Contribution of CYP2C19 and CYP3A4 to the formation of the active nortilidine from the prodrug tilidine

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The analgesic activity of tilidine is mediated by its active metabolite, nortilidine, which easily penetrates the blood–brain barrier and binds to the μ -opioid receptor as a potent agonist.
- Tilidine undergoes an extensive first-pass metabolism, which has been suggested to be mediated by CYP3A4 and CYP2C19; furthermore, strong inhibition of CYP3A4 and CYP2C19 by voriconazole increased exposure of nortilidine, probably by inhibition of further metabolism.
- The novel CYP2C19 gene variant CYP2C19*17 causes ultrarapid drug metabolism, in contrast to the *2 and *3 variants, which result in impaired drug metabolism.

WHAT THIS STUDY ADDS

- Using a panel study with CYP2C19 ultrarapid and poor metabolizers, a major contribution of polymorphic CYP2C19 on tilidine metabolic elimination can be excluded.
- The potent CYP3A4 inhibitor ritonavir alters the sequential metabolism of tilidine, substantially reducing the partial metabolic clearances of tilidine to nortilidine and nortilidine to bisnortilidine, which increases the nortilidine exposure twofold.
- The lowest clearance in overall tilidine elimination is the *N*-demethylation of nortilidine to bisnortilidine. Inhibition of this step leads to accumulation of the active nortilidine.

AIMS

To investigate *in vivo* the effect of the CYP2C19 genotype on the pharmacokinetics of tilidine and the contribution of CYP3A4 and CYP2C19 to the formation of nortilidine using potent CYP3A4 inhibition by ritonavir.

METHODS

Fourteen healthy volunteers (seven CYP2C19 poor and seven ultrarapid metabolizers) received ritonavir orally (300 mg twice daily) for 3 days or placebo, together with a single oral dose of tilidine and naloxone (100 mg and 4 mg, respectively). Blood samples and urine were collected for 72 h. Noncompartmental analysis was performed to determine pharmacokinetic parameters of tilidine, nortilidine, bisnortilidine and ritonavir.

RESULTS

Tilidine exposure increased sevenfold and terminal elimination half-life fivefold during ritonavir treatment, but no significant differences were observed between the CYP2C19 genotypes. During ritonavir treatment, nortilidine area under the concentration–time curve was on average doubled, with no differences between CYP2C19 poor metabolizers [2242 h ng ml⁻¹ (95% confidence interval 1811–2674) vs. 996 h ng ml⁻¹ (95% confidence interval 872–1119)] and ultrarapid metabolizers [2074 h ng ml⁻¹ (95% confidence interval 1353–2795) vs. 1059 h ng ml⁻¹ (95% confidence interval 789–1330)]. The plasma concentration–time curve of the secondary metabolite, bisnortilidine, showed a threefold increase of time to reach maximal observed plasma concentration; however, area under the concentration–time curve was not altered by ritonavir.

CONCLUSIONS

The sequential metabolism of tilidine is inhibited by the potent CYP3A4 inhibitor, ritonavir, independent of the CYP2C19 genotype, with a twofold increase in the exposure of the active nortilidine.

Introduction

Being classified as a World Health Organization class II analgesic, the synthetic opioid tilidine is used for treatment of moderate or strong pain or for long-term treatment of patients with chronic pain [1–3]. In Germany, it is marketed as a fixed combination of tilidine and the opioid antagonist naloxone to prevent abuse. Tilidine is a classical prodrug, with the therapeutic activity being elicited by its oxidative metabolite, nortilidine [4, 5], which easily penetrates the blood–brain barrier and binds to the μ -opioid receptor as an agonist with a 100-fold higher μ -receptor affinity than tilidine itself [4, 6]. Recently, it was demonstrated that tilidine is a substrate of cytochrome P450 (CYP) isozymes, especially CYP3A4 and CYP2C19 [7]. Tilidine undergoes a so-called sequential metabolism; two-thirds of an administered dose is converted to nortilidine, while about 50% of the formed nortilidine is further metabolized to bisnortilidine before leaving the metabolizing organ [8]. In a recently published study, the simultaneous inhibition of CYP3A4 and CYP2C19 by voriconazole resulted in a 20-fold increase of tilidine exposure [9]. However, the anticipated reduction of nortilidine formation did not result in a reduced exposure of the active metabolite, nortilidine; instead, a threefold increase was observed. This was explained by the inhibition of both metabolic steps of the sequential tilidine metabolism by voriconazole [9].

To date, it is unknown which enzyme(s) are involved in the second metabolic step (formation of bisnortilidine from nortilidine). From the data of the voriconazole interaction study, it can be suggested that at least CYP3A4 and CYP2C19 are involved [9]. The relative contribution of both enzymes to the sequential metabolism is unknown, nor is the relevance of the known CYP2C19 genetic polymorphism. The novel described CYP2C19 gene variant CYP2C19*17 causes ultrarapid drug metabolism [10, 11], which is in contrast to the *2 or *3 variants that result in impaired drug metabolism [12]. This ultrarapid drug metabolism is caused by enhancement of the expression of CYP2C19 [11]. Therefore, these mutations in the CYP2C19 gene might have an impact on tilidine sequential metabolism, resulting in large interindividual variability. The contribution of CYP3A4 to this sequential metabolism is also of interest and can be quantified using a potent CYP3A4 inhibitor, such as ritonavir [13, 14]. Hence, in this randomized, placebo-controlled, double-blind, cross-over study we investigated the contribution of CYP3A4 and CYP2C19 to the overall metabolism of tilidine in humans by using ritonavir to inhibit CYP3A4 potentially in subjects stratified according to their CYP2C19 genotype (poor and ultrarapid metabolizers).

Methods

The study was approved by the Competent Authority in Germany (BfArM) (EudraCT no. 2007-004666-41) and the Ethics Committee of the Medical Faculty of the University

of Heidelberg. It was conducted at the Department of Clinical Pharmacology and Pharmacoepidemiology in accordance with the standards of Good Clinical Practice (as defined in the ICH E6 Guideline for Good Clinical Practice), in agreement with the Declaration of Helsinki and the specific legal requirements in Germany.

Study population

Fourteen healthy male ($n = 6$) and female ($n = 8$) Caucasian participants, stratified according to the CYP2C19 genotype, were enrolled in the study; there were seven poor metabolizers (*2/*2 or *2/*3; PM) and seven ultrarapid metabolizers (*17/*17; UM). They were between 23 and 48 years old, with an average age of 29 ± 6.9 years. All participants were mentally and physically healthy as defined by medical history, physical examination, electrocardiogram and routine laboratory analyses that included haematology, blood chemistry, serology and a urine drug screening. With the exception of oral contraceptives, none of the participants took any continuous medication for 2 months prior to or during the study. All participants were nonsmokers. Women were required to be nonpregnant and nonlactating and agreed to use appropriate barrier contraception throughout the study. Further exclusion criteria included any intake of a substance known to induce or inhibit drug-metabolizing enzymes or transport system enzymes within a period of less than 10 times the respective elimination half-life, any condition which could modify absorption, distribution, metabolism or excretion of the drug regimen under investigation, allergies (except for mild forms of hay fever) or history of hypersensitivity reactions, smoking, excessive alcohol drinking (more than approximately 20 g alcohol per day), blood donation within the last 2 months, participation in a study within the last 2 months, positive drug screening or known or admitted drug abuse and inability to communicate well with the investigator. All participants gave their written informed consent before any study measures were carried out.

Study design, blood sampling and urine collection

A randomized, double-blind, placebo-controlled, cross-over study was conducted. The randomization scheme was generated by using the website Randomization.com (<http://www.randomization.com>). Each subject received ritonavir 300 mg (Norvir® 100 mg; Abbott, Wiesbaden, Germany) or placebo (P-Tabletten weiß 10 mm Lichtenstein; Winthrop, Fürstfeldbruck, Germany) every 12 h starting on the evening of day 1 (~19.00 h) until the morning of day 4 (total of six doses). One hour after the intake of the second ritonavir or placebo dose (day 2), a single oral dose of 100 mg tilidine–naloxone solution (1.44 ml; Valoron N®; Pfizer Pharma GmbH, Karlsruhe, Germany) was given together with 200 ml table water. On days 2 and 16, blood samples (7.5 ml each) were collected before the intake of tilidine and 0.25, 0.5, 0.75, 1.0, 1.25, 1.5,

Table 1

The day-to-day and within-day results of three quality control samples of tilidine, nortilidine, bisnortilidine and ritonavir in plasma and urine

Plasma					
Compound	C (ng ml ⁻¹)	Day to day (n = 20) Accuracy (%)	CV (%)	Within day (n = 6) Accuracy (%)	CV (%)
Tilidine	3.4	99.0	7.1	94.2	5.3
	74.4	99.2	10.0	96.0	9.0
	143.0	95.7	7.0	99.2	3.2
Nortilidine	3.8	95.4	5.3	102.1	6.9
	74.4	100.2	8.9	98.6	7.8
	143.0	100.8	7.9	100.9	10.2
Bisnortilidine	3.4	105.2	8.3	100.4	6.5
	81.3	103.3	11.2	94.1	9.2
	156.0	101.6	9.6	98.1	7.7
Ritonavir	35.9	101.7	8.4	100.3	2.0
	2910.0	104.0	7.2	91.5	4.9
	6050.0	101.7	7.2	97.7	10.5
Urine					
Compound	C (ng ml ⁻¹)	Day to day (n = 10) Accuracy (%)	CV (%)	Within day (n = 6) Accuracy (%)	CV (%)
Tilidine	34.0	97.8	8.4	94.6	5.0
	149.0	109.4	10.2	98.1	2.8
	287.0	104.6	5.6	97.7	4.4
Nortilidine	36.5	105.4	7.7	105.6	10.1
	160.0	102.8	9.2	101.9	9.2
	308.0	94.9	6.0	92.4	8.1
Bisnortilidine	40.0	113.5	6.8	109.4	5.4
	163.0	101.5	11.3	105.4	10.0
	313.0	107.0	7.5	99.4	5.7

Abbreviations: C, concentration; CV, coefficient of variation.

1.75, 2.0, 2.25, 2.75, 3.0, 3.5, 4.0, 4.5, 5, 6, 8, 10, 12, 24, 36, 48 and 72 h after administration of tilidine. Blood samples were immediately centrifuged at 2500g at 4°C and separated; plasma was stored at -20°C until analysis. Urine was collected for 72 h in three fractions on study days 2, 3 and 4; an aliquot of 10 ml was kept frozen at -20°C until analysis. After a washout period of 10 days, the procedure was repeated with ritonavir and placebo interchanged. During the study day, standardized lunch and dinner meals were served. Alcohol, grapefruit juice and caffeinated beverages were not allowed throughout the study.

Determination of tilidine, its metabolites and ritonavir in plasma

Study plasma samples (100 µl), calibration samples (range 1–250 ng ml⁻¹ for tilidine and metabolites, 10–10 000 ng ml⁻¹ for ritonavir) and quality control samples were transferred into 400 µl of acetonitrile, which contained the internal standard tramadol (100 ng ml⁻¹) and cyclopropyl ritonavir (1000 ng ml⁻¹). The samples were vortexed for 30 s and subsequently centrifuged (10 min, 16 000g, 10°C). From the clear supernatant, 450 µl was transferred into glass tubes. The organic phase was blown down to dryness with a gentle stream of nitrogen in a water bath at 40°C, and the residues were reconstituted

with 200 µl (5 mM ammoniumacetate/acetonitrile 1/1, v/v). Ten microlitres of this solution was injected into the LC/MS/MS (liquid chromatography-tandem mass spectrometry). Details of the LC/MS/MS instruments have been previously described [7]. The mass spectrometer was programmed to admit the protonated molecules [M + H]⁺ at *m/z* 274.1 (tilidine), 260.1 (nortilidine), 246.0 (bisnortilidine) and 264.0 (tramadol) via the first quadrupole (Q1), with collision-induced fragmentation at Q2 and monitoring the product ions via Q3 at *m/z* 155.0 (tilidine, nortilidine and bisnortilidine) and *m/z* 8.0 for tramadol. Peak area ratios obtained from the monitored ions were used for construction of calibration curves, using weighted linear least-squares regression. Each analytical run included seven calibration samples at concentrations of 1–250 ng ml⁻¹ for tilidine, nortilidine and bisnortilidine, respectively, and three quality control samples at concentrations of about 3, 75 and 150 ng ml⁻¹; exact concentrations are specified in Table 1. The quality control samples were measured in duplicate. Data collection, peak integration and calculations were performed using Analyst version 1.4.2 software (Applied Biosystems, Darmstadt, Germany). Prior to the quantification of the study samples, the method was validated in accordance with the US Food and Drug Administration guidelines [15]. Calibration for all of the three drugs (tilidine, nortilidine and bisnortilidine) was linear in the

range of 1–250 ng ml⁻¹. The coefficient of correlation (r^2) was always greater than 0.99. The lower limit of quantification was 1 ng ml⁻¹ for all of the drugs. The day-to-day and within-day results of the quality control samples are given in Table 1. The extraction recovery for all of the three drugs ranged between 94 and 109% at three quality control concentrations. The extraction recovery for the internal standard was 89.6% at the concentration of 100 ng ml⁻¹.

For the determination of ritonavir in plasma, 10 µl of the same reconstituted extracts was injected onto the same analytical column, but with different mobile phase and different mass spectrometry conditions, in that the mobile phase consisted of acetonitrile/0.1 M ammoniumacetate (60/40, v/v). The mass spectrometer was adjusted to admit the protonated molecules [M + H] at m/z 721.4 (ritonavir) and 747.0 (internal standard) via the first quadrupole (Q1), with collision-induced fragmentation at Q2 and monitoring the product ions via Q3 at m/z 296.2 for ritonavir and m/z 322.3 for the internal standard. Calibration for ritonavir was linear in the range 10–10 000 ng ml⁻¹. The coefficient of correlation (r^2) was always greater than 0.99. The lower limit of quantification was 10 ng ml⁻¹. The day-to-day and within-day results of the quality control samples are given in Table 1. The extraction recovery for ritonavir was 90–109% at three quality control concentrations. The extraction recovery for the internal standard was 113% at the concentration of 1000 ng ml⁻¹.

Determination of tilidine and its metabolites in urine

Urine samples (500 µl), calibration samples (calibration range 10–500 ng ml⁻¹) and quality control samples were transferred to 500 µl borate buffer (pH 9) containing the internal standard tramadol (100 ng ml⁻¹), and 500 µl water was added. For the cleavage of the glucuronides, the water was substituted by glucuronidase solution (2000 units per 500 µl), and the samples were placed in a water bath at 37°C for 48 h.

For liquid/liquid extraction, 5 ml of tert-butyl methyl ether was added, and the tubes were placed on an overhead shaker for 10 min. After centrifugation (10 min, 3000g) 4 ml of the supernatant was transferred into glass tubes, and the organic phase was reduced to dryness using nitrogen at 40°C. The residues were reconstituted with 500 µl (5 mM ammonium acetate/acetonitrile 1/1, v/v). Ten microlitres of this solution was injected into the LC/MS/MS system. The conditions of LC and MS/MS were the same as described for the plasma samples.

Calibration for all compounds (tilidine, nortilidine and bisnortilidine) was linear in the range 10–500 ng ml⁻¹. The coefficient of correlation (r^2) was always greater than 0.99. The lower limit of quantification was 10 ng ml⁻¹ for each compound. The day-to-day and within-day results of the quality control samples are given in Table 1. The extraction recovery for the three drugs ranged between 98 and 117%

at the three different quality control concentrations. The extraction recovery for the internal standard was 112% at the concentration of 100 ng ml⁻¹.

Calculation and statistic analysis

A power calculation was performed using PS Power and Sample Size Calculations [16]. Owing to the low frequency of CYP2C19 PMs (2%) an *a priori* sample size of seven UM and PM subjects was fixed. With an anticipated difference in tilidine clearance of 50% between the genotypes and the known variability [9], there is only a low power of 0.435 to detect a difference between population means of the PM and UM groups. However, in conditions of CYP3A4 inhibition and thereby considerable reduction of variability, the power will increase to more than 0.9 with the given sample size.

Data are presented as means $\pm$ SD. In addition, the 95% confidence intervals are given in the tables. Noncompartmental analysis was performed by use of WinNonlin software (version 5.2; Pharsight, Mountain View, CA, USA) to determine the following pharmacokinetic parameters of tilidine, nortilidine and bisnortilidine: maximal observed plasma concentration (C_{max}), time to reach maximal observed plasma concentration (t_{max}), terminal elimination half-life ($t_{1/2}$), area under the concentration–time curve (AUC) from 0 to 72 h (AUC_{0-72}) and AUC from 0 to infinity ($AUC_{0-\infty}$), extrapolated by use of the linear trapezoidal rule. Ritonavir AUC_{τ} ($\tau = 12$ h) was determined after the second ritonavir dose. The renal clearances (CL_{ren}) of tilidine, nortilidine and bisnortilidine were determined as the amount of tilidine, nortilidine and bisnortilidine excreted in urine from 0 to 72 h (Ae_{0-72}) divided by the corresponding AUC_{0-72} values. Partial metabolic clearances were calculated as the sum of metabolites formed via the metabolic pathway of interest excreted in urine from 0 to 72 h (Ae_{0-72}) divided by the AUC_{0-72} of the parent drug. Differences in these pharmacokinetic parameters between placebo and ritonavir treatment were assessed by use of the nonparametric Wilcoxon matched pairs signed rank test, between the CYP2C19 genotypes by the Mann–Whitney *U*-test (GraphPad InStat 3; GraphPad Inc., San Diego, CA, USA). A *P* value less than 0.05 was considered to be significant.

Results

Tilidine

After oral administration of 100 mg tilidine as a solution, maximal plasma concentrations of tilidine were reached quickly after 28 min in both CYP2C19 UM and PM participants. No significant differences were observed for any tilidine pharmacokinetic parameter between UM and PM participants (Table 2).

During treatment with ritonavir, significant changes occurred in both genotypes. Plasma concentrations were much higher during ritonavir treatment (Figure 1). The C_{max}

Table 2

Mean pharmacokinetic parameters (95% confidence intervals) after noncompartmental analysis of tilidine during ritonavir and placebo administration in 14 healthy individuals

	Ultrarapid metabolizers (n = 7)			Poor metabolizers (n = 7)		
	Placebo	Ritonavir	P value	Placebo	Ritonavir	P value
C _{max} (ng ml ⁻¹)	63.6 (39.0–88.2)	183 (146–221)	0.0156	75.3 (16.8–134)	222 (62.9–382)	0.0156
t _{max} (h)	0.46 (0.30–0.62)	0.61 (0.48–0.73)	0.072	0.46 (0.38–0.55)	0.68 (0.39–0.97)	0.250
AUC _{0–∞} (h ng ml ⁻¹)	109 (78–140)	750 (461–1038)	0.0156	159 (75–243)	1100 (51–2148)	0.0156
t _{1/2} (h)	3.58 (2.73–4.43)	19.06 (10.8–27.3)	0.0156	6.09 (3.25–10.55)	21.19 (9.11–33.3)	0.0469
CL/F (ml min ⁻¹)	16 287 (12 611–19 964)	2744 (1322–4166)	0.0156	17 097 (3013–31 181)	2878 (1065–4691)	0.0156
Ae _{0–72} (μg)	77 (–23 to 177)	1680 (631–2730)	0.0156	162 (23–301)	1524 (380–2667)	0.0156
CL _{ren} (ml min ⁻¹)	14.8 (–3.4 to 33.0)	39.9 (20.6–59.3)	0.156	14.7 (1.2–28.2)	26.3 (20.7–32.0)	0.219

Differences between placebo and ritonavir treatment were assessed by use of the Wilcoxon matched pairs signed rank test with *P* values reported. Abbreviations: C_{max}, maximal observed plasma concentration; t_{max}, time to reach maximal observed plasma concentration; AUC_{0–∞}, AUC from zero to infinity; t_{1/2}, terminal elimination half-life; CL/F, apparent oral clearance; Ae_{0–72}, amount excreted in urine from 0 to 72 h; and CL_{ren}, renal clearance.

Figure 1

Pharmacokinetic profiles (means ± SD) of tilidine (upper panels), nortilidine (middle panels) and bisnortilidine (lower panels) after oral administration of 100 mg tilidine solution to seven CYP2C19 ultrarapid metabolizer subjects (UM; left panels) and seven CYP2C19 poor metabolizer subjects (PM; right panels) during placebo (circles) or ritonavir treatment (squares)

Table 3

Mean pharmacokinetic parameters (95% confidence intervals) after noncompartmental analysis of nortilidine during ritonavir and placebo administration in 14 healthy individuals

	Ultrarapid metabolizers (<i>n</i> = 7)		<i>P</i> value	Poor metabolizers (<i>n</i> = 7)		<i>P</i> value
	Placebo	Ritonavir		Placebo	Ritonavir	
C_{max} (ng ml⁻¹)	181.8 (127.9–235.8)	188.3 (162.6–214.0)	0.183	167.4 (106.7–228.2)	116.2* (82.8–149.7)	0.0781
t_{max} (h)	0.82 (0.61–1.04)	1.21 (0.93–1.50)	0.134	0.96 (0.55–1.38)	3.29 (1.41–5.17)	0.109
AUC_{0–∞} (h ng ml⁻¹)	996 (872–1119)	2242 (1811–2674)	0.0156	1059 (789–1330)	2074 (1353–2795)	0.0469
t_{1/2} (h)	5.78 (4.39–7.16)	10.54 (8.58–12.49)	0.0156	7.53 (6.63–8.43)	16.87 (10.80–22.94)	0.0156
Ae_{0–72} (μg)	3653 (2454–4852)	8932 (5130–12 734)	0.0496	3382 (2493–4271)	6980 (4620–9340)	0.1563
CL_{ren} (ml min⁻¹)	63.2 (42.9–83.5)	73.7 (41.9–105.5)	0.688	56.5 (44.9–68.2)	60.6 (49.1–72.1)	0.938

Differences between placebo and ritonavir treatment were assessed by the Wilcoxon matched pairs signed rank test. Abbreviations are as in the footnote to Table 1. **P* < 0.05 UM vs. PM.

Table 4

Mean pharmacokinetic parameters (95% confidence intervals) after noncompartmental analysis of bisnortilidine during ritonavir and placebo administration in 14 healthy individuals

	Ultrarapid metabolizers (<i>n</i> = 7)		<i>P</i> value	Poor metabolizers (<i>n</i> = 7)		<i>P</i> value
	Placebo	Ritonavir		Placebo	Ritonavir	
C_{max} (ng ml⁻¹)	161.6 (122.0–201.1)	104.0 (86.8–121.2)	0.0156	125.6 (98.3–152.9)	59.0* (33.6–84.4)	0.0156
t_{max} (h)	2.75 (0.86–4.64)	6.61 (4.12–9.10)	0.0313	2.14 (0.74–3.54)	9.82 (4.47–15.2)	0.0223
AUC_{0–∞} (h ng ml⁻¹)	1901 (1678–2124)	2480 (2154–2806)	0.0313	1833 (1518–2148)	1834* (1382–2287)	0.8125
t_{1/2} (h)	8.91 (6.29–11.5)	14.3 (11.1–17.6)	0.0156	9.66 (7.81–11.5)	20.7 (10.1–31.4)	0.0156
Ae_{0–72} (μg)	5277 (3971–6583)	6824 (4477–9171)	0.2969	4932 (3953–5911)	5205 (3570–6840)	0.8125
CL_{ren} (ml min⁻¹)	48.6 (35.5–61.7)	50.5 (33.3–67.6)	0.2969	48.9 (37.6–60.1)	58.5 (42.9–74.1)	0.1094

Differences between placebo and ritonavir treatment were assessed by the Wilcoxon matched pairs signed rank test. Abbreviations are as in the footnote to Table 1. **P* < 0.05 UM vs. PM.

increased threefold, AUC_{0–∞} sevenfold, terminal elimination half-life increased on average to 20 h, and the amount excreted unchanged in urine increased more than 10-fold but was still below 2% of the administered dose (Table 2). The apparent oral clearance of tilidine decreased significantly during ritonavir treatment to 17% of the clearance during placebo (Table 2).

Nortilidine

Maximal plasma concentrations of the active nortilidine after oral administration of 100 mg tilidine as a solution were observed about 30 min after maximal tilidine plasma concentrations in both CYP2C19 UM and PM participants. Nortilidine maximal concentrations were almost threefold higher than tilidine concentrations (Table 3). No significant differences were observed for any pharmacokinetic parameter of nortilidine during placebo between UM and PM participants (Table 3).

Ritonavir treatment resulted in a significant prolongation of *t*_{max} of nortilidine (placebo, 0.89 ± 0.47 h; ritonavir, 2.25 ± 2.17 h; *P* = 0.0338; *n* = 14; Figure 1). The *C*_{max} of nortilidine was not significantly different between placebo and ritonavir treatment in both CYP2C19 genotypes. The AUC_{0–∞} and terminal elimination half-life of nortilidine were increased twofold during ritonavir treatment, but

were not different between the CYP2C19 genotypes (Table 3). The only significant difference during ritonavir treatment between UM and PM participants was a 40% lower *C*_{max} in CYP2C19 PM subjects (*P* < 0.05; Table 3).

Bisnortilidine

Maximal plasma concentrations of the secondary metabolite, bisnortilidine, after oral administration of 100 mg tilidine as a solution were observed at an average 2.5 h, with large variability. Bisnortilidine concentrations in plasma were in the same range as nortilidine plasma concentrations (Figure 1). During placebo, no significant differences of bisnortilidine pharmacokinetics were observed between UM and PM participants (Table 4).

During ritonavir treatment, *t*_{max} of bisnortilidine was significantly prolonged and *C*_{max} was significantly decreased in both phenotypes (Table 4). However, in UM subjects during ritonavir treatment, AUC_{0–∞} increased by 30% compared with placebo (*P* < 0.05), whereas in PM subjects no difference was observed (Table 4). Bisnortilidine terminal elimination half-life was increased almost twofold, with no differences between the CYP2C19 genotypes (Table 4). The *C*_{max} and AUC_{0–∞} of bisnortilidine during ritonavir were significantly different between the CYP2C19 genotypes, being lower in PM participants (Table 4).

Table 5

Mean partial metabolic clearances (95% confidence intervals) after administration of 100 mg tilidine during ritonavir and placebo in 14 healthy individuals

CL_{met} (ml min ⁻¹)	Ultrarapid metabolizers (n = 7)		P value	Poor metabolizers (n = 7)		P value
	Placebo	Ritonavir		Placebo	Ritonavir	
To nortilidine	3065 (2036–4093)	706 (436–975)	0.0156	3661 (1388–5933)	712 (208–1216)	0.0156
To bisnortilidine	210 (145–276)	110 (73–146)	0.0313	230 (151–310)	127 (82–173)	0.0156
To nortilidine conjugate	14 (2–26)	14 (–7 to 34)	0.6875	22 (13–31)	11 (–10 to 32)	0.2188
To bisnortilidine conjugate	54 (34–74)	43 (29–57)	0.2969	64 (46–81)	77* (57–97)	0.1094

Differences between placebo and ritonavir treatment were assessed by the Wilcoxon matched pairs signed rank test. Abbreviation: CL_{met} , partial metabolic clearance. * $P < 0.05$ UM vs. PM.

Clearances

Renal clearances of tilidine, nortilidine and bisnortilidine after oral administration of 100 mg tilidine as a solution were lowest for tilidine and about 60 ml min⁻¹ for nortilidine and bisnortilidine, with no differences between the *CYP2C19* genotypes, and were also unchanged during ritonavir treatment (Tables 2–4). Elimination of tilidine via metabolism showed distinct differences of the metabolic pathways involved. Partial metabolic clearance of tilidine (the first *N*-demethylation step) was high (>3 l min⁻¹), which was not different between the *CYP2C19* genotypes (Table 5). Further metabolism of nortilidine via *N*-demethylation was less than 10% of the first *N*-demethylation and also not different between the *CYP2C19* genotypes (Table 5). Conjugation did not occur for tilidine; it was only marginal for nortilidine and somewhat higher for bisnortilidine, with partial metabolic clearance less than 100 ml min⁻¹ (Table 5). Ritonavir treatment significantly affected only the *N*-demethylation pathways; the conjugation reactions were not changed significantly. The partial metabolic clearance of tilidine to nortilidine was reduced by 75–80%, and the partial metabolic clearance of nortilidine to bisnortilidine was halved (Table 5), with no differences between the *CYP2C19* genotypes.

Pooled analysis

As there were almost no differences in all obtained pharmacokinetic parameters between the *CYP2C19* genotypes, the pharmacokinetic data of *CYP2C19* UM and PM subjects were pooled in order to assess the effect of ritonavir on tilidine elimination more profoundly. The clearances and urinary excretion after 100 mg tilidine alone and in combination with 300 mg ritonavir twice daily are shown in Figure 2. Ritonavir reduced the apparent oral clearance of tilidine from 16.9 to 2.81 l min⁻¹, resulting in a sevenfold increase of tilidine exposure.

Ritonavir

Ritonavir plasma concentrations 1 h before tilidine administration were 1.68 µg ml⁻¹ (95% confidence interval 1.19–2.18), which was 12 after the first ritonavir dose. Ritonavir AUC_{τ} was determined to be 64.0 h µg ml⁻¹ (95% confi-

dence interval 49.8–78.3). Trough concentrations after the following ritonavir doses were on average above 2 µg ml⁻¹. No significant differences were observed between the *CYP2C19* genotypes.

Safety and tolerability

No serious adverse drug events occurred during the study. All adverse drug events were mild or moderate and transient. After administration of tilidine with placebo, the most common adverse drug events were tiredness (100%), feeling of drunkenness (57%), mild and transient dizziness (71%), feeling of warmth (29%), headache (50%), vomiting (43%), nausea (43%), itching (36%) and adynamia (7%). After administration of ritonavir, the incidence of adverse drug events was increased. The most frequent adverse drug events were mild tiredness (92%), nausea (71%), vomiting (64%), headache (57%), feeling of drunkenness (50%), dizziness (50%), feeling of warmth (36%), itching 29% (UM, 50%; PM, 14%), adynamia (28%) and limb paraesthesia (43%). Intermittent nausea occurred on average 4.6 h after administration of tilidine, and seven participants had to be treated with dimenhydrinate because of recurrent vomiting.

Discussion

The understanding of the formation and subsequent elimination of the active metabolite nortilidine is important for provision of rational pain therapy with tilidine. Based on *in vitro* data [7] and the results of an *in vivo* study with the antifungal voriconazole [9], the contribution of CYP3A4 and CYP2C19 to the formation of the active metabolite nortilidine was to be investigated in this study. For this purpose, CYP2C19 poor and ultrarapid metabolizers were exposed to tilidine. However, no major differences between CYP2C19 UM and PM subjects were observed for tilidine, nortilidine and bisnortilidine pharmacokinetics, suggesting a negligible contribution of CYP2C19 to the metabolic formation of the active nortilidine and the overall elimination of tilidine. When CYP3A4 activity was inhibited by ritonavir, no differences of tilidine pharmaco-

Figure 2

Partial metabolic clearances and urinary excretion of tilidine and metabolites after oral administration of 100 mg tilidine solution to 14 healthy subjects during placebo (left) or ritonavir treatment (right)

kinetics were observed between the *CYP2C19* genotypes. However, nortilidine C_{max} , bisnortilidine C_{max} and AUC were significantly lower during ritonavir treatment in *CYP2C19* PM subjects. Regarding the partial metabolic clearances, there were no *CYP2C19* genotype differences during both placebo and ritonavir treatment. Thus, the results after ritonavir treatment revealed only a minor contribution of *CYP2C19* to tilidine metabolic elimination.

As ritonavir is a potent inhibitor of *CYP3A4* [13], *CYP2D6* [17] and *CYP2C8* [18], and only *CYP3A4* was identified *in vitro* to carry out tilidine *N*-demethylation [7], *CYP3A4* is the major enzyme responsible for tilidine metabolism and also formation of the active metabolite, nortilidine. This interaction would have major consequences if the exposure of the active nortilidine is consequently decreased; a reduction or even a loss of the analgesic effect would result. However, similar to the

results obtained with voriconazole [9], ritonavir caused not a decrease but a twofold increase of nortilidine AUC, again independent of the *CYP2C19* genotype. This is mainly due to an inhibition of nortilidine metabolism to bisnortilidine; the partial metabolic clearance was reduced by 50%, with t_{max} being significantly prolonged (three- to fourfold), although the AUC of bisnortilidine was unchanged during ritonavir treatment. No effects of ritonavir on the conjugation pathways of nortilidine and bisnortilidine were observed; the partial metabolic clearances were rather small and well below 100 ml min⁻¹ (Figure 2).

Ritonavir significantly increased overall urinary excretion of tilidine and its metabolites from 17.5 ± 5.4 to $26.3 \pm 14.6\%$. This can be attributed mainly to nortilidine and tilidine; bisnortilidine and its conjugate were not affected by ritonavir (Figure 2). As nortilidine *N*-demethylation is much slower than nortilidine formation, the more than

doubled urinary excretion of unchanged nortilidine is comprehensible. Surprisingly, the total amount of tilidine and its metabolites recovered in urine over 72 h is well below 30% of the administered dose, even in conditions of CYP3A4 inhibition by ritonavir. This is in contrast to the previously published almost complete urinary excretion of an orally given dose of tilidine (90% in urine, 10% in faeces) [19, 20]. It has been suggested that more than 10 unconjugated metabolites might be formed; however, their structure was not elucidated due to methodological problems [20]. The amounts excreted in urine in our study (tilidine, 0.12%; nortilidine, 3.7%; and bisnortilidine, 5.7%) are well in agreement with data from Vollmer *et al.* [5] after oral administration of 50 mg tilidine (tilidine, <0.1%; nortilidine, 2.9%; and bisnortilidine, 5.0%) and even after intravenous administration of 50 mg tilidine (tilidine, 1.6%; nortilidine, 3.7%; and bisnortilidine, 5.1%). Consequently, the main proportion of an administered tilidine dose will be excreted in urine as unknown metabolites. Conjugated metabolites of the known metabolites only account for about 8% of the dose (Figure 2).

In this study, a marketed fixed combination of tilidine and naloxone (12.5:1) was used. We cannot exclude a triplicate drug interaction, because naloxone may influence gastrointestinal motility. As naloxone is nearly completely conjugated by glucuronosyltransferases, at least theoretically, site-dependent metabolic elimination routes for tilidine in the intestine may have been influenced.

Although ritonavir caused a major interaction with tilidine, there would probably be no clinical consequences, or only minor ones. The increase of tilidine exposure, which was even more pronounced in a study with voriconazole [9], did not cause a corresponding increase of adverse effects. Instead of a decrease, both ritonavir and voriconazole increased the exposure of the active nortilidine twofold and 2.5-fold, respectively. This might explain the higher number of adverse effects in this study during ritonavir treatment, although it cannot be excluded that this might also be caused by ritonavir itself.

In conclusion, polymorphic CYP2C19 has no relevant influence on the pharmacokinetics and metabolism of the weak opioid, tilidine. It is proposed that inhibitors or inducers of this polymorphic enzyme will not affect the disposition of tilidine in man. The sequential first-pass metabolism to bisnortilidine with nortilidine as an intermediate metabolite (the active principle) seems to be mainly dependent on CYP3A4. This can be inhibited very effectively by ritonavir, which is one of the most potent CYP3A4 inhibitors known. Inhibition of both *N*-demethylation steps results in accumulation of the active metabolite, nortilidine, because its metabolism to bisnortilidine seems to be carried out at a lower rate. Therefore, it is quite likely that drug interactions with tilidine have not been recognized because the inhibiting effect of other (weaker) CYP3A4 inhibitors will result in a less than twofold increase of nortilidine exposure. However, with strong CYP3A4 inhibitors

the interaction with tilidine might potentially be clinically important.

Competing Interests

There are no competing interests to declare.

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