software package WinNonlin Professional, version 5.1 (Pharsight, Mountain View, CA). The area under the plasma concentration–time curve extrapolated to infinity ($AUC_{0-\infty}$) of escitalopram and demethylescitalopram was calculated using the linear trapezoidal method. The maximum plasma concentration (C_{max}) and time to maximum plasma concentration (t_{max}) of escitalopram and demethylescitalopram were read directly from the data. The terminal elimination half-life ($t_{½}$) of escitalopram and demethylescitalopram was calculated as: $t_{½}=\ln 2 / \lambda$, where λ is the terminal slope of the log plasma concentration versus the time plot, calculated by linear regression.

Pharmacodynamic data analysis The area under effect curve (AUEC) was calculated for the MAX and REL AMPL parameters by linear interpolation using the non-compartmental model 220 for drug effect in the software package WinNonlin. In an attempt to minimise errors in the analysis of pupil data, AUEC was calculated solely for the period before drug intake (0 h) until 8 h after drug intake.

Statistical methods

Sample-size calculation Sample size calculation was based on the primary outcome: differences in AUC for escitalopram between CYP2C19 EM and PM. Based on an inter-individual coefficient of variance for AUC of 40%, it was estimated that a true difference of 67% could be detected, given a two-sided level of significance (α) of 0.05 and a power (β) of 80%, by using eight individuals in each group.

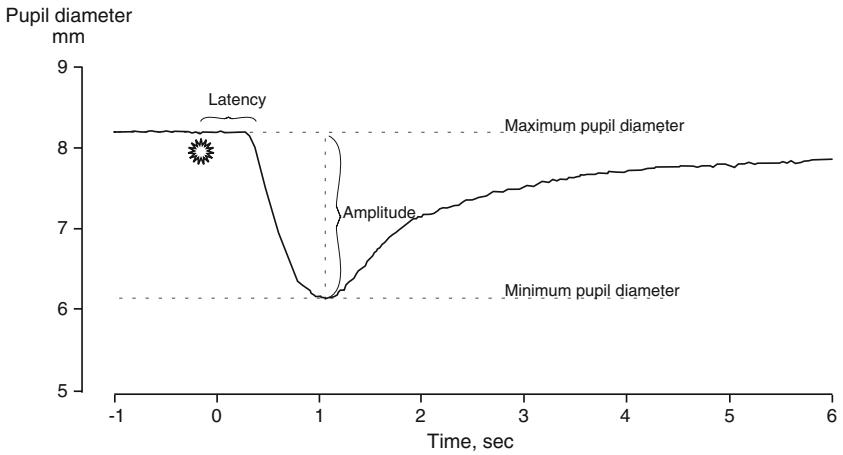

Fig. 1 Schematic drawing of pupil diameter versus time. A single light stimulus is given at time=0 s

Pharmacokinetics and pharmacodynamics Data are presented as medians and ranges, unless otherwise indicated. Before statistical analysis, all data except t_{max} were transformed to the natural logarithm to create a Gaussian distribution. Parameters were transformed back to the original scale when the effects were described.

Statistical inferences of phenotype were analysed by an unpaired *t* test and are presented as geometric mean ratios with 95% confidence intervals (CI) and associated *P* values. Inference tests on t_{max} were analysed by Hodges–Lehmann estimates of median differences with 95% CI. Statistical inferences of treatment on pupil parameters were analysed by a paired *t* test and are presented as geometric mean ratios with 95% CI and associated *P* values. Statistical analyses were performed using GraphPad QuickCalcs (http://graphpad.com/quickcalcs/index.cfm) (GraphPad Software, San Diego, CA), StatXact-3 (Cytel Software, Cambridge, MA) and Microsoft Office Excel 2007 (Microsoft Denmark ApS, Hellerup, Denmark).

Results

Pharmacokinetics The pharmacokinetic parameters of escitalopram and demethylescitalopram and statistical inference are listed in Table 1. The median plasma concentration *versus* time profile after a single dose of 10 mg escitalopram is presented in Fig. 2. The median escitalopram plasma concentration *versus* time profile after the last of multiple doses of 10 mg escitalopram is presented in Fig. 3.

The single dose $AUC_{0-\infty}$ and the multiple dose AUC_{0-24} were statistically significantly at 1.8-fold higher in the PM than in the EM. The oral clearance (CL/F) was about 0.5-fold lower and the $t_{½}$ was 1.5-fold higher in the PM. The phenotype did not affect the maximum plasma concentrations after a single dose, but after eight daily doses, the median C_{max} was 1.6 fold (95% CI 1.11–2.25; P=0.015) higher in the PM.

In both phenotype groups, the AUC_{0-24} for multiple doses tended to be higher than the single-dose $AUC_{0-\infty}$. The

Table 1 Median and range pharmacokinetic values for escitalopram and demethylescitalopram after the oral administration of 10 mg escitalopram as a single and multiple dose, with statistical inference of the ratio between poor (PM) and extensive metabolisers (EM) of cytochrome P450 2C19

Component/treatment	PM (n=5)	EM (n=8)	Statistical inference	P value
Escitalopram/single dose of escitalopram				
C_{max}, nmol/L	46.0 (33.6–71.5)	38.7 (29.7–75.24)	1.16 (0.79–1.70)[b]	0.41
t_{max}, h	4.0 (2.0–4.1)	3.5 (2.0–8.0)	0.01 (−3.00–1.05)[c]	0.98
$AUC_{0\rightarrow\infty}$, nmol h/L[a]	2195 (1618–3295)	1082 (885–2182)	1.82 (1.26–2.64)[b]	0.0046
$AUC_{0\rightarrow24}$, nmol h/L	811 (613–1023)	593 (488–968)	1.26 (0.95–1.69)[b]	0.10
$t_{½}$, h[a]	38 (26–52)	21 (17–31)	1.67 (1.25–2.23)[b]	0.0026
CL/F, L/h[a]	14.0 (9.4–19.0)	28.6 (14.1–34.8)	0.54 (0.38–0.80)[b]	0.0046
V_z/F, L[a]	702 (530–968)	789 (579–1029)	0.91 (0.70– 1.19)[b]	0.48
Escitalopram/multiple doses of escitalopram				
C_{max}, nmol/L	153 (95–194)	91 (61–136)	1.58 (1.11–2.25)[b]	0.015
t_{max}, h	3.1 (2.0–6.0)	2.6 (2.0–6.0)	0.05 (−1.03–1.97)[c]	0.85
$AUC_{0\rightarrow24}$, nmol h/L	2785 (1972–3800)	1501 (1094–2383)	1.80 (1.30–2.47)[b]	0.0020
$t_{½}$, h	35 (34–61)	28 (23–31)	1.49 (1.18–1.88)[b]	0.0029
CL/F, L/h	11.1 (8.1–15.6)	20.6 (12.9–28.2)	0.56 (0.40–0.77)[b]	0.0020
V_z/F, L	704 (532–898)	893 (578–1145)	0.83 (0.63–1.09)[b]	0.16
Demethylescitalopram/multiple doses of escitalopram				
C_{max}, nmol/L	23.7 (13.7–25.8)	23.9 (21.6–33.9)	0.80 (0.61–1.04)	0.095
t_{max}, h	4.8 (0.9–6.1)	5.1 (2.2–12.2)	−1.12(−6.23–1.98)	0.52
$AUC_{0\rightarrow24}$, nmol h/L	486 (284–505)	485 (437–726)	0.79 (0.61–1.02)	0.066
$t_{½}$, h	65 (45–72)	38 (29–50)	1.62 (1.28–2.04)	0.0008

C_{max}, Maximum observed plasma concentration; t_{max}, time to C_{max}; $AUC_{0-\infty}$, area under concentration-time curve from time zero to infinity; AUC_{0-24}, area under concentration-time curve from time zero to 24 h; $t_{½}$, apparent elimination half-life in plasma; CL/F, oral clearance; V_z/F, volume of distribution

[a] Sampling period only 24 h

[b] Geometric mean ratio (PM/EM) (95% confidence interval) and *P* value

[c] Hodges-Lehmans estimates of median difference (95% confidence interval) and *P* value

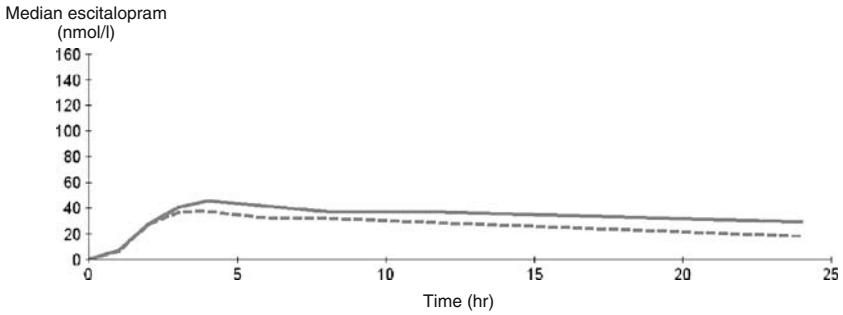

Fig. 2 Median plasma concentrations of escitalopram versus the time profile after a single dose of 10 mg escitalopram. *Broken line* Cytochrome P450 2C19 (CYP2C19) extensive metaboliser (EM), *solid line* CYP2C19 poor metaboliser (PM)

difference was statistically significant in the EM group (P=0.007), but not in the PM group (P=0.181).

The PK parameters of demethylescitalopram after a single dose of 10 mg escitalopram were not calculated as more than half of the plasma samples had concentrations below the LOQ, and the $t_{½}$ could only be estimated in three of the 13 subjects.

There were no statistically significant differences in C_{max}, t_{max} or $AUC_{0\text{-}24}$ between the PM and EM after multiple dosing of escitalopram. The $t_{½}$ was statistically significantly longer in the PM than in the EM (65 vs. 38 h, respectively).

After multiple doses of escitalopram, the demethylescitalopram was 34% (range 26–60%) that of escitalopram, measured as the $AUC_{0\text{-}24}$ in the EM group, compared to 17% (range 9–23%) in the PM group. The differences between phenotype groups were statistically significantly different, with a geometric mean ratio of 0.44 (95% CI 0.30–0.64; P=0.0006).

Pharmacodynamics Pupillometry data is presented as the AUEC median and range values together with statistical inferences of the difference between treatment with placebo and escitalopram in Table 2.

The $AUEC_{REL\ AMPL}$ was significantly decreased during treatment with both single and multiple doses of escitalopram compared to the placebo. This effect persisted when subjects were divided in phenotype groups, but the effect was not statistically significant in PM in the multiple-dose regimen. The effect was equal in both phenotype groups, as seen by the overlapping 95% CI in Table 2.

The $AUEC_{max}$ was not affected by escitalopram treatment compared to placebo.

Safety and tolerability A total of eight subjects reported adverse events during one of the treatment phases: six reported more than one adverse event, and two did so during the placebo phase. None of the adverse effects were serious, and all disappeared shortly after discontinuation of treatment. The results of all clinical laboratory tests carried out at the follow-up visits were considered to be within the normal range.

Discussion

Our results are the first to demonstrate in a formal panel study of healthy phenotyped subjects that the CYP2C19 phenotype significantly affects the PK of escitalopram. Drug exposure measured as the AUC was 1.8-fold higher in PM than in EM both after a single dose and at steady state. The higher drug exposure is probably due to an impaired metabolism, as seen by the significant difference in total body clearance, and not due to differences in drug absorption, as indicated by the lack of difference in C_{max} following a single dose of escitalopram.

We were unable to identify any comparable EM/PM panel studies of escitalopram in the literature, but the topic has indirectly been addressed in a Swedish panel study of EM and PM treated with racemic citalopram 2×10 mg/day for 7 days [9]. These researchers estimated a mean $AUC_{0\text{-}12}$= 530 nmol/L h in the EM group versus a mean $AUC_{0\text{-}12}$=

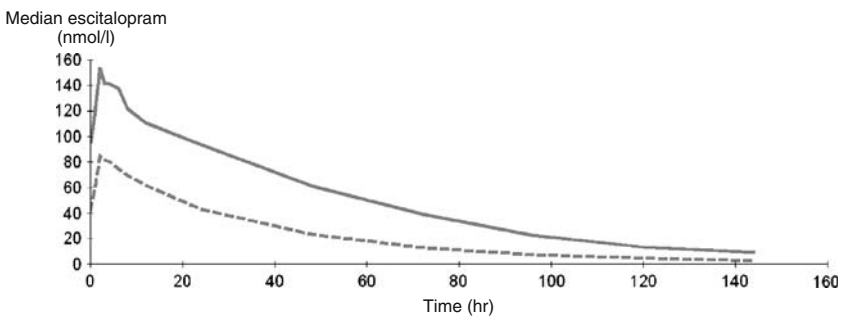

Fig. 3 Median plasma concentrations of escitalopram versus time profile after multiple doses of 10 mg escitalopram. *Broken line* CYP2C19 EM, *solid line* CYP2C19 PM

Table 2 Median and range of the area under the effect curve (AUEC) for the pupillometry data, following the administration of single or multiple oral doses of 10 mg escitalopram or placebo, with statistical inference of the ratio between treatments

Treatment	Escitalopram	Placebo	Statistical inference[a]	*P* value
Single dose, all (n=13)				
$AUEC_{MAX}$	3521 (3098–3897)	3463 (2907–4027)	1.01 (0.98–1.05)	0.51
$AUEC_{REL\ AMPL}$	163 (114–204)	192 (148–223)	0.88 (0.84–0.93)	0.0004
Multiple doses, all (n=13)				
$AUEC_{MAX}$	3464 (2837–3794)	3497 (2940–3985)	0.97 (0.94–1.01)	0.14
$AUEC_{REL\ AMPL}$	170 (112–210)	191 (152–226)	0.91 (0.86–0.96)	0.0019
Single dose, poor metabolisers (n=5)				
$AUEC_{MAX}$	3531 (3098–3897)	3522 (3420–4027)	1.00 (0.95–1.06)	0.88
$AUEC_{REL\ AMPL}$	160 (156–180)	183 (169–198)	0.89 (0.81–0.97)	0.023
Multiple doses, poor metabolisers (n=5)				
$AUEC_{MAX}$	3533 (2837–3794)	3592 (3387–3985)	1.00 (0.97–1.05)	0.62
$AUEC_{REL\ AMPL}$	163 (153–198)	189 (170–194)	0.94 (0.86–1.03)	0.12
Single dose, extensive metabolisers (n=8)				
$AUEC_{MAX}$	3479 (3256–3767)	3451 (2907–3581)	1.02 (1.96–1.08)	0.52
$AUEC_{REL\ AMPL}$	169 (114–204)	195 (148–223)	0.89 (0.80–0.96)	0.012
Multiple doses, extensive metabolisers (n=8)				
$AUEC_{MAX}$	3435 (2901–3653)	3434 (2940–3645)	0.95 (0.90–1.01)	0.071
$AUEC_{REL\ AMPL}$	175 (112–210)	193 (152–223)	0.88 (0.88–0.96)	0.009

$AUEC_{MAX}$, Area under effect-time curve for maximum pupil diameter; $AUEC_{REL\ AMPL}$, area under effect-time curve for relative light reflex amplitude

[a] Geometric mean ratio of AUEC (escitalopram treatment)/AUEC (placebo treatment), with the 95% confidence interval given in parenthesis

830 nmol/L h in the PM group [9]. These two mean values indicate a 1.6-fold higher AUC in the PM than in the EM, which is in good agreement with our data. In a recent study, the PK of escitalopram was determined in a group of 17 healthy subjects after multiple doses of escitalopram 10 mg/day [18]. The results are fairly consistent with our results obtained in the group of EM subjects. The discrepancy in AUC and CL/F may have been caused by the fact that three subjects in the earlier study were CYP2C19 PM whose CL/F ranged from 10 to 12 L/h, which is in good agreement with the CL/F range (8–16 L/h) found in our study. The exact times of blood sampling after the drug has been administered are crucial when estimating C_{max} and t_{max}, and differences in these parameters are, therefore, not of great significance. In a study based on data from therapeutic drug monitoring (TDM) files of a Norwegian psychiatric population, a 5.7-fold increase of dose-adjusted serum concentrations of escitalopram was found in subjects homozygous for defective *CYP2C19* alleles compared to subjects carrying two functional alleles [19]. This difference from our findings may be due to differences in sample size or populations. Results based on TDM material tend to overestimate the difference in drug exposure between phenotypes, as the material is assumed to be affected by selection bias: samples from patients that have therapeutic failure are probably more likely to be EM, whereas PM patients probably more often receive TDM due to adverse drug reactions. In our study, there were no differences in CYP2C19 metabolic capacity, measured as $MR_{omeprazole}$, between the randomly selected screened population (n=306) and the study population (n=13), separated in phenotype groups. This indicates that our estimates of the impact of CYP2C19 on escitalopram PK are reliable and can be extrapolated to the general population.

The difference between $AUC_{0\text{-}24}$ (multiple dose) and $AUC_{0\text{-}\infty}$ (single dose) found in this study is most likely explained by the short blood sampling period after the single dose administration; consequently, more than 50% of the individually estimated $AUC_{0\text{-}\infty}$ were extrapolated.

Most of the PK values of demethylescitalopram, except $t_{½}$, were identical in the EM and PM. However, given the relatively long $t_{½}$, steady state of demethylescitalopram could not be expected, and the PK values reported here are to be taken with caution.

There was a discrepancy between the CYP2C19 geno- and phenotype for one subject; the omeprazole MR was 6.44 and the subject was thereby classified as PM by phenotype. The genotype is limited to the selected alleles tested for, and the *1 phenotype is consequently not irrefutable evidence for a functional allele but only that no non-functional allele was detected. Hence, this subject may be a carrier of a non-functional allele that we did not test for.

We found no effect of escitalopram on the pupil diameter. This was surprising, considering the mydriatic effects demonstrated in a study of citalopram [12] and studies of other SSRIs [12, 20, 21]. The mydriatic effect of SSRIs has previously been explained by an increased sympathetic outflow of noradrenaline [12, 21, 22], but this may not be the case. Several SSRIs, including escitalopram and citalopram, have actually been demonstrated to decrease the firing activity of the noradrenergic neurons [23, 24]. There is a significant difference in the effect on

noradrenergic neurotransmission between escitalopram and citalopram that may be of a mechanistic nature and not simply a difference in potency [23], and this difference could explain the discrepancy in pupillary response between 10 mg escitalopram and 20 mg citalopram [12]. However, in a subsequent study, we actually found that escitalopram 20 mg/day at steady state significantly increased the pupil diameter in 15 subjects, all CYP2C19 and CYP2D6 EM, and that the lack of response in the present study may be a simple question of dose dependence (manuscript submitted). Further, the central effect of SSRIs may be an interplay with a local effect in the eye: in recent studies, several serotonin receptor subtypes have been identified in the human eye by mRNA techniques [24, 25]. The exact functions of these receptors are still to be determined but as some of the receptors are found on the dilator muscle of the pupil [24], a local mydriatic effect of SSRIs can not be excluded.

In conclusion, the CYP2C19 polymorphism significantly affects the metabolism of escitalopram. However, our findings do not justify dose adjustment based on CYP2C19 phenotypes. The puzzling results from pupillometry can be due to an interplay between a central noradrenergic effect and a local serotonergic effect. At present, pupillometry cannot be recommended as a serotonergic biomarker.

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