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PHARMACOKINETICS AND DISPOSITION

Ticlopidine inhibits both *O*-demethylation and renal clearance of tramadol, increasing the exposure to it, but itraconazole has no marked effect on the ticlopidine-tramadol interaction

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Abstract
Purpose We assessed possible drug interactions of tramadol given concomitantly with the potent CYP2B6 inhibitor ticlopidine, alone or together with the potent CYP3A4 and P-glycoprotein inhibitor itraconazole.
Methods In a randomized, placebo-controlled cross-over study, 12 healthy subjects ingested 50 mg of tramadol after 4 days of pretreatment with either placebo, ticlopidine (250 mg twice daily) or ticlopidine plus itraconazole (200 mg once daily). Plasma and urine concentrations of tramadol and its active metabolite *O*-desmethyltramadol (M1) were monitored over 48 h and 24 h, respectively.
Results Ticlopidine increased the mean area under the plasma concentration-time curve ($AUC_{0-\infty}$) of tramadol by 2.0-fold (90 % confidence interval (CI) 1.6–2.4; $p<0.001$) and C_{max} by 1.4-fold ($p<0.001$), and reduced its oral and renal clearance ($p<0.01$). Ticlopidine reduced the AUC_{0-3} of M1 ($p<0.001$) and the ratio of the $AUC_{0-\infty}$ of M1 to that of tramadol, but did not influence the $AUC_{0-\infty}$ of M1. Tramadol or M1 pharmacokinetics did not differ between the ticlopidine alone and ticlopidine plus itraconazole phases.
Conclusions Ticlopidine increased exposure to tramadol, reduced its renal clearance and inhibited the formation of M1, most likely via inhibition of CYP2B6 and/or CYP2D6. The addition of itraconazole to ticlopidine did not modify the outcome of the drug interaction. Concomitant clinical use of ticlopidine and tramadol may enhance the risk of serotonergic effects, especially when higher doses of tramadol are used.

Keywords Ticlopidine · Tramadol · Cytochrome P-450 CYP2B6 · Itraconazole · *O*-demethylation · Renal clearance

The contributions of Nora M. Hagelberg and Tuukka Saarikoski in the study were equal

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Introduction

Tramadol is a widely used analgesic agent with multiple pharmacological mechanisms of action. It has efficacy in the treatment of acute and chronic pain conditions, including neuropathic pain [1–4]. Still, guidelines for the treatment of neuropathic pain rank tramadol as a second- or third-line drug mainly because of its side effect profile and potentially harmful interactions with serotonergic drugs [5–7]. The risk of

interactions is perceived to be elevated in patients who use multiple medications for pain control or antidepressants for the treatment of comorbid depression [8]. In a Finnish study, every fifth inpatient using tramadol took concomitant medication with potential to inhibit the conversion of tramadol to its pharmacologically active *O*-desmethyl metabolite [9].

After oral administration, tramadol is rapidly absorbed with an oral bioavailability of approximately 70 % [10]. Tramadol undergoes extensive oxidative metabolism. *O*-demethylation of tramadol to *O*-desmethyltramadol (M1) is mediated by cytochrome P450 (CYP) 2D6 and possibly by 2B6 enzymes [11–14]. *N*-demethylation via CYP3A4 and CYP2B6 yields *N*-desmethyltramadol (M2) [14, 15]. Approximately 90 % of a tramadol dose is excreted in the urine, mainly as metabolites, and 12–32 % is excreted in the urine in unchanged form [13, 16]. Inhibitors of CYP2D6 have been shown to increase the plasma concentrations of tramadol and to decrease the formation of M1 [17].

Tramadol exerts its analgesic effects via opioidergic and monoaminergic mechanisms [18]. Racemic tramadol is a weak and (+)-M1 a potent μ-opioid receptor agonist, with K_i values of 2.4 μM and 0.0034 μM, respectively [19]. (+)-Tramadol inhibits the neuronal reuptake of serotonin (5-HT) and (−)-tramadol inhibits norepinephrine reuptake [20, 21]. Inhibition of 5-HT uptake into blood platelets may impair haemostasis [22]. In the central nervous system, increased synaptic concentrations of 5-HT may cause neuromuscular and autonomic hyperreactivity and mental symptoms [23]. Although previous literature suggests that pharmacological effects of the enantiomers differ, pharmacodynamic profiling of tramadol is very complex, and the relative contribution of tramadol and its metabolites remains to be explored.

Ticlopidine is an antiaggregation agent clinically used for the prevention of thrombotic events. It is a potent inhibitor of CYP2B6 [24–26] and it also inhibits CYP1A2, CYP2C19 and CYP2D6 enzymes [27–30]. Itraconazole is a triazole antifungal drug. It is a potent inhibitor of CYP3A4 and P-glycoprotein in vivo in humans [31, 32] and it inhibits CYP2B6 at least in vitro [33]. While CYP2D6 is primarily responsible for M1 formation and M2 formation is mainly catalyzed by CYP2B6 and CYP3A4 [13, 14], we investigated the possible drug interactions of tramadol with ticlopidine alone and together with the CYP3A4 inhibitor itraconazole. As the parent tramadol and its M1 metabolite are pharmacologically active, their plasma and urine pharmacokinetics were of our primary interest, completed by limited pharmacodynamic measurements.

Materials and methods

The study was conducted according to the revised Declaration of Helsinki, and approved by the ethics committee of the Hospital District of Southwest Finland and by the Finnish National Agency for Medicines (EudraCT 2010-020617-82). It was registered in the ClinicalTrials.gov clinical trials database under code number NCT01214941.

Subjects

Twelve healthy, non-smoking volunteers (six men and six women, age range 19–25 years, body mass index (BMI) range 19–27 kg/m^2) enrolled in the study after giving written informed consent. Only subjects with low risk for developing opioid abuse as assessed by a Finnish translation of the Abuse Questions [34] were accepted. None of the subjects were under regular medication including herbal remedies or oral contraceptives. The subjects were ascertained to be in good health by clinical examination, medical history and routine laboratory tests including an ECG before entering the study. Urine drug screens and pregnancy tests were negative. Female subjects used reliable non-hormonal contraception during the study. Consumption of grapefruit juice was not allowed on days 1–5, and coffee, tea, alcohol and cola drinks were not allowed on day 4.

Study outline

The study was conducted in a randomized, balanced, placebo-controlled, single-blinded, cross-over manner with three phases separated by wash-out periods of at least 6 weeks. The subjects took either oral placebo, oral ticlopidine or a combination of oral ticlopidine and itraconazole for 5 days. On day 4, the subjects ingested a single 50 mg tablet of tramadol. Venous blood samples (10 mL) to determine the concentrations of tramadol and M1 were drawn into tubes containing ethylenediaminetetraacetatic acid (EDTA) immediately before and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24 and 48 h after ingestion of tramadol. Plasma was separated within 30 min and stored at −70 °C until analysis. Plasma concentrations of ticlopidine, itraconazole and OH-itraconazole were determined from the plasma samples taken just before tramadol administration. Similar samples for the determination of 5-HT and its main metabolite 5-hydroxyindoleacetic acid (5-HIAA) in whole blood were drawn before and 4 and 8 h after tramadol. Urine was collected for 24 h after tramadol ingestion. Subjective and analgesic drug effects were assessed before and 1, 2, 3, 4, 5, 6, 8, 10 and 12 h after tramadol, and adverse effects were evaluated before and 3 and 6 h after tramadol administration.

Study drug administration

Dosing of placebo (phase 1) and ticlopidine (Ticlid® 250 mg tablet, Roche, Nutley, NJ) (phases 2 and 3) was done at 7:00 a.m. and 7:00 p.m. Itraconazole (Sporanox®; Jansen-

Cilag, Latina, Italy) 200 mg was administered in the morning (7:00 a.m., phase 3). The drugs were self-administered except on day 4 when they were administered by the investigators at the study facility with 150 mL of water. No specific dietary instructions were given for dosing of pretreatments at home. Compliance was assessed by determination of plasma ticlopidine and itraconazole concentrations. On day 4, 1 h after the last dose of premedication, a single oral dose of 50 mg of tramadol (Tramal®, Orion Pharma, Espoo, Finland) was ingested with 150 mL of water at 8:00 a.m. During all phases, subjects fasted overnight before the administration of tramadol and continued fasting until standardized meals were served 4 and 8 h after tramadol ingestion.

Determination of plasma and urine drug concentrations

Determination of tramadol and M1 concentrations

The concentrations of tramadol and M1 in plasma and urine were measured by use of an API 3,000 liquid chromatography-tandem mass spectrometry system (AB Sciex, Toronto, ON, Canada) as previously described [35] with minor modifications. Prior to analysis, precipitation of plasma proteins was performed with acetonitrile, excluding the 48-h samples with very low drug concentrations, where MCX solid phase extraction was employed (Waters, Milford, MA, USA). Chromatography was performed on an Atlantis T3 analytical column (2.1×100 mm; Waters) using 10 mM formic acid with 0.1 % (v/v) trifluoroacetic acid (channel A) and methanol (channel B) as the mobile phase. Tramadol-d_6 and M1-d_6 served as internal standards. The mass spectrometer was operated in positive multi-reaction monitoring (MRM+) detection mode with electrospray ionization (ESI). The selected ion transitions used for quantitation were as follows: m/z 264→58 for tramadol and m/z 250→58 for M1, and m/z 270→64 and m/z 256→64 for the internal standards. The limit of quantitation for plasma tramadol and M1 was 0.05 ng/mL, and the interday coefficients of variation (CV) were below 6 % at relevant concentrations for both analytes.

Determination of plasma ticlopidine concentrations

Plasma ticlopidine concentrations were determined using a Waters Quattro Micro triple quadrupole mass spectrometer equipped with a Z-spray electrospray source (Waters, Milford, MA, USA) as described previously [36].

Determination of plasma itraconazole and OH-itraconazole concentrations

Plasma itraconazole and OH-itraconazole concentrations were determined with ketoconazole as the internal standard and high-performance liquid chromatography (HPLC) with UV detection, as described earlier [37].

Whole blood 5-HT and 5-HIAA concentrations

Concentrations of 5-HT and 5-HIAA were analyzed with HPLC and coulometric electrochemical detection using a procedure modified from previously published methods [38, 39]. In brief, the instrumentation included an ESA Coulochem Model 5100 A detector equipped with a Model 5011 analytical cell (operated at+0.40 V for oxidation; ESA Inc., Bedford, MA, USA) and a Beckman Ultrasphere ODS 250×4.6 mm analytical column. The mobile phase consisted of a sodium phosphate/citric acid buffer with 14 % (v/v) methanol as organic modifier and 0.015 % (w/v) heptanesulphonic acid (Fluka, Buchs, Switzerland) as ion pairing reagent. Homovanillyl alcohol (Sigma-Aldrich, St. Louis, MO, USA) was used as an internal standard. Blood samples (300 μL) were diluted with water (900 μL) and proteins were precipitated by 10 % zinc sulphate solution (200 μL), with vigorous mixing and centrifugation. The clear supernatant (700 μL) was transferred to a clean tube and mixed with 10 μL of a 20 % solution of NaOH. After vigorous mixing and centrifugation, the supernatant was transferred to autosampler vials, from which 20 μL were injected into the HPLC. Limits of reliable detection were 30 nM for 5-HT and 3 nM for 5-HIAA. The intra-assay CVs of both analytes were < 5 % in the relevant concentrations and the inter-assay CVs were about 5 % for 5-HT and < 15 % for 5-HIAA. All samples from one experimental session were analyzed in one batch.

Pharmacokinetic calculations

Peak plasma concentrations (C_{max}) and times to peak concentration (t_{max}) of tramadol and M1 were observed directly from the data. Individual terminal log-linear phases of the plasma concentration-time curves were identified visually. The elimination rate constant (λ_z) was determined by regression analysis of the log-linear part of the curve. Elimination half-life ($t_{½}$) was calculated from the equation $\ln 2/\lambda_z$. Areas under the drug plasma concentration-time curves ($AUC_{0-\infty}$) were calculated using the linear trapezoidal rule for successive increasing concentration values and logarithmic trapezoidal rule when values were decreasing. The apparent oral clearance (CL/F) and the apparent volume of distribution (V_z/F) of tramadol were calculated using standard noncompartmental methods. The metabolite M1 to parent tramadol AUC ratios (AUC_m/AUC_p) were also calculated. The AUC_{0-3} was calculated for M1 to assess the initial formation of M1. The renal clearance of tramadol (Cl_r) was calculated from the formula Cl_r = amount excreted within 24 h in urine/AUC_{0-24}. The pharmacokinetic data

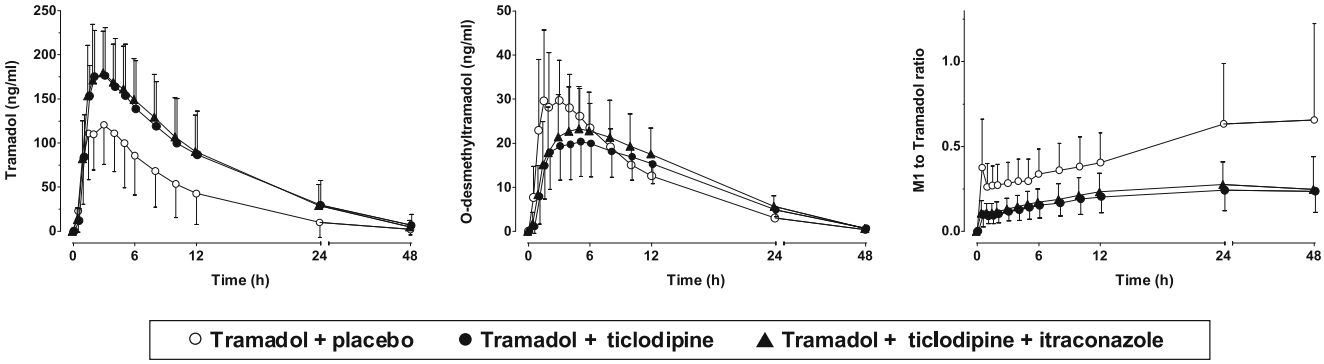

Fig. 1 Mean plasma (SD) concentrations of tramadol and *O*-desmethyltramadol (M1) and the M1 to tramadol ratio in 12 healthy volunteers after 50 mg oral tramadol on the fourth day of pretreatment with placebo, ticlopidine (250 mg twice daily) or a combination of ticlopidine and itraconazole (200 mg once daily) for 5 days

were analyzed using the WinNonlin pharmacokinetics program (version 4.1; Pharsight, Mountain View, CA, USA).

Pharmacodynamic measurements

Subjects answered questionnaires on specific adverse symptoms. These symptoms were dizziness, dryness of mouth, weakness, fever, headache, impaired coordination, increased sweating, nausea, restlessness, tremor, muscle stiffness, tiredness and sleep disturbance. Subjects scored their possible symptoms as mild (1), moderate (3) or severe (5).

Subjective effects of tramadol were evaluated using 100-mm visual analogue scales for the following items: drowsy/alert, poor/good performance, no/strong drug effect, relaxed/anxious, unpleasant/pleasant feeling, no/very strong nausea, calm/restless. Pupil size was measured with Cogan's pupillometer [40], central coordination of extraocular muscles was evaluated with the Maddox wing test [41], and central processing of sensory information was estimated by using the digit symbol substitution test [42].

The analgesic effect of tramadol was evaluated using the cold pressor test. The subject was asked to immerse the hand contralateral to the cannulated arm into ice-cold water (0–2 °C) and to report when the cold sensation became painful. The first report of pain was defined as the cold pain threshold (s). Cold pain intensity was assessed at 30 and 60 s. A verbal numerical scale 0–100 (0 = no pain; 100=intolerable pain) was used. If the pain became intolerable, the subject was instructed to withdraw his/her hand from the water.

The area under the effect-time curve ($AUEC_{0\text{-}12}$) was determined for 12 h for each pharmacodynamic variable except the adverse symptoms.

Statistical analysis

On the basis of previous studies, we calculated that 10 subjects would be needed to detect a 30 % difference in the $AUC_{0\text{-}\infty}$ of tramadol at a power of 80 % and two-sided significance of $p<0.05$ [43]. Twelve subjects were enrolled to allow for a 20 % drop-out rate.

The data were evaluated for normality of distribution using probit plots and the Shapiro–Wilk's W-test. Prior to statistical analysis, the data were log-transformed to correct for non-normality but reported as nontransformed results. Geometric mean ratios with 90 % confidence intervals were calculated for the pharmacokinetic variables. Differences in pharmacokinetic parameters and $AUEC_{0\text{-}12}$ values between the study phases were compared using analysis of variance for repeated measurements. A posteriori testing was performed with Tukey's test. *P*-values<0.05 were regarded as statistically significant. Results are expressed as mean ± SD and geometric mean ratios with 90 % confidence intervals except for T_{max} which is expressed as median (range). Data were analyzed using SYSTAT for Windows (version 10.2; Systat Software, Richmond, CA, USA) and GraphPad Prism 5 for Windows (GraphPad Software, San Diego, CA, USA) statistical programs.

Results

Pharmacokinetics

The mean plasma concentrations of tramadol and M1, and the ratio of M1 to tramadol in plasma during the placebo, ticlopidine, and itraconazole and ticlopidine phases are shown in Fig. 1. The effects of ticlopidine, and ticlopidine and itraconazole on the pharmacokinetics of tramadol and M1 are summarized in Table 1.

Effect of ticlopidine Ticlopidine increased the mean $AUC_{0\text{-}\infty}$ of tramadol by 2.0-fold (90 % confidence interval (CI) 1.6–2.4; $p<0.001$) and its C_{max} by 1.4-fold (1.2–1.6; $p<0.001$). Ticlopidine reduced the $AUC_{0\text{-}3}$ of M1 ($p<0.001$) and the ratio of the $AUC_{0\text{-}\infty}$ of M1 to that of tramadol (by 49 %; $p<0.001$) but did not influence the total $AUC_{0\text{-}\infty}$ of M1 when

Table 1 Pharmacokinetic parameters of tramadol and its primary metabolite *O*-desmethyltramadol (M1) after oral administration of 50 mg tramadol on the fourth day of pretreatment with oral placebo, ticlopidine alone (250 mg twice daily) or ticlopidine together with itraconazole (200 mg once daily) in 12 healthy volunteers

Parameter	Placebo	Ticlopidine	95 % CI of difference between phases	Ticlopidine + Itraconazole	95 % CI of difference between phases	Geometric mean ratio (90 % CI) for	
						Ticlodipine/Placebo	Ticlodipine + Itraconazole/ Placebo
Tramadol							
$AUC_{0-\infty}$ (ng· h/mL)	1,351±1,055	2,603±1,642**	738, 1,766	2,600±1,302**	898, 1,600	1.97 (1.63, 2.40)	2.08 (1.84, 2.36)
C_{max} (ng/mL)	142±48	193±54**	21, 81	188±49**	21, 73	1.39 (1.22, 1.58)	1.36 (1.20, 1.53)
t_{max} (h)	2.1±1.0	2.4±1.0		2.5±0.8			
$t_{1/2}$ (h)	5.4±2.6	8.0±3.5**	1.7, 3.5	7.6±2.2**	1.5, 2.9	1.49 (1.34, 1.67)	1.46 (1.34, 1.58)
CL/F (L/min)	0.85±0.44	0.43±0.25**	−0.64, −0.19	0.39±0.18**	−0.64, −0.28	0.51 (0.42, 0.62)	0.48 (0.42, 0.54)
CL_r (L/min)	0.17±0.10	0.09±0.04*	−0.14, −0.02	0.08±0.03**	−0.16, −0.03	0.58 (0.41, 0.82)	0.49 (0.35, 0.69)
V_z/F (L)	333±98	254±80**	−114.3, −43.9	230±56**	−138, −68	0.76 (0.69, 0.83)	0.70 (0.65, 0.75)
O-desmethyltramadol							
$AUC_{0-\infty}$ (ng· h/mL)	367±87	371±94	−69, 78	419±152	−49, 152	1.00 (0.84, 1.21)	1.10 (0.89, 1.36)
AUC_{0-3} (ng· h/mL)	66±32	35±16**	−44, −18	37±21*	−45, −13	0.54 (0.43, 0.67)	0.56 (0.41, 0.76)
C_{max} (ng/mL)	34±10	21±8**	−17, −9	24±10**	−14, −5	0.60 (0.53, 0.69)	0.68 (0.59, 0.79)
t_{max} (h)	2.7±2.0	5.3±2.6*		5.4±2.5*			
$t_{1/2}$ (h)	6.6±3.2	9.5±5.0*	1.5, 4.5	8.0±2.4	0.3, 2.6	1.45 (1.29, 1.63)	1.27 (1.13, 1.43)
CL_r (L/min)	0.21±0.09	0.21±0.06	−0.04, 0.03	0.21±0.06	−0.06, 0.05	1.05 (0.87, 1.29)	1.05 (0.75, 1.48)
AUC_m/AUC_p	0.35±0.14	0.21±0.16**	−0.23, −0.07	0.19±0.09**	−0.20, −0.11	0.51 (0.41, 0.63)	0.53 (0.47, 0.60)
AUC_m/AUC_p(0–3 h)	0.27±0.12	0.11±0.06**	−0.21, −0.12	0.12±0.06**	−0.20, −0.11	0.37 (0.33, 0.42)	0.41 (0.36, 0.46)

$AUC_{0-\infty}$=area under plasma concentration time curve extrapolated to infinity, AUC_{0-3}=area under plasma concentration time curve from 0 to 3 h, C_{max} = peak plasma concentration, t_{max} = time to peak concentration, $t_{1/2}$ = terminal elimination half-life, CL/F = apparent oral clearance, CL_r = renal clearance, V_z/F = apparent volume of distribution. AUC_m/AUC_p = $AUC_{0-\infty}$ of *O*-desmethyltramadol/$AUC_{0-\infty}$ of tramadol

Significantly different *($p<0.01$) or **($p<0.001$) from the placebo phase

Table 2 Excretion of tramadol and *O*-desmethyltramadol (M1) in urine during the first 24 h after ingestion of 50 mg tramadol after pretreatment with placebo, ticlopidine alone (250 mg twice daily) and ticlopidine together with itraconazole (200 mg once daily)

Parameter	Placebo	Ticlopidine	Ticlopidine + Itraconazole
Tramadol 0–12 h (mg)	6.71±3.10	7.01±3.79	6.43±1.90
Tramadol 12–24 h (mg)	4.22±3.54	5.30±3.95	3.91±2.97
Tramadol 0–24 h (mg)	10.93±5.01	12.31±5.62	10.34±3.71
M1 0–12 h (mg)	3.03±1.38	2.62±1.24	3.16±1.19
M1 12–24 h (mg)	1.41±0.50	2.03±1.64	1.69±0.81
M1 0–24 h (mg)	4.43±1.53	4.64±1.38	4.85±1.06

compared to placebo (p=0.94). Ticlopidine increased the mean $t_{½}$ of tramadol from 5.4 h to 8.0 h (p<0.001) and decreased its apparent oral clearance by 49 % (p<0.001). The mean trough concentration (C_{trough}) of ticlopidine before the first dose on day 4 was 133 ng/mL (range 66–290 ng/mL).

Effect of ticlopidine and itraconazole Ticlopidine and itraconazole increased the mean $AUC_{0-\infty}$ of tramadol by 2.1-fold (90 % CI 1.8–2.4; p<0.001) but did not influence the $AUC_{0-\infty}$ of M1 when compared to placebo (p=0.35). Ticlopidine and itraconazole decreased the $AUC_{0-\infty}$ of M1 to $AUC_{0-\infty}$ of tramadol ratio by 47 % when compared to placebo (p<0.001). Ticlopidine and itraconazole increased $t_{½}$ of tramadol from 5.4 h to 7.6 h (p<0.001) and decreased its oral clearance by 52 % (p<0.001). The mean C_{trough} of ticlopidine, itraconazole and OH-itraconazole before the first dose on day 4 were 119 ng/mL (range 65–200 ng/mL), 38.4 ng/mL (range 8.7–70 ng/mL) and 57.4 ng/mL (range 0.9–161 ng/mL), respectively.

Effects on renal excretion of tramadol About 22 % of the oral tramadol dose was excreted unchanged in urine within 24 h during the placebo phase. Renal clearance of the parent drug was reduced significantly by the concomitant use of ticlopidine alone (42 %, p<0.01) or together with itraconazole (51 %, p<0.001) when compared to the placebo phase (Table 1). Ticlopidine alone or together with itraconazole did not affect the renal clearance of M1 or the cumulative amount of tramadol or M1 excreted in urine when compared to placebo (Table 2).

Pharmacodynamics

Although tramadol was in general well tolerated, almost every subject experienced some mild or moderate adverse effects. Eleven subjects reported symptoms after tramadol ingestion during the placebo phase, nine subjects during the ticlopidine phase, and 10 subjects during the ticlopidine and itraconazole phase. The most frequent adverse effect was drowsiness, followed by dry mouth, nausea and headache.

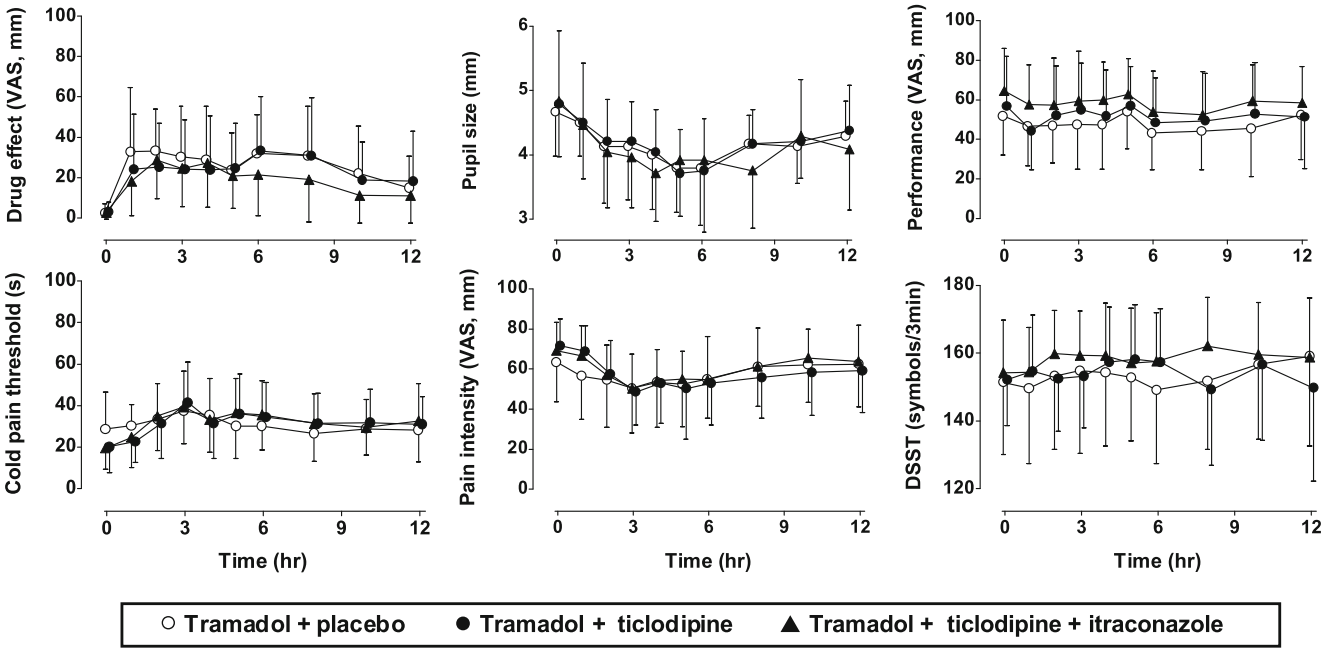

Fig. 2 Means (SD) of self-reported "drug effect," self-reported "impaired performance," pupil diameter, cold pain threshold, cold pain intensity at 60 s and number of digits substituted in 3 min (DSST) in 12 healthy volunteers after 50 mg oral tramadol following pretreatment with placebo, ticlopidine (250 mg twice daily) or a combination of ticlopidine and itraconazole (200 mg once daily) for 5 days

The most pronounced analgesic effect in the cold pressor test was observed 3 h after the ingestion of tramadol (Fig. 2). The $AUEC_{0\text{-}12}$ of cold pain intensity and threshold were unaffected by ticlopidine alone or together with itraconazole. The $AUEC_{0\text{-}12}$ for drowsiness was increased during the ticlopidine and ticlopidine plus itraconazole phases ($p<0.05$ and $p<0.001$, respectively) when compared to placebo. During the ticlopidine plus itraconazole phase, the $AUEC_{0\text{-}12}$ was increased for "impaired performance" ($p<0.05$) and was decreased for "drug effect" ($p<0.05$). There were no differences in the other pharmacodynamic variables between the phases (Fig. 2).

Whole blood 5-HT and 5-HIAA

Whole blood concentrations of 5-HT and 5-HIAA after tramadol ingestion were relatively stable and not influenced by ticlopidine, or by ticlopidine plus itraconazole (Fig. 3).

Discussion

Drug–drug interactions of tramadol can be clinically important as they may expose the patient to harmful effects or decreased analgesia. The results of this study show that a pharmacokinetic interaction of tramadol with ticlopidine markedly increased the $AUC_{0\text{-}\infty}$ of tramadol, prolonged its $t_{½}$ and decreased its oral and renal clearances. Although the total $AUC_{0\text{-}\infty}$ of the active metabolite M1 did not change, the formation rate of M1 was reduced by ticlopidine. Concomitant use of itraconazole and ticlopidine did not influence the pharmacokinetics of tramadol when compared to ticlopidine alone.

Ticlopidine is a potent inhibitor of CYP2B6 and it also inhibits CYP2C19, CYP1A2 and CYP2D6 [24–30, 44]. In the present study, the initial $AUC_{0\text{-}3}$ of M1 and the ratio of plasma M1 to tramadol concentrations were reduced, indicating a diminished formation clearance of M1 from tramadol. However, the total $AUC_{0\text{-}\infty}$ of this active M1 metabolite did not change during ticlopidine pretreatment because the $AUC_{0\text{-}\infty}$ of tramadol was increased two-fold by ticlopidine. Although CYP2D6 is the enzyme mainly responsible for M1 formation, CYP2B6 may also contribute to it, at least in vitro [14]. Considering the relative hepatic abundancies of CYP2D6 and CYP2B6 and the 50-fold higher efficiency of CYP2D6 to catalyze M1 formation in comparison to CYP2B6 [14], the reduced formation rate of M1 by ticlopidine seen in the present clinical experiment may be explained by inhibition of the CYP2B6, CYP2D6 or both enzymes during ticlopidine use [13, 14].

Itraconazole is a potent inhibitor of CYP3A4 and P-glycoprotein [31, 32]. Itraconazole has significant drug–drug interactions with many substrates of CYP3A4 such as triazolam, midazolam, quinidine and oxycodone [31, 32, 45–47]. In vivo studies have suggested that itraconazole can increase plasma concentrations of P-glycoprotein substrates such as digoxin, fexofenadine and paroxetine [48–50]. Although tramadol is a substrate of CYP3A4, combining the potent CYP3A4 inhibitor itraconazole together with ticlopidine did not influence the pharmacokinetics of tramadol in the present study when compared to ticlopidine alone, suggesting that CYP3A4 is of limited importance in the metabolism of tramadol in humans. Comprehensive assessment of metabolic alterations of tramadol by itraconazole was, however, not possible as we did not quantify M2 concentrations in the present study.

CYP2D6 plays a major role in the *O*-demethylation of tramadol to the active metabolite M1. The potent CYP2D6 inhibitor paroxetine has increased the AUC of (+)-tramadol by 37 % and that of (−)-tramadol by 32 %, and decreased the AUC of (+) and (−)-M1 by 67 % and 40 %, respectively [17]. Similarly, the potent CYP2D6 inhibitor quinidine inhibited in a human liver microsome assay only partially (about 80 %) the metabolism of tramadol to M1 [14]. It is of note that paroxetine did not totally prevent the formation of M1, suggesting a role for CYP2B6 and possibly other enzymes in M1 formation in humans [17].

Previous studies have shown that 12–32 % of a single oral dose of tramadol is excreted unchanged in urine within 24–72 h [13, 16]. In the present study, more than 20 % of the oral tramadol dose was excreted into urine in unchanged form within 24 h. Interestingly, ticlopidine reduced markedly

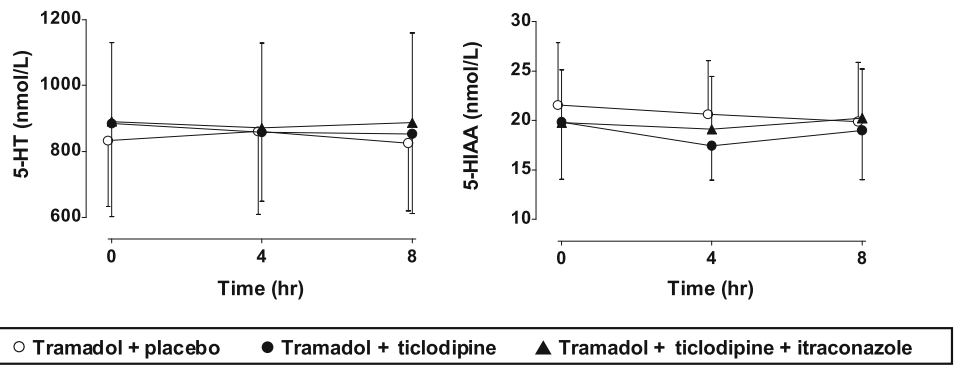

Fig. 3 Mean (SD) concentrations of 5-HT and 5-HIAA in whole blood of 12 healthy volunteers after 50 mg oral tramadol after pretreatment with placebo, ticlopidine (250 mg twice daily) or a combination of ticlopidine and itraconazole(200 mg once daily) for 5 days

(42 %) the renal clearance of the parent drug but not that of M1, contributing to the elevated plasma tramadol concentrations. An inhibition of one or several of the renal transporters that contribute to the renal excretion of tramadol would be compatible with this explanation. Another explanation for the elevated concentrations might be a decrease in the apparent volume of distribution during the ticlopidine phase. Although tramadol and M1 are not substrates for P-glycoprotein, other efflux pumps have been suggested to be involved in the renal excretion of tramadol or M1 [51] and further studies are needed to clarify the exact mechanisms.

The cold pressor test has been shown to be sensitive to tramadol analgesia [12, 17]. In the present study, the decrease in cold pressor pain intensity peaked at 3 h after tramadol intake. However, the analgesic effect of tramadol was unaffected by ticlopidine alone or together with itraconazole. It is possible that the cold pressor test was too insensitive to capture a 50 % exposure reduction of M1, which is mainly responsible for the opioid analgesia of tramadol. Influences of pretreatments on other pharmacodynamic effects of tramadol in the present study were minor and possibly affected by the single-blinded study design.

In our earlier studies we have faced previously unrecognized, drastic increases in the AUC of several victim drugs, which have a limited therapeutic index, such as midazolam, triazolam, quinidine and oxycodone, when itraconazole or other potent inhibitors of their metabolism have been administered concomitantly [31, 32, 45–47]. For safety reasons, we used only a small, 50-mg dose of tramadol in our present tramadol interaction study because the magnitude of possible interaction was not known beforehand and the safety of the healthy subjects could not be compromised even in the case of drastic interaction.

(+)-Tramadol is a potent inhibitor of the 5-HT transporter [20]. Patients who use tramadol may be exposed to excessive serotonergic effects especially if tramadol is taken concomitantly with antidepressants [23]. In the present study, we did not observe any symptoms or signs of increased serotonergic effects. After tramadol, whole blood concentrations of 5-HT and 5-HIAA were not altered and the frequency of adverse (possibly serotonergic) effects (such as tremor and nausea) was not influenced by ticlopidine alone or together with itraconazole. However, conclusions about the clinical safety of the concomitant use of tramadol with ticlopidine cannot be drawn on the basis of this study, as we only employed small single doses of tramadol and the study was conducted in young healthy individuals.

In conclusion, ticlopidine and concomitant use of ticlopidine and itraconazole doubled the exposure to tramadol and reduced the ratio of M1 to tramadol, suggesting inhibition of M1 formation, possibly via CYP2B6 and/or CYP2D6 inhibition. Ticlopidine markedly reduced the renal clearance of tramadol but not of M1. The pharmacokinetic changes were not accompanied by marked subjective drug effects, and the analgesic effect of tramadol was not influenced. Still, the concomitant clinical use of tramadol and ticlopidine might involve an increased risk of serotonergic adverse effects when higher tramadol doses are used.

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References

1. Moore RA, McQuay HJ (1997) Single-patient data meta-analysis of 3453 postoperative patients: oral tramadol versus placebo, codeine and combination analgesics. Pain 69:287–294
2. Harati Y, Gooch C, Swenson M, Edelman S, Greene D, Raskin P, Donofrio P, Cornblath D, Sachdeo R, Siu CO, Kamin M (1998) Double-blind randomized trial of tramadol for the treatment of the pain of diabetic neuropathy. Neurology 50:1842–1846
3. Boureau F, Legallicier P, Kabir-Ahmadi M (2003) Tramadol in post-herpetic neuralgia: a randomized, double-blind, placebo-controlled trial. Pain 104:323–331
4. Malonne H, Coffiner M, Sonet B, Sereno A, Vanderbist F (2004) Efficacy and tolerability of sustained-release tramadol in the treatment of symptomatic osteoarthritis of the hip or knee: a multicenter, randomized, double-blind, placebo-controlled study. Clin Ther 26:1774–1782
5. Attal N, Cruccu G, Baron R, Haanpää M, Hansson P, Jensen TS, Nurmikko T (2010) EFNS guidelines on the pharmacological treatment of neuropathic pain: 2010 revision. Eur J Neurol 17:1113–1123
6. Dworkin RH, O'Connor AB, Audette J, Baron R, Gourlay GK, Haanpää ML, Kent JL, Krane EJ, Lebel AA, Levy RM, Mackey SC, Mayer J, Miaskowski C, Raja SN, Rice AS, Schmader KE, Stacey B, Stanos S, Treede RD, Turk DC, Walco GA, Wells CD (2010) Recommendations for the pharmacological management of neuropathic pain: an overview and literature update. Mayo Clin Proc 85:S3–S14
7. Haanpää ML, Gourlay GK, Kent JL, Miaskowski C, Raja SN, Schmader KE, Wells CD (2010) Treatment considerations for patients with neuropathic pain and other medical comorbidities. Rev Mayo Clin Proc 85:S15–S25
8. Roughead EE, McDermott B, Gilbert AL (2007) Antidepressants: prevalence of duplicate therapy and avoidable drug interactions in Australian veterans. Aust N Z J Psychiatry 41:366–370
9. Tirkkonen T, Laine K (2004) Drug interactions with the potential to prevent prodrug activation as a common source of irrational prescribing in hospital inpatients. Clin Pharmacol Ther 76:639–647
10. Lintz W, Barth H, Osterloh G, Schmidt-Böthelt E (1986) Bioavailability of enteral tramadol formulations. 1st communication: capsules. Arzneimittelforschung 36:1278–1283
11. Paar WD, Frankus P, Dengler HJ (1992) The metabolism of tramadol by human liver microsomes. Clin Investig 70:708–710
12. Poulsen L, Arendt-Nielsen L, Brøsen K, Sindrup SH (1996) The hypoalgesic effect of tramadol in relation to CYP2D6. Clin Pharmacol Ther 60:636–644

13. Paar WD, Poche S, Gerloff J, Dengler HJ (1997) Polymorphic CYP2D6 mediates O-demethylation of the opioid analgesic tramadol. Eur J Clin Pharmacol 53:235–239
14. Subrahmanyam V, Renwick AB, Walters DG, Young PJ, Price RJ, Tonelli AP, Lake BG (2001) Identification of cytochrome P-450 isoforms responsible for cis-tramadol metabolism in human liver microsomes. Drug Metab Dispos 29:1146–1155
15. Wu WN, McKown LA, Liao S (2002) Metabolism of the analgesic drug ULTRAM (tramadol hydrochloride) in humans: API-MS and MS/MS characterization of metabolites. Xenobiotica 32:411–425
16. Lintz W, Erlacin S, Francus E, Uragg H (1981) Biotransformation of tramadol in man and animal. Arzneimittelforschung 31:1932–1943
17. Laugesen S, Enggaard TP, Pedersen RS, Sindrup SH, Brøsen K (2005) Paroxetine, a cytochrome P450 2D6 inhibitor, diminishes the stereoselective O-demethylation and reduces the hypoalgesic effect of tramadol. Clin Pharmacol Ther 77:312–323
18. Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL (1992) Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic. J Pharmacol Exp Ther 260:275–285
19. Gillen C, Haurand M, Kobelt DJ, Wnendt S (2000) Affinity, potency and efficacy of tramadol and its metabolites at the cloned human μ-opioid receptor Naunyn-Schmiedeberg's. Arch Pharmacol 362:116–121
20. Driessen B, Reimann W (1992) Interaction of the central analgesic, tramadol, with the uptake and release of 5-hydroxytryptamine in the rat brain in vitro. Br J Pharmacol 105:147–151
21. Driessen B, Reimann W, Giertz H (1993) Effects of the central analgesic tramadol on the uptake and release of noradrenaline and dopamine in vitro. Br J Pharmacol 108:806–811
22. Maurer-Spurej E, Pittendreigh C, Solomons K (2004) The influence of selective serotonin reuptake inhibitors on human platelet serotonin. Thromb Haemost 91:119–128
23. Gillman PK (2005) Monoamine oxidase inhibitors, opioid analgesics and serotonin toxicity. Br J Anaesth 95:434–441
24. Richter T, Műrdter TE, Heinkele G, Pleiss J, Tatzel S, Schwab M, Eichelbaum M, Zanger UM (2004) Potent mechanism-based inhibition of human CYP2B6 by clopidogrel and ticlopidine. J Pharmacol Exp Ther 308:189–197
25. Turpeinen M, Tolonen A, Uusitalo A, Jalonen J, Pelkonen O, Laine K (2005) Effect of clopidogrel and ticlopidine on cytochrome P450 2B6 activity as measured by bupropion hydroxylation. Clin Pharmacol Ther 77:553–559
26. Turpeinen M, Raunio H, Pelkonen O (2006) The functional role of CYP2B6 in human drug metabolism: substrates and inhibitors in vitro, in vivo and in silico. Curr Drug Metab 7:605–714
27. Turpeinen M, Nieminen R, Juntunen T, Taavitsainen P, Raunio H, Pelkonen O (2004) Selective inhibition of CYP2B6-catalyzed bupropion hydroxylation in human liver microsomes in vitro. Drug Metab Dispos 32:626–631
28. Ha-Duong N-T, Dijols S, Macherey A-C, Goldstein JA, Dansette PM, Mansuy D (2001) Ticlopidine as a selective mechanism-based inhibitor of human cytochrome P450 2C19. Biochemistry 40:12112–12122
29. Ko JW, Desta Z, Soukhova NV, Tracy T, Flockhart DA (2000) In vitro inhibition of the cytochrome P450 (CYP450) system by the antiplatelet drug ticlopidine: potent effect on CYP2C19 and CYP2D6. Br J Clin Pharmacol 49:343–351
30. Hagihara K, Nishiya Y, Kurihara A, Kazui M, Farid NA, Ikeda T (2008) Comparison of human cytochrome P450 inhibition by the thienopyridines prasugrel, clopidogrel, and ticlopidine. Drug Metab Pharmacokinet 23:412–420
31. Olkkola KT, Ahonen J, Neuvonen PJ (1996) The effects of the systemic antimycotics, itraconazole and fluconazole, on the pharmacokinetics and pharmacodynamics of intravenous and oral midazolam. Anesth Analg 82:511–516
32. Kaukonen KM, Olkkola KT, Neuvonen PJ (1997) Itraconazole increases plasma concentrations of quinidine. Clin Pharmacol Ther 62:510–517
33. Walsky RL, Astuccio AV, Obach RS (2006) Evaluation of 227 drugs for in vitro inhibition of cytochrome P450 2B6. J Clin Pharmacol 46:1426–1438
34. Michna E, Ross EL, Hynes WL, Nedeljkovic SS, Soumekh S, Janfaza D, Palombi D, Jamison RN (2004) Predicting aberrant drug behavior in patients treated for chronic pain. J Pain Symptom Manage 28:250–258
35. Patel BN, Sharma N, Sanyal M, Shrivastav PS (2009) An accurate, rapid and sensitive determination of tramadol and its active metabolite O-desmethyltramadol in human plasma by LC-MS/MS. J Pharm Biomed Anal 49:354–366
36. Peltoniemi MA, Saari TI, Hagelberg NM, Reponen P, Turpeinen M, Laine K, Neuvonen PJ, Olkkola K (2011) Exposure to oral S-ketamine is unaffected by itraconazole but greatly increased by ticlopidine. Clin Pharmacol Ther 90:296–302
37. Gubbins PO, Gurley BJ, Bowman J (1998) Rapid and sensitive high performance liquid chromatographic method for the determination of itraconazole and its hydroxy-metabolite in human serum. J Pharm Biomed Anal 16:1005–1012
38. Anderson GM, Young JG, Cohen DJ, Schlicht KR, Patel N (1981) Liquid-chromatographic determination of serotonin and tryptophan in whole blood and plasma. Clin Chem 27:775–776
39. Scheinin M, Karhuvaara S, Ojala-Karlsson P, Kallio A, Koulu M (1991) Plasma 3,4-dihydroxyphenylglycol (DHPG) and 3-methoxy-4-hydroxyphenylglycol (MHPG) are insensitive indicators of alpha 2-adrenoceptor mediated regulation of norepinephrine release in healthy human volunteers. Life Sci 49:75–84
40. Cogan DG (1941) Simplified entopic pupillometer. Am J Ophthalmol 24:1431–1433
41. Hannington-Kiff JG (1970) Measurement of recovery from outpatient general anaesthesia with a simple ocular test. Br Med J 3:132–135
42. Stone BM (1984) Pencil and paper tests—sensitivity to psychotropic drugs. Br J Clin Pharmacol 18:15S–20S
43. Kirchheiner J, Keulen JT, Bauer S, Roots I, Brockmoller J (2008) Effects of the CYP2D6 gene duplication on the pharmacokinetics and pharmacodynamics of tramadol. J Clin Psychopharmacol 28:78–83
44. Kot M, Daniel MA (2009) Effect of diethyldithiocarbamate (DDC) and ticlopidine on CYP1A2 activity and caffeine metabolism: an in vitro comparative study with human cDNA-expressed CYP1A2 and liver microsomes. Pharmacol Rep 61:1216–1220
45. Varhe A, Olkkola KT, Neuvonen PJ (1994) Oral triazolam is potentially hazardous to patients receiving systemic antimycotics ketoconazole or itraconazole. Clin Pharmacol Ther 56:601–607
46. Backman JT, Kivistö KT, Olkkola KT, Neuvonen PJ (1998) The area under the plasma concentration-time curve for oral midazolam is 400-fold larger during treatment with itraconazole than with rifampicin. Eur J Clin Pharmacol 54:53–58
47. Saari TI, Grönlund J, Hagelberg NM, Neuvonen M, Laine K, Neuvonen PJ, Olkkola KT (2010) Effects of itraconazole on the pharmacokinetics and pharmacodynamics of intravenously and orally administered oxycodone. Eur J Clin Pharmacol 66:387–397
48. Partanen J, Jalava KM, Neuvonen PJ (1996) Itraconazole increases serum digoxin concentration. Pharmacol Toxicol 79:274–276
49. Shimizu M, Uno T, Sugawara K, Tateishi T (2006) Effects of itraconazole and diltiazem on the pharmacokinetics of fexofenadine, a substrate of p-glycoprotein. Br J Clin Pharmacol 61:538–544
50. Yasui-Furukori N, Saito M, Niioka T, Inoue Y, Sato Y, Kaneko S (2007) Effect of itraconazole on pharmacokinetics of paroxetine: the role of gut transporters. Ther Drug Monit 29:45–48
51. Kanaan M, Daali Y, Dayer P, Desmeules J (2009) Uptake/efflux transport of tramadol enantiomers and O-desmethyl-tramadol: focus on p-glycoprotein. Basic Clin Pharmacol Toxicol 105:199–206