

RESEARCH PAPER

The effects of CYP2D6 and CYP3A activities on the pharmacokinetics of immediate release oxycodone

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Background and purpose: There is high interindividual variability in the activity of drug-metabolizing enzymes catalysing the oxidation of oxycodone [cytochrome P450 (CYP) 2D6 and 3A], due to genetic polymorphisms and/or drug–drug interactions. The effects of CYP2D6 and/or CYP3A activity modulation on the pharmacokinetics of oxycodone remains poorly explored.

Experimental approach: A randomized crossover double-blind placebo-controlled study was performed with 10 healthy volunteers genotyped for CYP2D6 [six extensive (EM), two deficient (PM/IM) and two ultrarapid metabolizers (UM)]. The volunteers randomly received on five different occasions: oxycodone 0.2 mg·kg⁻¹ and placebo; oxycodone and quinidine (CYP2D6 inhibitor); oxycodone and ketoconazole (CYP3A inhibitor); oxycodone and quinidine+ketoconazole; placebo. Blood samples for plasma concentrations of oxycodone and metabolites (oxymorphone, noroxycodone and noroxymorphone) were collected for 24 h after dosing. Phenotyping for CYP2D6 (with dextromethorphan) and CYP3A (with midazolam) were assessed at each session.

Key results: CYP2D6 activity was correlated with oxymorphone and noroxymorphone AUCs and C_{max} (−0.71 < Spearman correlation coefficient ρ_s < −0.92). Oxymorphone C_{max} was 62% and 75% lower in PM than EM and UM. Noroxymorphone C_{max} reduction was even more pronounced (90%). In UM, oxymorphone and noroxymorphone concentrations increased whereas noroxycodone exposure was halved. Blocking CYP2D6 (with quinidine) reduced oxymorphone and noroxymorphone C_{max} by 40% and 80%, and increased noroxycodone AUC_∞ by 70%. Blocking CYP3A4 (with ketoconazole) tripled oxymorphone AUC_∞ and reduced noroxycodone and noroxymorphone AUCs by 80%. Shunting to CYP2D6 pathway was observed after CYP3A4 inhibition.

Conclusions and implications: Drug–drug interactions via CYP2D6 and CYP3A affected oxycodone pharmacokinetics and its magnitude depended on CYP2D6 genotype.

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Abbreviations: AUC, area under the concentration curve; CL/F, oral clearance; C_{max}, maximum concentration; CV, coefficient of variation; CYP, cytochrome P450; EM, extensive metabolizer; MOR, μ opioid receptor; MR, metabolic ratio; PM, poor metabolizer; T_{1/2z}, terminal elimination half-life; T_{max}, time to C_{max}; UM, ultrarapid metabolizers; V_z/F, volume of distribution

Introduction

Oxycodone is a potent semi-synthetic μ opioid agonist analgesic in clinical use since 1916 (Falk, 1917). It has therefore bypassed the now conventional pharmacological studies assessing specifically its pharmacokinetics and pharmacodynamics. A high interindividual variability in the activity of cytochrome P450 (CYP) 2D6 and CYP3A, the major

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drug-metabolizing enzymes responsible for oxycodone oxidation, has been extensively described and is due to drug–drug interactions and/or genetic polymorphisms for CYP2D6 (Samer *et al.*, 2005). The activity of CYP2D6 may also influence risks for substance dependence (Tyndale *et al.*, 1997). The possible role of an active metabolite in the pharmacodynamics of oxycodone has been questioned, but whether CYP2D6 genetic polymorphism results in phenotypic differences in the antinociceptive response has not been fully investigated (Kaiko *et al.*, 1996; Heiskanen *et al.*, 1998; Lalovic *et al.*, 2006; Samer *et al.*, 2006). The importance of CYP3A mediated effects on the pharmacokinetics and pharmacodynamics of oxycodone needs further investigation.

In vitro and *in vivo* studies have demonstrated that oxycodone metabolism proceeds through four metabolic pathways: N-demethylation, O-demethylation, 6-ketoreduction and glucurono-conjugation (Cone *et al.*, 1983). CYP3A catalyses the N-demethylation to the major (80%) circulating metabolite, noroxycodone whereas CYP2D6 catalyses the O-demethylation to oxymorphone which accounts for 10% of oxycodone metabolites (Lalovic *et al.*, 2004). The additional O-demethylation of noroxycodone to the didemethylated

metabolite, noroxymorphone, is also catalysed by CYP2D6 (Lalovic *et al.*, 2004) (Figure 1). These metabolites have varying potencies and affinities for the μ opioid receptor (MOR; nomenclature follows Alexander *et al.*, 2009). Oxymorphone is 14 times more potent than oxycodone (Chen *et al.*, 1991). Its affinity for the MOR is 40- and threefold higher than oxycodone and morphine respectively. The affinity of noroxymorphone at the MOR is three- and 10-fold higher than oxycodone and noroxycodone respectively. MOR affinities for each oxycodone metabolite can be therefore summarized, in order of highest to lowest, as oxymorphone > morphine > noroxymorphone > oxycodone > noroxycodone.

Literature on the significance of the CYP3A pathway on the pharmacokinetics of oxycodone is sparse. Co-administration of the CYP3A inducer rifampicin has been associated with negative urine oxycodone screening (Lee *et al.*, 2006) and a dramatic reduction in oxycodone exposure in healthy volunteers (Nieminen *et al.*, 2009). The consequences of CYP3A inhibition have not been extensively described. In healthy volunteers, it was recently shown that voriconazole leads to increased oxycodone exposure (Hagelberg *et al.*, 2009). In the case of codeine, Caraco *et al.* (1996) suggested that CYP3A

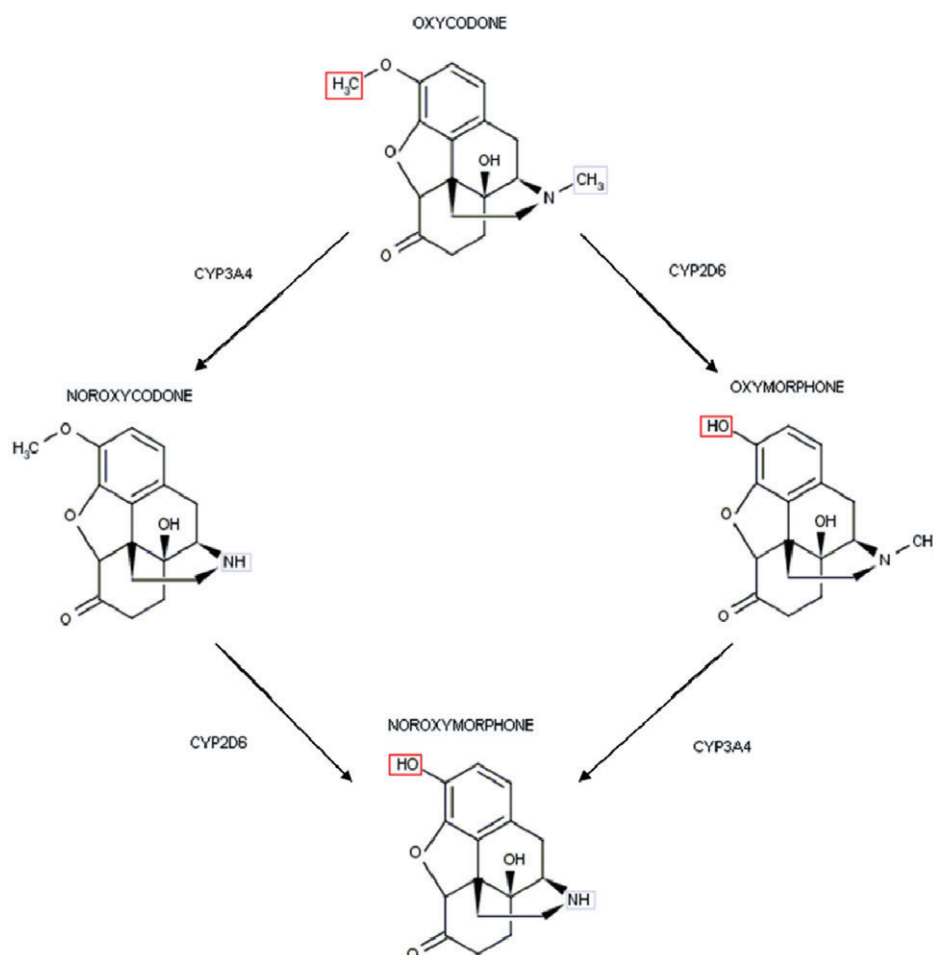


Figure 1 CYP2D6-mediated O-demethylation and CYP3A-mediated N-demethylation of oxycodone and metabolites. CYP3A catalyses the N-demethylation to the major (80%) circulating metabolite noroxycodone whereas CYP2D6 catalyses the O-demethylation to oxymorphone which accounts for 10% of oxycodone metabolites. The additional O-demethylation of noroxycodone to the didemethylated metabolite noroxymorphone is also catalysed by CYP2D6. CYP, cytochrome P450.

inhibition results in a shunt to the CYP2D6 pathway. Further, inhibition of CYP3A by drugs, in the context of CYP2D6 genetic polymorphism, can result in serious clinical consequences even after small codeine doses (Gasche *et al.*, 2004). Therefore, the role of CYP3A in oxycodone metabolism needs to be further investigated.

We demonstrate in this exploratory study with 10 healthy volunteers that both CYP2D6 and CYP3A activities (modulated by selective inhibition) influenced the pharmacokinetics of oxycodone and its metabolites and that the magnitude of the effect was modulated by the CYP2D6 genotype.

Methods

This five-arm randomized double-blind double-dummy crossover placebo-controlled study was approved by the institutional ethical committee of the Anaesthesiology, Pharmacology and Intensive care Department, Geneva University Hospitals, notified to the Swiss Agency for therapeutic products (Swissmedic) and performed according to Good Clinical Practices.

Ten healthy non-smoking male volunteers were included who had no history of illicit drugs consumption or opioid contraindications, had normal medical examination, ECG, liver and kidney functions and were not taking any medications.

The Latin squares method was used for randomization. The five crossover phases were separated by at least 1 week, as a washout period. The fasting volunteers received a single oral dose of the CYP2D6 blocker quinidine (100 mg), the CYP3A4 blocker ketoconazole (400 mg) or matching placebos. Microdoses of midazolam (75 µg) and dextromethorphan (2.5 mg) were given orally 1 h later. A single oral dose of oxycodone hydrochloride (Oxynorm®, Mundipharma) (0.2 mg·kg⁻¹; drops) or matching placebo was given 1 h later. Naloxone (0.8 mg) was injected intravenously 1.5 h after oxycodone or placebo.

Pharmacokinetic measurements

Blood samples (8 mL) for plasma concentration of oxycodone, oxymorphone, noroxycodone and noroxymorphone were drawn from an indwelling catheter in a left forearm vein before oxycodone administration and 0.5, 1, 1.5, 2, 3, 6 and 24 h after dosing. Blood samples were divided in two ethylenediamine-tetraacetic acid (EDTA) tubes, centrifuged at 3350×g for 10 min at 6°C and frozen at -20°C until analysis. Plasma concentrations of the analytes were determined by a validated assay using online column-switching liquid chromatography coupled to tandem mass spectrometric detection (CS-LC-MS/MS). Shortly, plasma proteins were precipitated by adding to 250 µL of plasma sample 500 µL of an acetonitrile/ethanol 1/1 solution containing the internal standard ISTD (oxycodone-d3, 2.5 ng·mL⁻¹). The mixture was vortexed and then centrifuged (15 min at 4°C, 15 000×g). 550 µL of the supernatant were transferred into a 1.5 mL Eppendorf-tube, and evaporated to dryness. The solid residue was reconstituted with 50 µL of 0.1% formic acid in water/acetonitrile 90/10 (v·v⁻¹) and injected on the CS-LC-MS/MS system. The

online column-switching was performed with a Prospekt 2 system (Spark, Emmen, the Netherlands). First, the analytes were trapped on a Hysphere Resin GP – 10–12 µm, cartridge. Then they were transferred from the trapping column to the analytical column (XTerra MS 4.6 × 50 mm, 3.5 µm, Waters) and separated in gradient mode (water/acetonitrile/0.1% formic acid) at 1 mL·min⁻¹. Mass spectrometric detection was performed on an 4000 Q TRAP (AB/MDS Sciex, Concord, ON) mass spectrometer in the selected reaction monitoring mode (dwell time = 75 ms). The following transitions and collision energies were selected: oxycodone *m/z* 316.1→*m/z* 241.1, 37 eV, oxymorphone *m/z* 302.3→*m/z* 198.2, 57 eV, noroxycodone: *m/z* 302.3→*m/z* 187.0, 30e, noroxymorphone *m/z* 288.1→*m/z* 213.2, 37 eV, oxycodone d3 *m/z* 319.3→*m/z* 244.3, 31 eV. Precision and accuracy of quality control samples was found to be better than 15%. The lower limit of quantification (LLOQ) using a plasma aliquot of 0.25 mL were found to be 100 pg·mL⁻¹ for oxycodone and oxymorphone, and 50 pg·mL⁻¹ for noroxycodone and noroxymorphone respectively.

Phenotyping

During the (pre-study) screening visit, a conventional dose (25 mg) of dextromethorphan was given to determine CYP2D6 activity. Microdoses of dextromethorphan (2.5 mg) and midazolam (75 µg) were given to determine CYP2D6 and CYP3A activities, respectively, during the five study sessions to avoid any pharmacodynamic interaction with oxycodone. For CYP2D6, deconjugated dextromethorphan and metabolites were assayed in urine collected during 8 h as described by Daali *et al.* (2008) and metabolic ratio between deconjugated dextromethorphan and dextroprhan was calculated. CYP3A phenotypes were measured using a previously validated method (Eap *et al.*, 2004). The 30 min total (unconjugated+conjugated) 1-OH- midazolam/midazolam ratios were determined in a single plasma sample using gas chromatography-negative chemical ionization mass spectrometry (GC-NCIMS).

Genotyping

Genomic DNA was extracted from whole blood and genetic variants of CYP2D6 (32 alleles) were identified by the gene AmpliChip™ CYP450 DNA microarray (Roche) as previously described (Rebsamen *et al.*, 2009). CYP2D6 alleles are associated with normal, absent, decreased or increased enzyme activity (<http://www.imm.ke.se/CYPalleles>). In our study, the following CYP2D6 alleles were identified: *1,*2,*35 associated with normal enzyme activity; *41 displaying decreased enzyme activity; *4,*5,*6 associated with the absence of activity and the *2xN gene duplication resulting in increased enzyme activity. According to the alleles' combinations observed, the CYP2D6 phenotype of each individual was predicted as follows: poor metabolizer (PM) if carrying two non-functional alleles; intermediate (IM), if carrying one non-functional allele and one associated with reduced activity; extensive (EM) if carrying at least one functional allele; ultra rapid (UM) if carrying at least three copies of a functional allele (see detailed predicted phenotype in Table 1).

Table 1 CYP2D6 genotype and measured CYP2D6 phenotype by dextromethorphan/dextrorphan (DEM/DOR) metabolic ratio, and CYP3A activity measured by 1-OH-midazolam/midazolam (1-OH MID/MID) metabolic ratio, according to the session/pretreatment with placebo, quinidine and/or ketoconazole

Volunteer	CYP2D6 genotype	Predicted phenotype	DEM/DOR metabolic ratio						1-OH MID/MID metabolic ratio				
			Pre-study	Oxy [#]	Pbo [#]	Keto [#]	Quin [#]	Keto+Quin [#]	Oxy	Pbo	Keto	Quin	Keto+Quin
1	*5/*35	EM	0.0165	0.0166	0.0042	0.0094	0.6040	0.1120	2.85	3.6	0.04	2.37	0.06
2	*41/*41xN	n.d.	0.0013	0.0008	0.0022	0.0018	0.0291	0.0267	3.86	2.61	0.20	2.82	0.16
3	*1/*41	EM	0.0017	0.0047	0.0006	0.0022	0.3971	0.4878	1.05	2.75	0.06	0.51	0.08
4	*5/*41	IM	0.22	0.0950	0.0898	0.0561	1.7437	3.3063	0.17	1.41	0.10	0.61	0.07
5	*4/*35	EM	0.031	0.0171	0.0123	0.0378	1.7918	0.7285	0.78	0.46	0.18	1.05	0.12
6	*2/*41	EM	0.0066	0.0044	0.0058	0.0105	0.1507	0.6122	3.12	3.78	0.12	3.95	0.08
7	*1/*4	EM	0.0065	0.0031	0.0054	0.0070	0.3915	1.2133	3.69	3.23	0.14	4.14	0.10
8	*1/*2xN	UM	0.0012	0.0006	0.0089	0.0027	0.0553	0.0458	4.25	3.55	0.14	3.15	0.07
9	*1/*6	EM	0.013	0.0081	0.0039	0.0039	0.8485	0.1502	3.90	3.24	0.06	2.73	0.22
10	*4/*4	PM	1.84	6.3962	4.5134	7.5278	6.3407	1.1127	5.81	2.64	0.07	4.14	0.08
Median			0.0098	0.0064	0.0056	0.0082	0.3943**	0.670**	3.404	2.993	0.107***	2.55	0.08**
IQ25			0.0029	0.0034	0.0040	0.0030	0.1501	0.205	1.498	2.615	0.063	0.719	0.073
IQ75			0.0274	0.0170	0.0115	0.0310	0.7874	1.185	3.894	3.475	0.145	3.070	0.115

[#]Using microdoses of dextromethorphan 2.5 mg.

Oxy, oxycodone with placebo; Pbo, placebo with placebo; Keto, oxycodone with ketoconazole; Quin, oxycodone with quinidine; Keto+Quin, oxycodone with ketoconazole and quinidine.

DEM/DOR, dextromethorphan/dextrorphan; 1-OH MID/MID, 1-hydroxy-midazolam/midazolam; n.d. not determined.

CYP2D6 phenotype according to DEM/DOR: PM > 0.3, 0.3 > IM > 0.03, 0.03 > EM > 0.003, 0.003 > UM.

Pairwise comparisons with Oxy: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Data analysis

Pharmacokinetic analysis. The maximum concentration (C_{max}), time to C_{max} (T_{max}), terminal elimination half-life ($T_{1/2z}$), area under the concentration curve (AUC) extrapolated to infinity AUC_{∞} (using linear trapezoidal method), AUC/dose and partial areas (AUC_{90} , AUC_{360} , AUC_{1440}) of oxycodone, oxymorphone, noroxycodone and noroxymorphone were estimated from their plasma concentration/time values, by use of non-compartmental methods (WinNonLin 4.01, Pharsight, Mountainview, California).

The software package SPSS 15 for Windows (Chicago, Illinois) was used for the statistical analysis. The normality of the data was assessed by a descriptive analysis and data were normalized by log transformation to allow the use of parametric tests if necessary. Mean values and standard deviations (SD) were used to summarize continuous variables. Multiple repeated analyses of variance (ANOVA) were used to test treatment differences depending on genotype whereas one-way ANOVA was used to test the impact of genotype on oxycodone treatment. Spearman correlation coefficients were used for the correlations between the pharmacokinetics and CYP2D6/3A activities. Two-tailed tests with a significance level of 5% were used in all statistical analyses.

Results

Descriptive population data

Ten healthy male volunteers were enrolled and completed the study (mean age 26 years) with a weight of 76.4 ± 8.1 kg and height of 181.2 ± 5.4 cm. The mean oral oxycodone dose was 15.3 mg (range 13–18 mg).

CYP2D6 phenotyping/genotyping. No difference was observed between the dextromethorphan/dextrorphan metabolic ratio

after 25 mg of dextromethorphan and after 2.5 mg of dextromethorphan during the placebo study session. Consistent correlations between genotype and phenotype were observed after conventional doses (25 mg) and microdoses (2.5 mg) of dextromethorphan (Table 1). One subject (#10) was genotyped and phenotyped as a CYP2D6 poor metabolizer (PM). He was homozygous for the deficient allele CYP2D6*4 and had a dextromethorphan/dextrorphan metabolic ratio above 0.3. One heterozygous CYP2D6*5/*41 subject (#4) had a dextromethorphan/dextrorphan of 0.22 and should be considered as IM. Nevertheless, we considered these two individuals as closely related deficient CYP2D6 metabolizers and called them PM for simplification. Two healthy volunteers (#2 and #8) carried gene duplications (CYP2D6*2xN, *41xN) and were phenotyped as UM (dextromethorphan/dextrorphan < 0.003). All other individuals were considered as EM, including #3, who was predicted EM from his genotype (CYP2D6*1/*41), but had slightly increased CYP2D6 activity in pre-study phenotyping.

CYP3A phenotyping. Mean 1-OH-midazolam/midazolam ratio (MR) was 2.727; SD 1.052. The administration of oxycodone alone did not statistically modify CYP3A phenotypes (MR 2.947; SD 1.771). Two volunteers (#4 and #5) had a repeated low CYP3A activity (Table 1).

Effect of drug blockage on CYP2D6 and CYP3A phenotypes

CYP2D6 phenotypes. The extent of phenocopying after oral quinidine varied according to CYP2D6 genotypes as demonstrated by a significant interaction between treatment and genotype in the analysis of variance (*P* = 0.0009).

As expected in PM for CYP2D6, no statistically significant differences were demonstrated within and between all treatment sessions. All but one EM (#6) were phenotyped as PM for CYP2D6 after quinidine (Table 1). The co-administration of

quinidine with ketoconazole had no further significant effect on dextromethorphan/dextrorphan MR values as compared with quinidine alone ($P = 0.567$) in EM. As expected, quinidine and quinidine+ketoconazole, significantly increased dextromethorphan/dextrorphan as compared with the other sessions, whereas no significant phenotypic differences were demonstrated between oxycodone and placebo (placebo) ($P = 0.1921$), and between oxycodone and ketoconazole ($P = 0.845$). In contrast, quinidine was unable to completely block CYP2D6 activity in UM who were phenotyped as EM and IM for CYP2D6 after quinidine (Table 1).

CYP3A4 phenotypes. After ketoconazole, CYP3A blockade was effective in all 10 volunteers, as demonstrated by the 1-OH-midazolam/midazolam MR values, all below 0.22 (Table 1). No differences between quinidine+ketoconazole and ketoconazole alone were demonstrated (0.104 vs. 0.112, $P = 0.374$). After both sessions, CYP3A activities were significantly lower than after oxycodone ($P = 0.0007$ and 0.004), quinidine ($P = 0.002$ and 0.017) and placebo ($P = 3.10^{-5}$ and 0.001), whereas no difference between oxycodone, quinidine and placebo were observed.

Comparisons of oxycodone and metabolite concentrations in plasma, without CYP inhibitors

The mean plasma concentration versus time curves of oxycodone and metabolites ($n = 10$) are presented in Figure 2A.

Mean oxycodone plasma concentrations were higher than noroxycodone for the first 10 h after dosing (20 h in UM) and mean oxycodone C_{max} was 2.5-fold higher than noroxycodone ($P < 0.0001$). Oxycodone AUC_{∞} was twice the size of the noroxycodone AUC_{∞} in UM (10123 vs. 5008 min·ng·mL⁻¹) but only slightly higher in EM and PM. Noroxycodone $T_{1/2}$ was twice that for oxycodone. Noroxymorphone and oxymorphone AUC_{∞} were eight and 38 times lower than oxycodone AUC_{∞} in UM, and six and 51 times lower in EM. In PM, oxymorphone and noroxymorphone plasma concentrations were extremely low. Oxymorphone AUC_{∞} was 97 times lower than oxycodone.

Impact of CYP2D6 genetic polymorphism on the pharmacokinetics of oxycodone and its metabolites

A moderate inter-individual variability was demonstrated in the pharmacokinetics of oxycodone and noroxycodone (coefficient of variation CV 25–35%) whereas the variability was considerably higher in the pharmacokinetics of the two CYP2D6-dependent metabolites oxymorphone and noroxymorphone (CV 35–105%). Oxycodone and metabolites exhibited CYP2D6 phenotypic-based differences in their pharmacokinetics (Table 2). The mean plasma concentration versus time curves of oxycodone and metabolites in CYP2D6 EM, UM and PM are presented in Figure 2B–E and the derived pharmacokinetic parameters in Table 2.

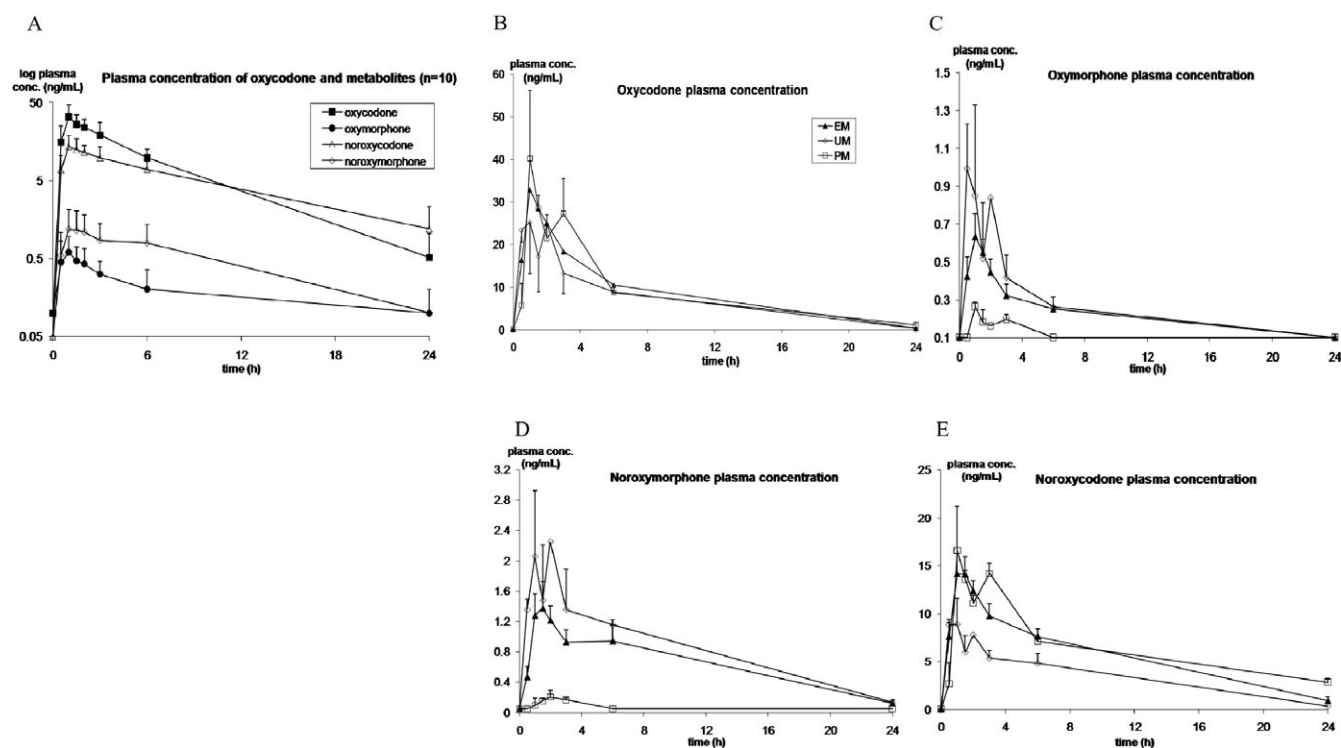


Figure 2 Mean (SD) plasma concentration-time profile of oxycodone and metabolites in 10 healthy volunteers and according to genotype in EM, UM and PM for CYP2D6, after a single $0.2 \text{ mg}\cdot\text{kg}^{-1}$ oral dose of immediate release oxycodone. The mean plasma concentration versus time curves of oxycodone and metabolites ($n = 10$) are presented in panel A (note the concentrations are shown on a log scale), where mean oxycodone plasma concentrations were higher than those of noroxycodone, in turn higher than noroxymorphone and oxymorphone. CYP2D6 phenotypic differences in the plasma concentrations of oxycodone, oxymorphone, noroxymorphone and noroxycodone are presented in panels B–E (concentrations on a linear scale). CYP, cytochrome P450; EM, extensive metabolizer; PM, poor metabolizer; UM, ultrarapid metabolizers.

Table 2 Mean (SD) estimates of pharmacokinetic parameters for oxycodone and its metabolites according to CYP2D6 genotype

	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ng·mL ⁻¹)	AUC_{90} (min·ng·mL ⁻¹)	AUC_{1440} (min·ng·mL ⁻¹)
Oxycodone	4.1 (0.8)	1.1 (0.3)	36 (11.2)	1882 (653)	12079 (3121)
UM	3.9 (0.5)	0.7 (0.3)*	29 (11.9)	1740 (823)	10033 (1952)
EM	3.8 (0.8)	1.2 (0.3)	37 (8.6)	1946 (647)	12486 (3169)
PM	5.2 (0.2)	1.2 (0.3)	41 (21.7)	1833 (1000)	12905 (4788)
Metabolites	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ng·mL ⁻¹)	AUC_{90} metabolite/parent drug	AUC_{1440} metabolite/parent drug
Oxymorphone	4.5 (2.1)	1.0 (0.3)	0.7 (0.3)	0.021	0.019
UM	4.4 (2.5)	0.7 (0.3)	1 (0.4)	0.036	0.031
EM	3.3 (1.7)	1.1 (0.4)	0.7 (0.3)	0.021	0.021
PM	6.8 (2.1)	1.0 (0.1)	0.3 (0.03)*	0.006**	0.003***
Noroxycodone	6.8 (2.8)	1.2 (0.4)	15(4.2)	0.435	0.642
UM	5.3 (0.3)	0.7 (0.3)*	11 (1.5)	0.361	0.484*
EM	5.0 (2.1)	1.4 (0.4)	16 (3.8)	0.456	0.653
PM	11.1(0.3)*	1.2 (0.3)	18 (4.7)	0.439	0.732
Noroxymorphone	12.3 (8.8)	1.7 (0.9)	1.4 (0.98)	0.038	0.066
UM	6.4 (0.8)	0.7 (0.3)*	2.2 (1)	0.073	0.117
EM	12.2 (9.5)	1.6 (0.8)	1.6 (0.8)	0.039	0.074
PM	-	2.6 (0.5)*	0.2 (0.1)**	0.003**	0.003***

Mean values (SD). UM, ultrarapid metabolizers; EM, extensive metabolizers; PM, deficient metabolizers for cytochrome P450 (CYP)2D6. CV, coefficient of variation; T_{max} , time to maximum concentration; C_{max} , maximum concentration; AUC_{90} , area under the plasma concentration curve (AUC) after 90 min; AUC_{1440} , area under the plasma concentration curve (AUC) after 1440 min; $T_{1/2}$, half-life. Comparisons with EM: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Oxycodone $T_{1/2}$ was prolonged (1.5-fold) in PM compared with EM and UM ($P = 0.042$ and $P = 0.17$ respectively) and oxycodone AUC_{∞} increased by 35% in PM compared with UM.

Oxymorphone exposure was markedly decreased in PM compared with EM and UM. Oxymorphone AUC_{90} was 83% lower in PM than UM ($P = 0.007$) and 73% lower than EM ($P = 0.013$). Oxymorphone C_{max} was 75% lower in PM than UM ($P = 0.007$) and 63% lower than EM ($P = 0.015$).

Noroxymorphone plasma concentrations were extremely low in PM. C_{max} decreased by 85% compared with EM ($P = 0.003$) and 90% to UM ($P = 0.004$), whereas noroxymorphone AUC_{1440} decrease was 25- and 30-fold respectively ($P = 1.3 \cdot 10^{-5}$ and $P = 2.8 \cdot 10^{-5}$). The opposite was true in UM where C_{max} increased by 39% and T_{max} decreased by 55% versus EM ($P = 0.058$) and PM ($P = 0.015$). Noroxymorphone $T_{1/2}$ was reduced by 50% in UM compared with EM.

A reduction in noroxycodone exposure was observed in UM compared with EM and PM. Indeed noroxycodone C_{max} was reduced by 36% ($P = 0.07$) and AUC_{∞} by 43% in comparison with EM ($P = 0.032$). Conversely, noroxycodone AUC_{∞} was more than twice as high in PM compared with UM ($P = 0.012$). However, no significant differences were observed between EM and PM in oxycodone pharmacokinetics.

The phenotypic differences in the pharmacokinetics of oxycodone and metabolites were confirmed by Spearman's rank correlations (Table 3). Strong significant relationships were demonstrated between CYP2D6 activities and the AUCs and C_{max} of CYP2D6-dependent metabolites, oxymorphone and noroxymorphone ($0.71 < \text{Spearman's correlation coefficient } \rho_s < 0.83$, $P < 0.05$) whereas no correlation between CYP3A activity and the pharmacokinetic parameters of oxymorphone and noroxymorphone were demonstrated. Strong negative correlations between noroxycodone AUC_{∞} , C_{max} and $T_{1/2}$, and CYP2D6 activities were also demonstrated ($P < 0.05$).

Effect of CYP2D6 and CYP3A4 blockade on the pharmacokinetics of oxycodone and metabolites

The effects of quinidine and/or ketoconazole pretreatment on the plasma concentrations of oxycodone and metabolites are described in Figure 3A–D and individual pharmacokinetic parameters are presented in Table 4.

Effect of quinidine. Overall, quinidine dramatically decreased oxymorphone C_{max} and AUC_{360} by 40% ($P = 0.01$ and 0.044) as compared with oxycodone alone. This effect was even more pronounced (80% decrease) on noroxymorphone C_{max} and AUC_{360} ($P = 0.0009$ and 0.0006). However, the blockade was not complete as oxymorphone and noroxymorphone were still detectable in the samples. A 1.5- and 8.5-fold increase in oxycodone AUC_{∞} ($P = 0.0004$) and AUC_{90} ($P = 5.10^{-8}$) were, respectively, observed after quinidine, and a 20–30% decrease in the clearance CL/F ($P = 0.0003$) and volume of distribution V_z/F ($P = 0.009$). An apparent compensatory 70% increase in noroxycodone AUC_{∞} was observed ($P = 0.0004$).

Differences among CYP2D6 genotypic groups were observed. The increase in noroxycodone exposure was more pronounced in UM than EM, and, in turn, in EM than in PM.

Effect of ketoconazole. After ketoconazole, both noroxycodone and noroxymorphone C_{max} decreased by 80% ($P = 10^{-7}$ and 0.0005), and their exposure was also reduced ($P = 0.007$ and 0.017). Significant differences between quinidine and ketoconazole for all the assessed pharmacokinetic parameters of noroxycodone were demonstrated, but no significant difference between quinidine and ketoconazole were found for the pharmacokinetic parameters of noroxymorphone.

A shunt to the CYP2D6 pathway was clearly observed after ketoconazole, although a 3.5-fold increase in oxymorphone AUC_{∞} ($P = 0.0004$); indeed, 1.5-fold increase in C_{max} and

Table 3 Spearman rank correlation (ρ_s) between CYP2D6 and CYP3A phenotypes* and the pharmacokinetic parameters of oxycodone and its metabolites according to pretreatment with placebo, ketoconazole or/and quinidine

PK parameters	Oxy						Keto						Quin						Keto+Quin					
	CYP2D6			CYP3A			CYP2D6			CYP3A			CYP2D6			CYP3A			CYP2D6			CYP3A		
	ρ_s	P-value	ρ_s	P-value	ρ_s	P-value	ρ_s	P-value	ρ_s	P-value	ρ_s	P-value	ρ_s	P-value	ρ_s	P-value	ρ_s	P-value	ρ_s	P-value	ρ_s	P-value		
Oxycodone	AUC ₉₀	0.079	0.829	0.297	0.405	-0.188	0.603	0.467	0.174	-0.430	0.215	-0.273	0.446	0.285	0.425	-0.031	0.933							
	C _{max}	-0.309	0.385	0.382	0.276	-0.818	0.004	0.115	0.751	0.176	0.627	-0.176	0.627	0.273	0.446	-0.720	0.019							
Noroxycodone	AUC ₉₀	-0.152	0.676	0.309	0.385	-0.333	0.347	0.042	0.907	-0.636	0.048	-0.115	0.751	-0.164	0.652	-0.579	0.080							
	C _{max}	-0.648	0.043	0.467	0.174	-0.943	0.005	0.543	0.266	-0.224	0.533	-0.139	0.701	0.536	0.215	0.432	0.333							
Oxymorphone	AUC ₉₀	0.782	0.008	-0.321	0.365	0.758	0.011	-0.176	0.627	0.030	0.934	-0.103	0.777	0.237	0.509	-0.429	0.216							
	C _{max}	0.830	0.003	-0.333	0.347	-0.583	0.099	-0.233	0.546	0.406	0.244	-0.503	0.138	-0.595	0.119	-0.293	0.482							
Noroxymorphone	AUC ₉₀	0.758	0.011	-0.273	0.446	0.733	0.016	-0.515	0.128	0.248	0.489	-0.588	0.074	-0.325	0.359	-0.724	0.018							
	C _{max}	0.806	0.005	-0.309	0.385	-0.829	0.042	0.143	0.787	0.576	0.081	-0.770	0.009	-0.252	0.482	-0.713	0.021							

C_{max}, maximum concentration; AUC_{∞/D}, area under the plasma concentration curve (AUC) extrapolated to infinity under dose; T_{1/2}, half-life. Sessions: Oxy, oxycodone+placebo; Keto, oxycodone+ketoconazole; Quin, oxycodone+quinidine; Keto+Quin, oxycodone+ketoconazole+quinidine. Statistically significant results are shown in bold. *CYP2D6 and CYP3A phenotypes, respectively, measured by dextromethorphan/dextropropranolol and 1-OH-midazolam/midazolam metabolic ratios.

fourfold longer T_{1/2} (not significant NS) were observed. Statistically significant differences were demonstrated between ketoconazole and quinidine in all the assessed pharmacokinetic parameters of oxymorphone.

An increased AUC_∞ for oxycodone (1.8-fold; $P = 0.004$) was observed after ketoconazole which was slightly higher than what was observed after quinidine (NS), but the magnitude of CL/F and Vz/F decreases were same as oxycodone alone ($P = 0.003$ and 0.075). No significant differences in the pharmacokinetics of oxycodone after quinidine and ketoconazole were demonstrated except for the AUC₉₀ ($P = 2.10^{-8}$) and T_{1/2} (4.3 vs. 5.5 h, $P = 0.009$).

Effect of ketoconazole+quinidine co-administration. Quinidine and ketoconazole co-administration had a cumulative effect on oxycodone exposure with a threefold higher AUC_∞ ($P = 2.10^{-10}$) and a 1.5-fold higher C_{max} ($P = 0.002$) and T_{1/2} ($P = 8.10^{-6}$) as compared with oxycodone alone, whereas oxycodone CL/F was reduced by 70% ($P = 7.10^{-7}$). In all assessed oxycodone pharmacokinetic parameters, significant differences were demonstrated between ketoconazole+quinidine and both quinidine and ketoconazole alone. Oxycodone C_{max} and AUC₃₆₀ were 1.7-fold higher after ketoconazole+quinidine than after quinidine ($P = 0.001$ and 2.10^{-5}) and one- to fourfold higher than after ketoconazole ($P = 0.001$ and 0.004).

Ketoconazole+quinidine reduced oxymorphone and noroxycodone C_{max} by 40% ($P = 0.009$) and 60% ($P = 10^{-6}$), respectively, as compared with oxycodone alone. Noroxycodone AUC₃₆₀ was also reduced by 60% ($P = 0.0003$), whereas no significant reduction in oxymorphone AUC_s were noticed versus oxycodone alone ($P > 0.05$). Ketoconazole+quinidine was less effective in reducing noroxycodone exposure than ketoconazole alone. Indeed noroxycodone C_{max} was 1.7-fold lower after ketoconazole than ketoconazole+quinidine ($P = 0.004$) and noroxycodone AUC_∞ was more than threefold lower ($P = 0.016$).

The addition of ketoconazole to quinidine had no significant effect on most pharmacokinetic parameters of oxymorphone, compared with those after quinidine alone, whereas it significantly reduced oxymorphone C_{max} and AUCs, compared with the effects of ketoconazole alone. As expected, the combination dramatically reduced noroxymorphone plasma concentrations to low to undetectable levels ($P < 0.0001$). Most of the assessed pharmacokinetic parameters of noroxymorphone were significantly lower after quinidine+ketoconazole than after ketoconazole or quinidine alone.

Effect of CYP2D6 and CYP3A4 blockade on the pharmacokinetics of oxycodone and metabolites

Spearman rank correlations. Spearman rank correlation coefficients (ρ_s) between CYP2D6 and CYP3A activities and the pharmacokinetic parameters of oxycodone and metabolites during the different sessions (oxycodone, oxycodone+ketoconazole, oxycodone+quinidine and oxycodone+ketoconazole+quinidine) are presented in Table 3.

After ketoconazole, most pharmacokinetic parameters of oxymorphone and noroxymorphone were highly correlated with CYP2D6 activity, compared with oxycodone alone

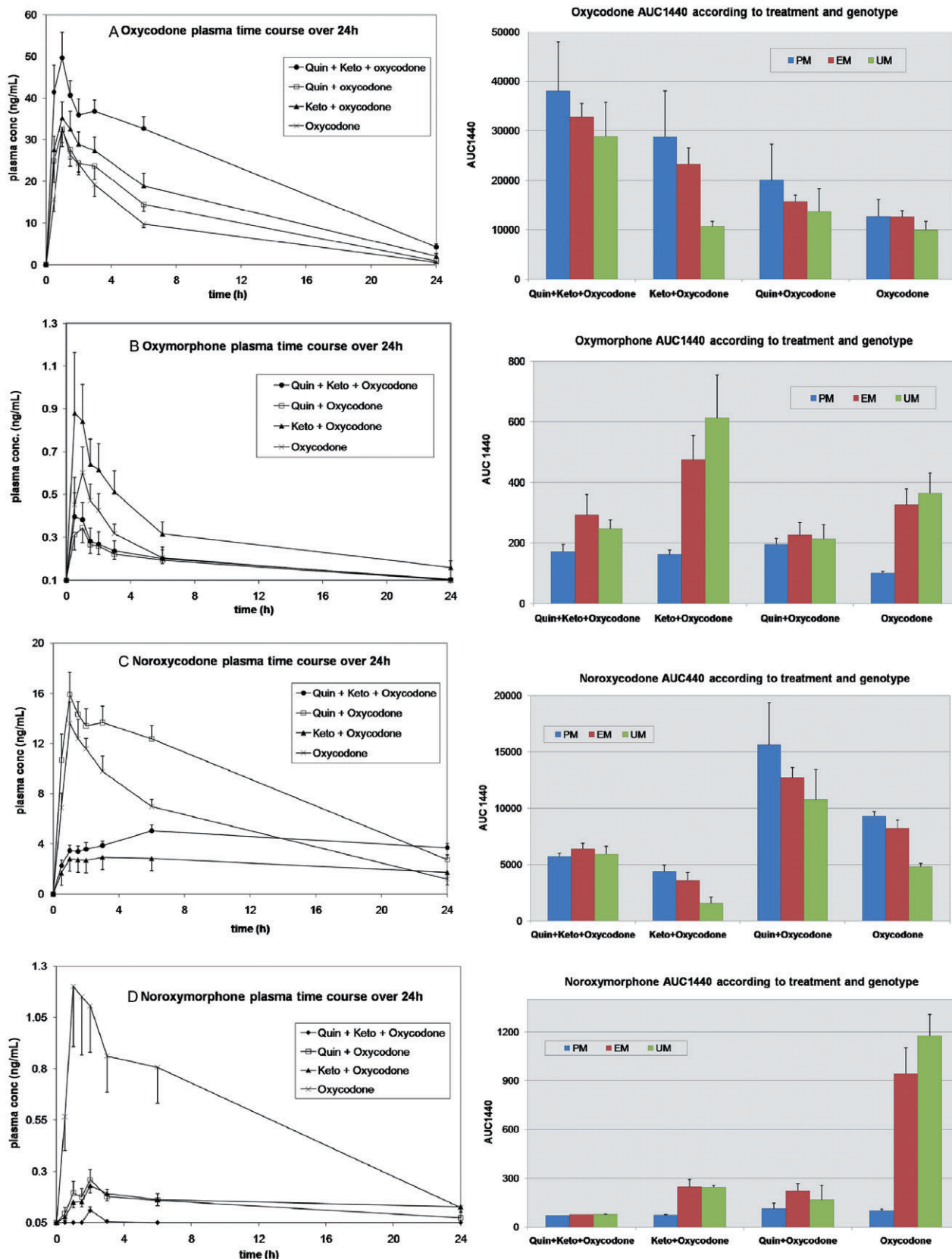


Figure 3 Mean (SEM) plasma concentration-time profile and AUC_{1,440} (SEM) of oxycodone and metabolites in 10 healthy volunteers after a single 0.2 mg·kg⁻¹ oral dose of immediate release oxycodone±quinidine±ketoconazole, according to CYP2D6 genotype. The effect of CYP2D6 and/or CYP3A inhibition by quinidine (Quin) or ketoconazole (K or Keto), as well as CYP2D6 genotype, on the plasma concentration-time profile and AUC_{1,440} of oxycodone, oxymorphone, noroxymorphone and noroxycodone are presented. AUC, area under the concentration curve; CYP, cytochrome P450.

Table 4 Mean (SD) pharmacokinetic parameters of oxycodone and its metabolites after inhibition of CYP2D6 (with quinidine) and CYP3A (with ketoconazole) in healthy male volunteers

	Session	$T_{1/2}$ (h)	T_{max} (min)	C_{max} (ng·mL ⁻¹)	AUC_{∞} (min·ng·mL ⁻¹)	AUC_{90} (min·ng·mL ⁻¹)
Oxycodone	Quin	4.3 (0.5)	82 (54)	36.4 (7.2)	16 461 (5324)***	16 126 (5102)***
	Keto	5.5 (1.3)**	86 (57)	39.2 (12.4)	21 391 (9410)**	2 383 (799)
	Keto+Quin	6.5 (1.4)**	62 (47)	56.9 (17.6)**	35 998 (9915)***	3 442 (1014)***
	Oxy	4.1 (0.8)	66 (19)	36 (11.2)	11 633 (3102)	1 882 (653)
Oxymorphone	Quin	6.6 (3.3)	91 (62)	0.4 (0.2)**	196 (59)	22 (16)*
	Keto	20.8 (24)**	46 (17)	1.0 (0.8)	755 (380)**	60 (47)*
	Keto+Quin	12.8 (12.7)*	37 (15)	0.4 (0.3)**	347 (201)	34 (18)
	Oxy	4.5 (2.1)	58 (20)	0.7 (0.3)	218 (113)	39 (25)
Noroxycodone	Quin	8.6 (1.4)	94 (59)	18 (4.4)	15 028 (4274)**	1 024 (356)
	Keto	19.9 (16.5)*	309 (412)**	3.4 (1.1)**	7 510 (9236)	179 (80)**
	Keto+Quin	49.7 (54.6)*	681 (562)**	5.4 (1.1)**	24 208 (25 329)*	232 (96)**
	Oxy	6.8 (2.8)	73 (25)	15 (4.2)	8 726 (30)	819 (252)
Noroxymorphone	Quin	10.4 (4.4)*	134 (85)*	0.3 (0.2)***	320 (187)*	12 (12)**
	Keto	43.3 (33.1)	121 (29)	0.2 (0.1)***	966 (629)***	8.4 (6.2)**
	Keto+Quin	–	130 (27)*	0.1 (0.1)***	–***	0.03 (0.01)***
	Oxy	12.3 (8.8)	99 (53)	1.4 (1)	1681 (1765)	72 (55)

Sessions: Oxy, oxycodone+placebo; Keto, oxycodone+ketoconazole; Quin, oxycodone+quinidine; Keto+Quin, oxycodone+ketoconazole+quinidine. $T_{1/2}$, half-life; T_{max} , time to maximum concentration; C_{max} , maximum concentration; AUC_{∞} , area under the plasma concentration curve (AUC) extrapolated to infinity; AUC_{90} , AUC for 90 min after dosing.

Comparisons with Oxy: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

($\rho_s < 0.75$), whereas it was only the case for oxycodone C_{max} ($\rho_s = 0.818$, $P = 0.004$) and both noroxycodone C_{max} ($\rho_s = 0.943$, $P = 0.005$) and T_{max} ($\rho_s = 0.673$, $P = 0.033$). Oxycodone $T_{1/2}$ was correlated with CYP3A activity ($\rho_s = 0.697$, $P = 0.025$) and a trend for oxycodone AUC_{∞} ($\rho_s = 0.612$, $P = 0.06$) was still observed.

After quinidine, the metabolites noroxymorphone and oxymorphone were no longer clearly correlated with CYP2D6 activity. Interestingly some pharmacokinetic parameters were now correlated with CYP3A activity such as noroxymorphone AUC_{360} ($\rho_s = -0.7818$, $P = 0.008$) and C_{max} ($\rho_s = -0.7697$, $P = 0.009$), as well as oxymorphone T_{max} ($\rho_s = 0.6606$, $P = 0.038$) and a trend for oxymorphone AUC_{360} ($\rho_s = -0.5879$, $P = 0.07$). Oxycodone and noroxycodone T_{max} were in turn strongly inversely correlated with CYP2D6 activity ($\rho_s = -0.903$, $P = 0.0003$, and $\rho_s = -0.8545$, $P = 0.002$), as well as noroxycodone AUC_{90} ($\rho_s = -0.6364$, $P = 0.048$).

After the combination of quinidine and ketoconazole, no correlations with CYP2D6 phenotype were demonstrated.

Discussion

This randomized double-blind placebo-controlled study assesses the effects of CYP2D6 and CYP3A activity on the pharmacokinetics of oxycodone and metabolites. We demonstrated a clear effect of drug–drug interaction (CYP2D6 or CYP3A blockade) mimicking deficient metabolism on the pharmacokinetics of oxycodone and the influence of CYP2D6 genetic polymorphism on the magnitude of this drug–drug interaction.

Effects of CYP2D6 genetic polymorphism on the pharmacokinetics of oxycodone and metabolites

Our study offers new insight in the pharmacokinetics of oxycodone and metabolites in previously genotyped and/or phe-

notyped subjects for CYP2D6. Differences in the time course of the plasma concentrations of oxycodone metabolites depending on CYP2D6 genotype were clearly observed. The limit of our analyses was that the sample size of the study was small. Two UM and 2 PM were included among 10 healthy volunteers. However, our data strongly suggested that CYP2D6 genotype affects oxycodone pharmacokinetics.

In CYP2D6 deficient metabolizers (PM), circulating oxymorphone was markedly lower than in EM and UM (62% and 75% C_{max} respective decrease, and 85% AUC_{1440} reduction). The reduction of noroxymorphone exposure was even higher (85–90% C_{max} decrease, and 24 and 32 times lower AUC_{1440} than EM and UM respectively). However, oxymorphone plasma concentrations were still measurable in PM. Other metabolic pathways may therefore be involved in the formation of oxymorphone when CYP2D6 is absent or non functional. In lymphoblast microsomes, it was demonstrated that aside from CYP2D6, both CYP2C19 and CYP1A1 (Lalovic *et al.*, 2004) may be involved in the formation of oxymorphone from oxycodone, although their contribution appears minor, at least at their constitutive level of expression.

The pharmacokinetics of oxycodone and metabolites was assessed in CYP2D6 UM. Oxycodone pharmacokinetic parameters were reduced as compared with EM (44% and 20% lower T_{max} and C_{max}), and oxycodone AUC_{∞} was 33% lower than PM. Inversely, higher plasma levels and exposure of both oxymorphone and noroxymorphone were observed whereas noroxycodone exposure and $T_{1/2}$ were halved in UM compared with both EM and PM.

A strong relationship between the CYP2D6 dependent metabolites, oxymorphone and noroxymorphone, and CYP2D6 activity was demonstrated. The latter accounts probably for the higher interindividual variability in the pharmacokinetics of CYP2D6 dependent metabolites, oxycodone and noroxymorphone (CV 35–105%), than oxycodone and noroxycodone (CV 25–35%). Noroxycodone pharmacokinetics was also inversely correlated with CYP2D6 activity, but not

with CYP3A phenotype. Indeed, when CYP2D6 was deficient, a shunt to metabolism via CYP3A was observed, and when CYP2D6 was hyperactive, less noroxycodone was produced. The shift to CYP3A pathway in CYP2D6 PM is consistent with an earlier study (Heiskanen *et al.*, 1998). The pharmacokinetic parameters of oxycodone in EM were also concordant with previous studies conducted in human healthy volunteers (Kaiko *et al.*, 1996; Mandema *et al.*, 1996; Heiskanen *et al.*, 1998; Lalovic *et al.*, 2006). Notable differences were however, demonstrated in some of the pharmacokinetic parameters of the metabolites as compared with a previous report (Lalovic *et al.*, 2006). Some factors may account for these differences. CYP2D6 genotyping/phenotyping was not performed in the previous report (Lalovic *et al.*, 2006) and we clearly demonstrate here that the pharmacokinetics of oxycodone metabolites varies with CYP2D6 phenotype. Also, our volunteers did not receive a fixed dose of oxycodone (15 mg) but a weight adjusted dose ($0.2 \text{ mg}\cdot\text{kg}^{-1}$). Finally, the administration of naloxone 90 min after oxycodone possibly modified the shape of pharmacokinetic curves with a possible enterohepatic recirculation.

Effects of inhibition of CYP2D6 and CYP3A4 metabolic pathways

We demonstrated that CYP2D6 blockade by quinidine had a considerable effect on the pharmacokinetic parameters of oxymorphone and noroxymorphone, reduced overall by 40% and 80% respectively. As plasma levels were however, still detectable, blockade by oral quinidine (100 mg) 2 h before oxycodone was not complete (Table 1). Other authors have used 200 mg of quinidine 3 h before 20 mg CR oxycodone and 100 mg after 6 h (Heiskanen *et al.*, 1998). With this regimen no oxymorphone was detected at any time point in 8/10 subjects, and the two remaining subjects had levels between 0.2 and $0.24 \text{ ng}\cdot\text{mL}^{-1}$. Other enzymatic pathways may also be involved in the formation of oxymorphone as described above in the case of our genotypic PM.

We assessed the effect of CYP3A inhibition by ketoconazole on the pharmacokinetic parameters of oxycodone and metabolites. After 400 mg of ketoconazole, noroxycodone and noroxymorphone exposures dramatically decreased by 80%. Again, we demonstrated that the blockade of one pathway led to a compensatory increase of the remaining pathway. Indeed, after quinidine, noroxycodone exposure rose by 70%, and after ketoconazole, oxymorphone exposure was 3.5-fold higher than after oxycodone alone. Furthermore both quinidine and ketoconazole increased the exposure to oxycodone (1.4- to 1.8-fold). The combination of ketoconazole and quinidine had a cumulative effect on the pharmacokinetics of oxycodone (threefold higher AUC_{∞} and 70% reduced CL/F). It was previously demonstrated that quinidine led to a compensatory 1.9-fold increase of noroxycodone AUC_{24} and 1.5-fold increase of noroxycodone C_{max} , as well as a non-significant 1.15-fold increase of oxycodone AUC_{24} (Heiskanen *et al.*, 1998).

Pharmacodynamic consequences of the pharmacokinetic effects of CYP2D6 and CYP3A modulation of oxycodone and its metabolites

The O-demethylated metabolites of oxycodone (oxymorphone and noroxymorphone) have higher potencies and

affinities for the μ opioid receptor (MOR) than do oxycodone and noroxycodone. Indeed the structure of oxycodone and oxymorphone bear the same chemical relation as codeine and its O-demethylated active metabolite, morphine (Chen *et al.*, 1991; Poyhia *et al.*, 1993). As a result of CYP2D6 O-demethylation, the hydroxyl group at the 3 position confers an increased analgesic potency to morphine and oxymorphone compared with their respective 3-methoxy congeners codeine and oxycodone (Cleary *et al.*, 1994). Oxymorphone has indeed a 40- and threefold higher affinity for the MOR than oxycodone and morphine, respectively, whereas noroxymorphone affinity for MOR is three- and 10-fold higher than oxycodone and noroxycodone respectively. Oxymorphone is moreover 14 times more potent than oxycodone (Chen *et al.*, 1991). It is also now recognized that codeine and tramadol O-demethylated metabolites are responsible for their analgesic action (Dayer *et al.*, 1988) The same variability would be expected with other opioids dependent on CYP pathways for their activation (Samer *et al.*, 2006). Several pieces of indirect evidence support a role for pharmacologically active metabolites in the anti-nociceptive effect of oxycodone in both rats (Lemberg *et al.*, 2006) and humans (Backlund *et al.*, 1997) and a few case reports have pointed out the importance of CYP2D6 phenotype in the pharmacology of oxycodone as a result of drug interactions or genetic polymorphisms. We have shown here that CYP2D6 activity modulated by genetic polymorphism or drug-drug interactions was highly correlated with oxymorphone and noroxymorphone AUC and C_{max} and a shunt to CYP2D6 pathway was observed after CYP3A4 inhibition. We have evidence (unpublished experiments; Samer *et al.*, 2010) that these pharmacokinetic data translate into pharmacodynamic effects of oxycodone (anti-nociceptive effect, psychomotor effect, pupil size, toxicity), where oxymorphone appears indeed to be the active metabolite responsible for the pharmacodynamic effects of oxycodone.

Micro-cocktail for CYP3A4 and CYP2D6 phenotyping and CYP2D6 genotyping

Our study demonstrated that low oral doses of midazolam (75 μg) and dextromethorphan (2.5 mg) can be used as a micro-cocktail to simultaneously assess CYP3A and CYP2D6 activities. The volunteers were both genotyped (AmpliChip) and phenotyped for CYP2D6 before the start of the study. Excellent concordance between phenotypes determined with microdoses of dextromethorphan and genotype-based predictions were obtained. Full correlations between CYP2D6 phenotypes with conventional and microdoses of dextromethorphan were also observed. Microdoses of dextromethorphan are therefore an accurate and safer alternative to assess CYP2D6 phenotypes, especially in CYP2D6 PM subjects. No differences in CYP2D6 and CYP3A phenotypes were demonstrated between oxycodone and placebo sessions, confirming that oxycodone has no influence on CYP2D6 and CYP3A activities. Two UM carried gene duplications. The well-described CYP2D6*2xN duplication is associated with the phenotype of an UM and our volunteer had a dextromethorphan/dextrorphan ratio <0.003 . Another volunteer (#2) carried a CYP2D6*41xN (multi-) duplication. The

predicted phenotype for a *41/*41xN individual carrying a real gene duplication ($n = 2$) is IM for CYP2D6 and was correlated with an IM measured phenotype in a previous report (Rebsamen *et al.*, 2009). However, the microarray-based technology cannot discriminate between real duplication and potential multi-duplication of CYP2D6*41 ($n > 2$ copies). Gaedigk *et al.* (2007) have questioned whether the lack of discrimination or positive identification of CYP2D6*17xN, *29xN and *41xN had any effect on accurate phenotype prediction, expecting that duplicated genes of reduced function with a total gene count superior or equal to 3 confer a phenotype at the fast end of the activity distribution. In our study this volunteer was phenotyped as an UM during three sessions with dextromethorphan/dextrorphan ratio <0.003 and his oxycodone pharmacokinetics were similar to the other genotyped UM, suggesting that he may carry more than three copies of the *41 allele. A similar discrepancy between measured and predicted phenotype was reported in a previous study where a *1/*41xN genotype resulted in an UM measured phenotype (Rebsamen *et al.*, 2009).

Microdoses of midazolam were used as previously described (Eap *et al.*, 2004). In our study, 1-OH-midazolam/midazolam ratios were overall lower than those observed by Eap *et al.* After oxycodone, mean 1-OH-midazolam/midazolam ratio was 2.947 (SD 1.771, interval 0.17–5.81) with no difference between oxycodone and placebo. Therefore oxycodone does not modify CYP3A phenotype. One volunteer had low 1-OH-midazolam/midazolam ratios during all the study sessions suggesting he could carry a CYP3A deficient allele. CYP3A blockade by a single oral dose of 400 mg ketoconazole was effective in all volunteers as demonstrated by the low 1-OH-midazolam/midazolam ratio (mean 0.79, SD 0.39) similar to the effect of ketoconazole 400 mg·day⁻¹ for 2 days used in the study of Eap *et al.* (2004) (mean 0.112, SD 0.054).

In conclusion, the complex pharmacology of oxycodone has been incompletely understood and studied. We assessed the effect of both CYP2D6 genetic polymorphism and CYP2D6 and CYP3A drug–drug interactions on the pharmacokinetics of oxycodone. We demonstrated that drug–drug interactions via CYP2D6 and CYP3A had an important effect on the pharmacokinetics of oxycodone that differed significantly depending on CYP2D6 genotype. The results of our exploratory randomized controlled study have to be confirmed in a larger sized prospective study.

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Conflicts of interest

None.

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