RESEARCH PAPER

Genetic polymorphisms and drug interactions modulating CYP2D6 and CYP3A activities have a major effect on oxycodone analgesic efficacy and safety

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Background and purpose: The major drug-metabolizing enzymes for the oxidation of oxycodone are CYP2D6 and CYP3A. A high interindividual variability in the activity of these enzymes because of genetic polymorphisms and/or drug–drug interactions is well established. The possible role of an active metabolite in the pharmacodynamics of oxycodone has been questioned and the importance of CYP3A-mediated effects on the pharmacokinetics and pharmacodynamics of oxycodone has been poorly explored.

Experimental approach: We conducted a randomized crossover (five arms) double-blind placebo-controlled study in 10 healthy volunteers genotyped for CYP2D6. Oral oxycodone (0.2 mg·kg^{-1}) was given alone or after inhibition of CYP2D6 (with quinidine) and/or of CYP3A (with ketoconazole). Experimental pain (cold pressor test, electrical stimulation, thermode), pupil size, psychomotor effects and toxicity were assessed.

Key results: CYP2D6 activity was correlated with oxycodone experimental pain assessment. CYP2D6 ultra-rapid metabolizers experienced increased pharmacodynamic effects, whereas cold pressor test and pupil size were unchanged in CYP2D6 poor metabolizers, relative to extensive metabolizers. CYP2D6 blockade reduced subjective pain threshold (SPT) for oxycodone by 30% and the response was similar to placebo. CYP3A4 blockade had a major effect on all pharmacodynamic assessments and SPT increased by 15%. Oxymorphone C_{max} was correlated with SPT assessment ($\rho_S = 0.7$) and the only independent positive predictor of SPT. Side-effects were observed after CYP3A4 blockade and/or in CYP2D6 ultra-rapid metabolizers.

Conclusions and implications: The modulation of CYP2D6 and CYP3A activities had clear effects on oxycodone pharmacodynamics and these effects were dependent on CYP2D6 genetic polymorphism.
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Introduction

The potent semi-synthetic μ-opioid agonist oxycodone is one of the leading opioids in the USA used in acute and/or intense pain settings as well as chronic cancer pain (Coluzzi and Mattia, 2005). Oxycodone abuse has been of growing concern and associated with a significant number of drug-related deaths (Davis *et al.*, 2003). The long-standing clinical use of oxycodone (Falk, 1917), and lack of sufficient analytical sensitivity to detect active metabolites explain it has bypassed the conventional pharmacological studies assessing its pharmacokinetics, pharmacodynamics and, more specifically, its mechanism of analgesic action.

Cytochrome P450 (CYP) 2D6 and CYP3A are two major drug-metabolizing enzymes responsible for the oxidation of oxycodone. A high interindividual variability in the activity of these enzymes, because of genetic polymorphisms and/or drug–drug interactions, is well established. The possible role of an active metabolite in the pharmacodynamics of oxycodone has been questioned (Lalovic *et al.*, 2006) and the importance of CYP3A-mediated effects on the pharmacokinetics and pharmacodynamics of oxycodone have been poorly explored.

CYP2D6 catalyses the O-demethylation of oxycodone to oxymorphone which accounts for 10% of oxycodone metabolites and CYP3A catalyses the N-demethylation of oxycodone to the major (80%) circulating metabolite, noroxycodone (Cone *et al.*, 1983). The additional O-demethylation of noroxycodone to noroxymorphone is also catalysed by CYP2D6 (Lalovic *et al.*, 2004). These metabolites have varying potencies and affinities for the μ-opioid receptor. The structures of oxycodone and oxymorphone have the same chemical relationship as codeine and its O-demethylated active metabolite, morphine (Chen *et al.*, 1991). 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). Indeed, oxymorphone is 14-fold more potent than oxycodone and its affinity for the μ-opioid receptor is, respectively, 40- and 3-fold higher than oxycodone and morphine (Childers *et al.*, 1979). The μ-opioid receptor affinity of noroxymorphone is 3- and 10-fold higher than that of oxycodone and noroxycodone respectively (Lalovic *et al.*, 2006).

It is now recognized that the O-demethylated metabolites of codeine and tramadol are responsible for their analgesic action. Codeine is an ineffective analgesic in CYP2D6 poor metabolizers (PM) because of deficient O-demethylation of codeine to morphine (Desmeules and Dayer, 1991) whereas the genotype of ultrarapid metabolizers (UM) has been associated with higher morphine exposure and serious safety concerns (Gasche *et al.*, 2004). The same variability would be expected with other opioids dependent on CYP pathways for their activation (Samer *et al.*, 2006; 2005). Several pieces of indirect evidence point to the role of pharmacologically active metabolites in the anti-nociceptive effect of oxycodone in rats (Lemberg *et al.*, 2006a) and humans (Backlund *et al.*, 1997). A few case reports have pointed out the importance of CYP2D6 phenotype in the pharmacology of oxycodone as a result of drug interactions (Otton *et al.*, 1993) or genetic

polymorphisms (De Leon *et al.*, 2003; Susce *et al.*, 2006). Pharmacokinetic–pharmacodynamic studies conducted thus far have not explored the role of an active metabolite in the analgesic properties of oxycodone (Kaiko *et al.*, 1996; Heiskanen *et al.*, 1998; Lalovic *et al.*, 2006) and the correlations between the pharmacokinetics of oxycodone and its metabolites (oxymorphone, noroxymorphone) and non-pain pharmacodynamic parameters have been inconsistent.

There is little published on the importance of the CYP3A pathway in oxycodone pharmacokinetics and pharmacodynamics and therefore this needs to be further investigated. Co-administration of the CYP3A inducer rifampin has been associated with negative urine oxycodone screening (Lee *et al.*, 2006), as well as with a dramatic reduction in oxycodone exposure and pharmacodynamic effects in healthy volunteers (Nieminen *et al.*, 2009). Inversely voriconazole increased oxycodone exposure despite modest pharmacodynamic changes (Hagelberg *et al.*, 2009). In the case of codeine, after 3 weeks of rifampin, codeine N-demethylation was enhanced and codeine's pupil, respiratory and psychomotor effects were significantly reduced (Caraco *et al.*, 1997). Conversely, CYP3A inhibition in the context of CYP2D6 genetic polymorphism has been associated with serious toxicities even after small codeine doses (Gasche *et al.*, 2004) and it has been suggested that CYP3A inhibition leads to a shunt to the CYP2D6 pathway.

The aim of this study was to assess the effect of CYP2D6 genetic polymorphism as well as CYP2D6 and CYP3A drug–drug interaction on the pharmacodynamics of oxycodone in healthy volunteers. Our results showed that both CYP2D6 genetic polymorphism and drug–drug interactions via CYP2D6 and CYP3A had major effects on the antinociceptive actions, pharmacodynamics and safety of oxycodone. Volunteers who were CYP2D6 UM experienced higher analgesic effects and toxicity whereas those who were CYP2D6 PM had reduced pharmacodynamic effects.

Methods

This study was approved by the institutional ethical committee of Geneva University Hospitals Anaesthesiology Pharmacology and Intensive Care Department, and notified to the Swiss Agency for therapeutic products (Swissmedic). The study was performed according to Good Clinical Practices.

Subjects
Ten healthy, non-smoker, male volunteers were included and completed the study. They had no history of illicit drug consumption or contraindication to opioids, had normal medical examination, electrocardiogram, liver and kidney functions, and were not taking concomitant medications.

Design
This 5-arm crossover study was randomized, double-blind, double-dummy and placebo-controlled. Latin squares method was used for randomization. Crossover phases were separated by at least 1 week washout period. Fasting

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volunteers were given a single oral dose of the CYP2D6 blocker quinidine 100 mg, the CYP3A4 blocker ketoconazole 400 mg or matching placebos. Oral microdoses of midazolam (75 µg) and dextromethorphan (2.5 mg) were given 1 h later (for phenotyping). One hour later, an oral dose of oxycodone hydrocholoride (Oxynorm®, Mundipharma, Basel, Switzerland) 0.2 mg·kg⁻¹ (drops) or matching placebo was administrated. Intravenous naloxone (0.8 mg, Opopharma, Zurich, Switzerland) was routinely injected 1.5 h after oxycodone or respective placebo.

Pharmacodynamic measurements

Volunteers were familiarized with test procedures before the start of the study. Pharmacodynamic measurements were performed in the same order, at baseline ($t = 0$) before oxycodone administration and 0.5, 1, 1.5, 2, 3, 6 h after dosing.

Nociceptive tests

The quantitative sensory testing and pain assessment were evaluated with previously well-described and validated tests (Sandrini *et al.*, 2005). Objective pain thresholds or nociceptive flexion reflex (NFR) were determined in response to single electrical stimulations (series of 30–40) of fixed duration (0.5 ms) randomly applied transcutaneously on the sural nerve with variable intensities (0–100 mA) and constant current stimulator voltage (100 mV) (Viking IV, Madison, WI, USA). The NFR was recorded by electromyography on the ipsilateral biceps femoris. NFR threshold was defined as the intensity of current eliciting 50% of positive responses determined by fitting the data to the equation of Hill using the Marquardt algorithm. Subjective pain thresholds (SPT) in response to experimental electrical stimulation were evaluated simultaneously with the NFR, using a numerical rating scale (0–10).

Pain tolerance thresholds were assessed by the cold pressor test (Jones *et al.*, 1988). Volunteers were asked to have their right hand immersed wide open in ice-water until unbearable pain sensation or 2 min maximum. Both the duration of immersion and the intensity of pain (rated on a visual rating scale coupled to a computer) were recorded, and the area under the effect curve was calculated (Douleur MS-DOS, Gillequin 2005).

Thermal perception and pain detection thresholds to cold and hot stimuli were assessed by a thermode operating on the Peltier principle placed in contact with the skin in the inside of the forearm (Nicolet Viking™ Sensation IV, Nicolet Biomedical Inc., Madison, WI, USA) (Fruhstorfer *et al.*, 1976; Yarnitsky and Ochoa, 1990). The thermode temperature was set to increase or decrease its temperature at a 0.5°C s⁻¹ speed from a 32°C neutral temperature.

Dark-adapted pupil diameter and light reflex were measured with a handheld digital pupillometer (NeurOptics PLR-100, San Clemente, CA, USA) as previously described (Noehr-Jensen *et al.*, 2009).

Psychomotor testing by mean of the digit symbol substitution test (DSST) was performed by recording the correct number of symbol substituted in 1 min (Hindmarch, 1980).

Blood pressure, heart rate and oxygen saturation were monitored. Sedation was assessed on a numerical scale and spontaneously reported adverse drug reactions (ADRs) were recorded (detailed description, number and severity).

Phenotyping

Microdoses of dextromethorphan (2.5 mg) and midazolam (75 µg) were used. CYP2D6 phenotype was calculated using the metabolic ratio between deconjugated dextromethorphan and dextrorphan which was assayed in urine (8 h collection) as previously described (Daali *et al.*, 2008). The 30 min total (unconjugated + conjugated) 1-OH-midazolam/midazolam plasma ratios were assayed by gas chromatography-negative chemical ionization mass spectrometry to assess CYP3A phenotypes using a previously validated method (Eap *et al.*, 2004).

Genotyping

Genomic DNA was extracted from whole blood and genetic variants of CYP2D6 (32 alleles) were detected using the AmpliChipTMCYP450 DNA microarray (Roche, Basel, Switzerland) as previously described (Rebsamen *et al.*, 2009). Individuals were predicted to be extensive (EM), deficient (IM/PM) or ultrarapid (UM) metabolizer for CYP2D6, based on previously described allelic variants (http://www.cypalleles.ki.se).

Analysis of the data and statistics

Time-effect curves were generated by arithmetic means of effect values at each sample time. Area under the drug effect-time curves (AUEC) were calculated with the trapezoidal method.

The statistical software package SPSS for Windows (version 15, Chicago, IL, USA) was used for the statistical pharmacokinetic and pharmacodynamic analysis. A descriptive analysis allowed the determination of the data normality. Mean values and SD were used to summarize continuous variables. If necessary, log transformations were used to normalize the data and allow the use of parametric tests. Multiple repeated ANOVA were used to assess pharmacodynamic differences according to treatment and genotype, as well as naloxone effects. One-way ANOVA was used to test the effect of genotype on oxycodone treatment. Spearman correlations were used to assess associations between CYP2D6/3A activities and oxycodone pharmacodynamics, and pharmacokinetic–pharmacodynamic correlations. Differences in the incidence and severity of ADRs between groups were detected with a χ^2 analysis. The statistical software S-plus for Windows was used to perform the univariable and multivariable analysis. A linear mixed effect model was used to identify the independent predictors (pharmacokinetics of oxycodone and metabolites) of each pharmacodynamic test. Two-tailed tests with a significance level of 5% were used in all statistical analysis.

Materials

The quinidine, ketoconazole, dextromethorphan, midazolam and placebo used in this study were provided by Geneva University Hospital Pharmacy; the oxycodone (Oxynorm®)

was from Mundipharma (Basel, Switzerland) and the naloxone (Narcan®) from Opopharma (Zurich, Switzerland). Standards for the assay of oxycodone and its metabolites were supplied by Cerilliant (TX, USA), and dextrorphan and 1-OH-midazolam were obtained from Roche (Basel, Switzerland)

Results

The characteristics of the ten healthy male volunteers enrolled in the study are presented in Table 1. The mean oral oxycodone dose was 15.3 mg (range 13–18 mg). Two volunteers genotyped CYP2D6*4/*4 and CYP2D6*5/*41 had deficient CYP2D6 activities. Two carried gene (multi-)duplications (*2xN and *41xN) and were phenotyped as UM with dextromethorphan/dextrorphan metabolic ratio lower than 0.003.

Effect of CYP2D6 phenotypic polymorphism on the pharmacodynamics of oxycodone

Antinociception. Cold pressor test, quantitative sensory testing and SPT to electrical stimulation in CYP2D6 extensive (EM), ultrarapid (UM) and deficient metabolizers (PM = PM + IM) are presented in Figure 1. Cold pressor test evaluations (peak effect E_{max} and $AUEC_{90}$) were highly correlated (Spearman) with CYP2D6 activity ($\rho_S = 0.816$, $P = 0.004$ and $\rho_S = 0.612$, $P = 0.059$) but not with CYP3A activity. Pain tolerance thresholds to cold pressor were 40% higher in UM than EM ($AUEC_{90}$ 3735 vs. 2710 s·min^{-1}, and E_{max} 61 vs. 43 s, Figure 1). Inversely they were 20–30-fold lower in PM than EM and UM respectively. The genotypic PM showed trivial antinociceptive effects after oxycodone ($AUEC_{90}$ 135 s·min^{-1}, ΔE_{max} 2 s).

The same phenotypic differences were observed in the evaluation of the SPT to electrical stimulation. These were strongly correlated with CYP2D6 activity ($AUEC_{90}$: $\rho_S = 0.745$, $P = 0.013$ and E_{max}: $\rho_S = 0.636$, $P = 0.047$) but not CYP3A ($\rho_S = -0.567$, $P = 0.081$ and $\rho_S = -0.43$, $P = 0.214$). Mean peak antinociceptive effects were 2.5-fold higher in UM than EM (34.6 vs. 14.1 mA), and more than fivefold higher than PM

(6.4 mA) (Figure 1B). Also mean $AUEC_{90}$ was sixfold higher in UM as compared with EM (2155 mA·min^{-1} vs 356 mA·min^{-1}) and 16-fold higher than PM (131 mA·min^{-1}).

Pupil size. Oxycodone had no significant effect on the pupil size of the PM ($\Delta AUEC_{90}$: –6 mm·min^{-1}, ΔE_{min}: –0.4 mm) whereas it induced a pupil constriction in all other volunteers (Figure 1). Mean pupil constriction $AUEC_{90}$ was 1.6-fold higher in UM than EM (–167 vs. –106 mm·min^{-1}, $P = 0.038$). Although not statistically significant, an inverse correlation was observed between peak pupil effects and CYP2D6 activity ($\rho_S = -0.576$, $P = 0.082$), but not with CYP3A.

Sedation, O_2 saturation and DSST. Higher sedation and respiratory depression were observed when CYP2D6 activity was high (Figure 1D). Also, CYP2D6 activity showed a trend towards an inverse correlation with the saturation $AUEC_{90}$ ($\rho_S = -0.612$, $P = 0.06$). Moreover, oxycodone had no effect on O_2 saturation $\Delta AUEC_{90}$ in PM (7.5% min) whereas it was twofold higher in UM than EM (–202.5% vs. –97.5% min). As compared with PM, saturation E_{min} were 5- and 7-fold higher in EM and UM (–2.5, –3.5 and –0.5%, respectively, PM vs. EM $P = 0.034$).

Inverse correlations between CYP2D6 activities and sedation $AUEC_{360}$ ($\rho_S = -0.697$, $P = 0.025$) were also demonstrated. In PM, oxycodone had no effect on sedation mean $\Delta AUEC$ (–18.75 U·min^{-1}). Moreover, sedation E_{max} were 2.75–3-fold higher in EM and UM than PM, and the $AUEC_{360}$ was three-fold higher in UM than EM (16.25 vs. 38.75 U·min^{-1}).

Finally, larger psychomotor effects were demonstrated in UM. The DSST $\Delta AUEC_{90}$ was 2.4-fold lower in UM than EM ($P = 0.031$) and 3.5-fold lower than PM. The DSST E_{max} was 4.3-fold lower in UM than EM (–2.6 vs. –11.5 U·min^{-1}, $P = 0.016$), and 3.3-fold lower than PM (–3.5 vs. –11.5 U·min^{-1}, $P = 0.052$).

Spontaneously reported ADRs. After oxycodone alone, all UM spontaneously reported several mild to severe side-effects (Table 2) whereas one EM only reported mild side-effects and no toxicity was reported among PM. The reported ADRs were the common µ-opioid-related side-effects.

Table 1 Healthy volunteers characteristics, oxycodone dose, CYP2D6 genotype, predicted and measured phenotype

Volunteer	Age (year)	Weight (kg)	Height (cm)	Oxycodone dose (mg)	CYP2D6 genotype	Predicted phenotype	DEM/DOR metabolic ratio	Measured CYP2D6 phenotype
1	28	68	182	13.5	*5/*35	EM	0.0165	EM
2	28	65	175	13	*41/*41xN	UM	0.0013	UM
3	22	67	176	13.5	*1/*41	EM	0.0017	EM
4	28	90	182	18	*5/*41	IM	0.22	IM
5	22	79	185	16	*4/*35	EM	0.031	EM
6	23	79	185	16	*2/*41	EM	0.0066	EM
7	21	79	182	16	*1/*4	EM	0.0065	EM
8	21	86	190	17	*1/*2xN	UM	0.0012	UM
9	21	77	183	15	*1/*6	EM	0.013	EM
10	48	74	172	15	*4/*4	PM	1.84	PM
Mean	26.2	76.4	181.2	15.3				
SD	8.2	8.1	5.4	1.7				

DEM/DOR, dextromethorphan/dextrorphan metabolic ratio; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UM, ultrarapid metabolizer for cytochrome P450 2D6.
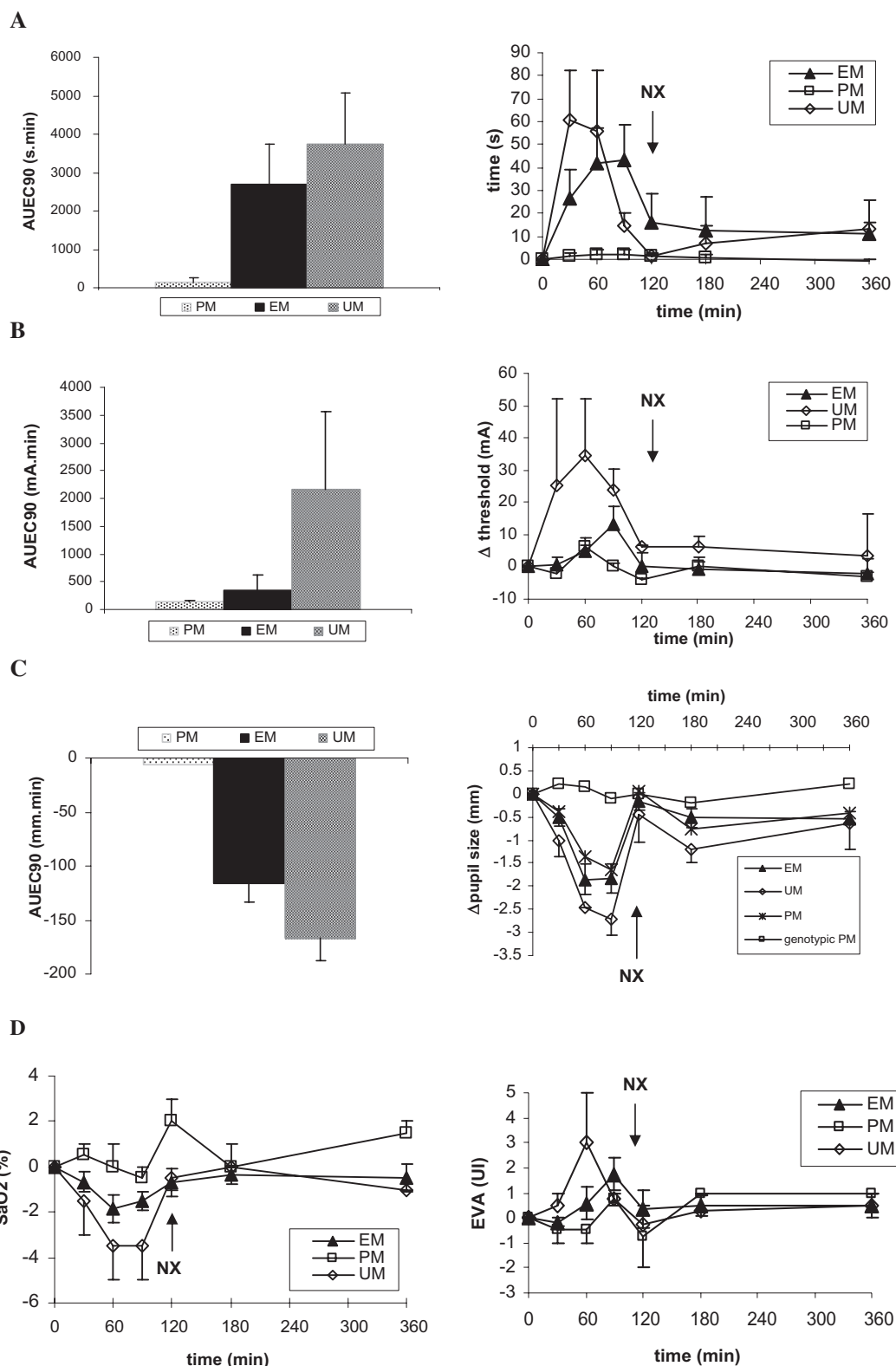
Figure 1 Pharmacodynamic assessments after oxycodone in CYP2D6 phenotypic groups (UM, EM and PM). The mean area under the time-effect curves over the 90 min after dosing with oxycodone (AUEC$_{90}$) and the time course over 6 h of the change from baseline values are shown on the left and right, respectively, in A–C. Note that all subjects received naloxone (NX; 0.8 mg i.v.) 90 min after oxycodone (arrow in time courses). In (A) data for each phenotypic group from the cold pressor test are shown and in (B) corresponding data from the subjective pain threshold after electrical stimulation and in (C) for the effects of oxycodone on pupil size are presented. In (D), the time courses for oxygen saturation (left) and for sedation (right) are presented. Phenotypic differences were demonstrated in the pharmacodynamic assessments. CYP2D6 PM had decreased pharmacodynamic effects whereas UM experienced increased effects, compared with EM for CYP2D6. Data shown are means ($\pm$ s.e.mean). EM, extensive metabolizer; PM, poor metabolizer; UM, ultrarapid metabolizer.

Table 2 Adverse drug reactions (ADRs) spontaneously reported at each session

ADRs	Total	Oxy	Quin + Oxy	Keto + Oxy	Keto + Quin + Oxy
Drowsiness	10	1*	–	3	6
Nausea	9	1*	–	5	3
Fullness euphoria	9	1*	1*	2	5
Vomiting	7	1*	1*	3	2
Light-headedness/dizziness	7	1 + 1*	1*	2	2
Distorted perception/slurred speech/visual hallucination	6	1*	–	1	4
Sweating	4		–	2	2
Headache	4	2*	–	–	2
Itching	1	–	–	–	1
Hypotension/unsteadiness	1	–	–	–	1
Flush	1	–	–	1	–
Desinhibition	1	–	–	–	1
Total	60	9	3	19	29

*Ultrarapid metabolizer for CYP2D6 only.
Oxy: placebo + oxycodone; Quin + Oxy: quinidine + oxycodone; Keto + Oxy: ketoconazole + oxycodone; Keto + Quin + Oxy: quinidine + ketoconazole + oxycodone.

Table 3 Mean pharmacodynamic assessments ($AUEC_{90}$ and E_{max}) after placebo, oxycodone, quinidine, ketoconazole and quinidine + ketoconazole

	E_{max}					$AUEC_{90}$				
	Pbo	Oxy	Quin + Oxy	Keto + Oxy	Keto + Quin + Oxy	Pbo	Oxy	Quin + Oxy	Keto + Oxy	Keto + Quin + Oxy
Objective (mA)	26.2	27.6	27.5	31.3*	33.8**	2090	2108	2036	2421*	2557**
Subjective (mA)	38.6	52.2*	41.2	56.5*	60.4**	2952	3869*	3264	4101*	4072**
Sensitive (mA)	41.5	54.8^T	45.9	58.8**	61.2**	2674	3494	2976	3591*	3942**
Affective (mA)	49.2	71.1^T	63.8	75.6*	81.5**	3951	5285*	5038*	5735**	5946**
Cold pressor (s)	71.9	99.5*	103*	108*	108*	6007	7881*	8190*	8736*	8952*
Pupil size (mm)	6.3	4.4**	4.5**	3.6**	3.7**	590	486**	491**	436**	445**
Saturation (%)	96.5	95.7*	95.6*	95.1*	94.7**	8757	8705	8717	8684	8648**
Somnolence (U)	1.4	3.4**	2.3	4.1**	4.1*	55.5	89.3*	87.8	149**	153**

*$P < 0.05$, **$P < 0.01$.
^{T}Trend $P < 0.07$, different from corresponding values with placebo.
The column headings show the treatments given, as follows; Pbo, placebo; Oxy: placebo + oxycodone; Quin + Oxy: quinidine + oxycodone; Keto + Oxy: ketoconazole + oxycodone; Keto + Quin + Oxy: quinidine + ketoconazole + oxycodone; E_{max}: peak pharmacodynamic effect; $AUEC_{90}$: area under the effect curve over 90 min.

Pharmacokinetic–pharmacodynamic correlations. Positive Spearman correlations between SPT $AUEC_{90}$ and both oxymorphone and noroxymorphone AUC_{90} and C_{max} were demonstrated, whereas no correlation with oxycodone and noroxycodone pharmacokinetics was demonstrated. Similarly, positive correlations between the objective testing of the NFR $AUEC_{90}$ and both oxymorphone and noroxymorphone C_{max} and AUC_{90} were demonstrated ($0.575 < \rho_S < 0.685$). Sedation was highly and negatively correlated with oxymorphone and noroxymorphone pharmacokinetic parameters ($-0.552 < \rho_S < -0.842$). Pupil size $AUEC_{90}$ and E_{max} were, however, correlated (trend) with both oxycodone AUC_{90} ($\rho_S = -0.587$, $P = 0.074$ and $\rho_S = -0.65$, $P = 0.041$) and noroxymorphone AUC_{90} ($\rho_S = -0.6$, $P = 0.066$ and $\rho_S = -0.589$, $P = 0.072$).

Effect of drug–drug interactions (CYP2D6 and 3A blockade) on the pharmacodynamics of oxycodone

Pharmacodynamic effects of oxycodone ± ketoconazole ± quinidine as compared with placebo. Statistically significant higher pharmacodynamic effects were observed after oxycodone than placebo in all the pharmacodynamics tests with the exception of the NFR (Table 3, Figure 2). All pharmacodynamic assessments were also significantly higher than placebo after CYP3A4 blockade. The effect of quinidine on the pharmacodynamics of oxycodone was not as clear-cut. There were no differences between quinidine and placebo in the objective NFR, SPT after electrical stimulation, as well as sedation. However, differences between quinidine and placebo were seen in the cold pressor test assessments ($AUEC_{90}$ and E_{max}) 1.5-fold higher after quinidine than placebo ($P = 0.026$ and 0.033) and pupil size $AUEC_{90}$ and E_{max} 1.2- and 1.4-fold lower than placebo ($P = 4.10$–10 and 4.10–8).

Effect of quinidine and ketoconazole inhibition on the pharmacodynamics of oxycodone. Conversely, ketoconazole increased the NFR $AUEC_{90}$ by 15% as compared with oxycodone alone ($P = 0.037$), and pupil peak-effects by 20% ($P = 0.004$). In addition, sedation $AUEC_{90}$ dramatically increased (80%) after selective CYP3A4 blockade ($P = 0.012$).

The co-administration of ketoconazole and quinidine induced a 20% increase of the NFR assessments ($P = 0.01$) and pupil peak-effects ($P = 0.0004$) as compared with oxycodone.
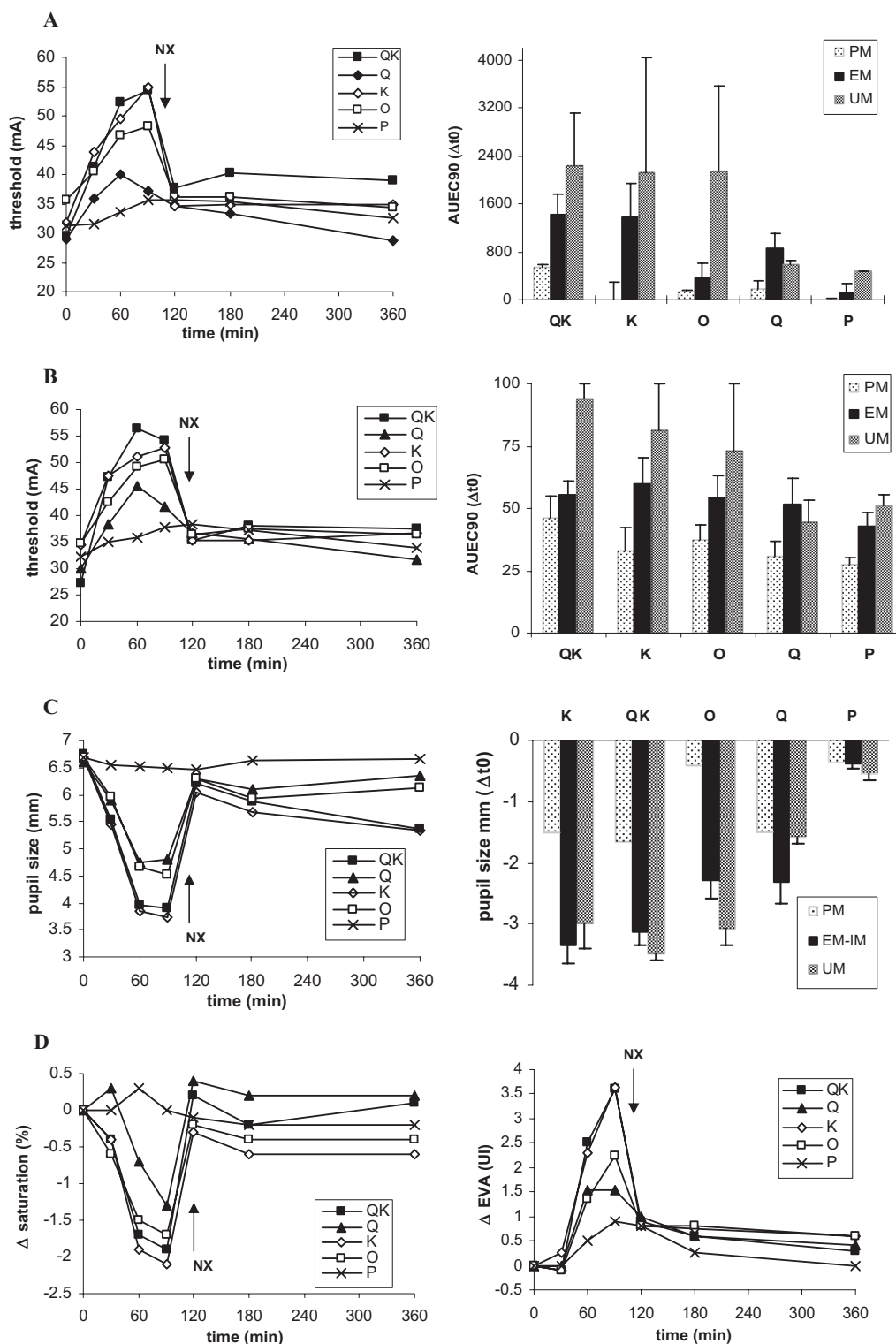
Figure 2 Pharmacodynamic assessments in the different treatment arms and phenotypic groups for CYP2D6 (UM, EM and PM). The pharmacodynamic variables were measured after different treatments: oxycodone alone (O); oxycodone and quinidine (Q), oxycodone and ketoconazole (K), oxycodone with quinidine and ketoconazole (QK) and placebo (P) and in each phenotypic group (UM, EM and PM). The left hand graphs show the mean time course of effects over 6 h and each subject received naloxone (NX; 0.8 mg, i.v.) at 90 min after oxycodone or placebo (arrow in time course). In (A), the time course (left) and the $AUEC_{90}$ (right) of the subjective pain threshold (SPT) is shown. In (B), the time course (left) and the E_{max} for the sensitive pain threshold after electrical stimulation are shown. In (C) the time course (left) and the minimum pupil size are shown. In (D), the time course of the oxygen saturation of blood (left) and the time course of sedation (right) are presented. CYP2D6 blockade with quinidine reduced pharmacodynamic assessments such as the SPT by 30%. CYP3A4 blockade with ketoconazole had a major effect on all pharmacodynamic assessments and the SPT increased by 15%. Data shown are means ($\pm$ s.e.mean). EM, extensive metabolizer; PM, poor metabolizer; UM, ultrarapid metabolizer.
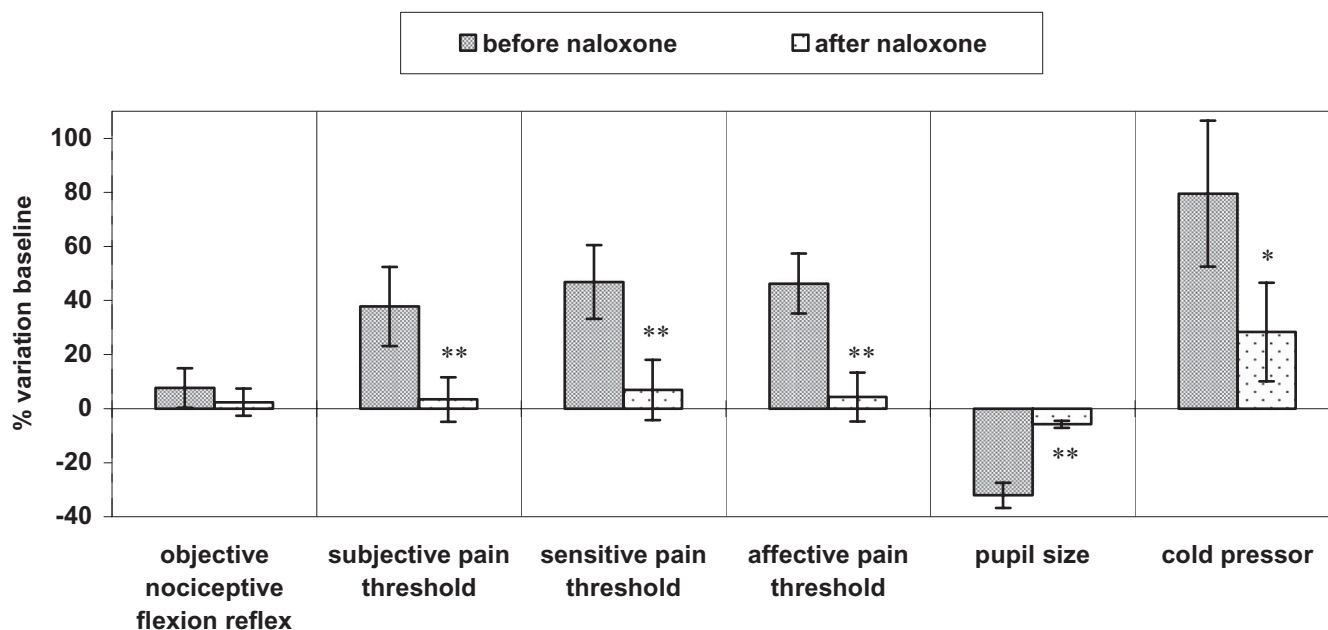
Figure 3 Antagonistic effects of naloxone on the pharmacodynamic effects of oxycodone. The effects of naloxone on the objective nociceptive flexion reflex, subjective, sensitive and affective pain threshold after electrical stimulation, cold pressor test and pupil size assessments were measured in all subjects. Naloxone significantly reversed the pharmacodynamic effects of oxycodone, irrespective of CYP2D6 genotype. Data shown are means ($\pm$ s.e.mean). **$P < 0.005$; *$P < 0.05$ significantly different from values before naloxone.

The saturation E_{max} was significantly lower after ketoconazole + quinidine (94.7% vs. 95.7%, $P = 0.015$) and the sedation $AUEC_{90}$ increased by 80% ($P = 0.001$).

Comparisons of the effect of quinidine and ketoconazole alone versus their combination on oxycodone pharmacodynamics. Significant differences between the combination of ketoconazole with quinidine and quinidine alone were observed for most pharmacodynamic tests assessed (Table 3, Figure 2). The opposite was not true. No differences between the combination of ketoconazole with quinidine and ketoconazole alone were observed for most assessed pharmacodynamic parameters. The only significant difference was observed on pupil size $AUEC_{90}$ (436 vs. 445 mm·min^{-1}, $P = 3 \times 10^{-5}$) and E_{min} (3.6 mm vs. 3.7 mm, $P = 5.8 \times 10^{-7}$).

Pharmacokinetic–pharmacodynamic analysis. We performed a multivariable analysis (linear mixed effect model) in order to identify independent predictors among possible explanatory pharmacokinetic variables (AUC_{90} of oxycodone, oxymorphone, noroxycodone and noroxymorphone) associated with pharmacodynamic outcome ($AUEC_{90}$). The only independent positive predictor of the SPT after electrical stimulation was oxymorphone $AUEC_{90}$ (parameter estimate 17.9, $P < 0.05$); the pain threshold AUEC increased by 17.9 units when the AUC_{90} of oxymorphone increased by 1 unit. Oxymorphone $AUEC_{90}$ was also the only independent predictor of the saturation $AUEC_{90}$ (–1.218). Interestingly, both oxycodone (–0.02) and oxymorphone (–0.55) were independent predictors of the pupil size $AUEC_{90}$ (associated with the decrease in the pupil size), whereas noroxycodone (0.028) was a positive predictor.

Toxicity. The ADRs spontaneously reported by the healthy volunteers are presented in Table 2. They were all opioid-

related and observed in 23 out of 50 sessions. CYP3A4 blockade dramatically increased the risk of adverse effect particularly in CYP2D6 UM. Eighteen ADRs were indeed reported after the co-administration of ketoconazole and oxycodone (with or without quinidine). Side-effects observed after quinidine were observed in UM only. A χ^2 analysis (Fisher exact test) was highly significant ($P = 0.00046$) when comparing the different treatments vs. number of ADRs, as well as the severity of the ADRs ($P < 0.00001$).

Effect of naloxone. Naloxone significantly reversed all oxycodone pharmacodynamic effects, irrespective of CYP2D6 genotype (Figure 3). Most reported ADRs improved within minutes after naloxone injection. Interestingly, we systematically observed withdrawal symptoms (shivering, cold and bad feeling, yawning, goose-flesh) within minutes after naloxone injection in EM and UM, but not in PM.

Discussion

We performed a randomized crossover double-blind placebo-controlled study aiming to assess the effect of CYP2D6 and CYP3A activities (drug inhibitions) on the pharmacokinetics and pharmacodynamics of oxycodone and metabolites and show for the first time the influence of CYP2D6 genetic polymorphism on the magnitude of these drug–drug interactions.

CYP2D6 genetic polymorphism and oxycodone pharmacodynamics

Oxycodone analgesic effects were assessed in the experimental pain tests in healthy volunteers, genotyped and phenotyped for CYP2D6. Differences depending on CYP2D6 activity

were demonstrated in the analgesic response (SPT after electrical stimulation and tolerance to cold pressor test) by strong and significant Spearman correlations ($\rho_S = 0.636$–0.745). CYP2D6 UM experienced a 1.5- to 6-fold increase of the analgesic effects as compared with EM (SPT after electrical stimulation, tolerance to cold pressor test, thermal pain threshold). Inversely, PM had a 2–20-fold reduction of the effects as compared with EM. This is in agreement with the few case reports describing reduced analgesic effect of oxycodone in PM (Maddocks *et al*., 1996; Myers *et al*., 2005; Susce *et al*., 2006; Foster *et al*., 2007).

Differences according to CYP2D6 phenotype were similarly demonstrated in the other assessed pharmacodynamic parameters (pupil size, saturation, sedation and psychomotor effects). Oxycodone had no significant effect on the pupil size of the genotypic PM whereas it induced a pupil constriction in all other volunteers. Pupil constriction was more pronounced in UM, although the inverse correlation ($\rho_S = -0.576$) between CYP2D6 phenotype and peak pupil size effects did not reach statistical significance. Likewise, oxycodone had no effect on the sedation and saturation assessment in PM, whereas they were higher in UM than EM. Inverse Spearman correlations were also demonstrated between saturation, sedation and CYP2D6 activity which confirms the important role of CYP2D6 in the effects of oxycodone.

Some authors have proposed that the role of oxymorphone in oxycodone pharmacodynamics is minor, although they did not investigate its antinociceptive effect (Kaiko *et al*., 1996; Heiskanen *et al*., 1998; Lalovic *et al*., 2006). Their conclusions were based on Pearson correlation coefficients (Kaiko *et al*., 1996), simple regression analysis (Heiskanen *et al*., 1998), or pharmacokinetic–pharmacodynamic modelling (Lalovic *et al*., 2006) in non-genotyped patients on some pharmacodynamic parameters (pupil size and subjective drug effects). To test these approaches on our results, we assessed correlations (Spearman) between pharmacokinetic and pharmacodynamic variables. The pharmacokinetic parameters for CYP2D6-dependent metabolites (oxymorphone and noroxymorphone), but not those for oxycodone or noroxycodone, were indeed positively correlated with the NFR, sedation and saturation. However, pupil size was correlated with oxycodone pharmacokinetic parameters, and psychomotor function (DSST) was significantly correlated with noroxycodone AUC_{90} ($\rho_S = 0.652$–0.7) and possibly oxycodone ($\rho_S = 0.579$). This is in agreement with the findings that pupil size and subjective pharmacodynamic variables (drug effect) were significantly correlated (Pearson) with oxycodone plasma concentration but not oxymorphone (Kaiko *et al*., 1996) (noroxymorphone and noroxycodone were not assessed). Oxycodone and oxymorphone pharmacokinetic parameters were correlated with subjective drug effects ratings and psychomotor effects in another study (Heiskanen *et al*., 1998) but the disadvantage was that the detection limit of the assay was twice as high as ours (Samer *et al*., 2010). In a pharmacokinetic–pharmacodynamic modelling of oxycodone and noroxymorphone plasma drug concentration vs. pupil constriction, oxycodone level was sufficient to explain the time course of the pupil size in 16 non-genotyped healthy volunteers (Lalovic *et al*., 2006), in accordance with our results. Other authors also recently concluded that oxycodone analgesia may depend on both oxycodone and its metabolite, oxymorphone (Zwisler *et al*., 2009).

CYP2D6 activity and oxycodone toxicity
Notable differences in the incidence of spontaneously reported ADRs were also observed with CYP2D6 genotype. All UM volunteers experienced side-effects more severe and frequent than those classified as EM, whereas genotypic PM did not report any side-effects after oxycodone alone. Neuropsychiatric side-effects were reported in one UM after oxycodone (De Leon *et al*., 2003), and in the case of codeine, the UM genotype had already been associated with increased morphine formation and severe toxicity (Gasche *et al*., 2004). Kaiko reported that healthy volunteers ($n = 10$) with no measurable oxymorphone had significantly higher opioid adverse effects in comparison with the subjects with measurable oxymorphone and lower oxycodone concentrations ($n = 19$). Their subjects were not genotyped and the assay sensitivity was probably not sufficient to detect oxymorphone plasma concentrations possibly minimizing CYP2D6 role in the efficacy and safety of oxycodone.

Effect of drug inhibition of CYP2D6 and 3A metabolic pathways
CYP2D6 inhibition had a major effect on the analgesic effect of oxycodone. Indeed, after inhibition of CYP2D6, the NFR assessment and the SPT after electrical stimulation results with oxycodone were not different from those with placebo. Moreover, as compared with oxycodone given alone, the addition of quinidine significantly reduced the peak effect by about 30%. In terms of pharmacokinetics, CYP2D6 inhibition by quinidine induced a significant increase in oxycodone exposure, and a decrease in oxymorphone and noroxymorphone exposure (Samer *et al*., 2010). Therefore, oxycodone does not appear to be the active molecule responsible for the analgesic effects. Our multivariable analysis confirmed the role of the metabolite oxymorphone as the only independent positive predictor of the SPT after electrical stimulation, sedation and oxygen saturation $AUEC_{90}$.

However, quinidine did not completely abolish all pharmacodynamic effects, in particular the pupil size and cold pressor test assessments. Pupil effects and subjective drug and psychomotor effects have been reported by other authors who suggested that oxycodone might be locally metabolized into oxymorphone in the brain (Heiskanen *et al*., 1998). Oxymorphone plasma levels were low in our study after quinidine, but still detectable, suggesting an incomplete blockade. Oxymorphone plasma concentrations might also not mirror brain concentrations which could be high enough at the blood brain barrier to exert effects. Also, quinidine does not readily penetrate the blood brain barrier and might be unable to block CYP2D6 in the CNS. This would explain the discrepancy between the pupil effects observed after quinidine blockade, but not in the genotypic PM. Quinidine might also increase penetration into the CNS of oxycodone, by inhibiting the multidrug transporter ABCB1. Another possibility is that oxycodone acts on the 5-hydroxytryptaminergic pathways as does tramadol. Oxycodone has indeed shown efficacy in neuropathic pain syndromes such as postherpetic neuralgia

and diabetic neuropathy, and the 'serotoninergic syndrome' has been reported after oxycodone and sertraline (Rosebraugh *et al*., 2001).

CYP3A inhibition induced a consistent and significant increase of oxycodone pharmacodynamic effects, compared with the placebo and oxycodone with placebo. The main observation was that CYP3A blockade dramatically increased the analgesic efficacy as well as the toxicity of oxycodone, especially in UM for CYP2D6. Side-effects (related to μ-opioid receptors) were only observed after CYP3A inhibition or in UM. Some authors have recently pointed out the importance of CYP3A pathway in the pharmacokinetics and pharmacodynamics of oxycodone after induction by rifampin (Nieminen *et al*., 2009) and in the same fashion, increased toxicity has been reported after codeine when the CYP3A4 pathway was blocked (Gasche *et al*., 2004). This further emphasizes the role of CYP2D6 pathway and oxymorphone on the pharmacodynamics, analgesic efficacy and safety of oxycodone.

Both CYP2D6 and CYP3A are indeed involved in the biotransformation of oxycodone. CYP3A is the major metabolic pathway that is responsible for the N-demethylation of oxycodone to noroxycodone, and oxymorphone to noroxymorphone. Conversely, CYP2D6 catalyses the O-demethylation of oxycodone into oxymorphone, and of noroxycodone into noroxymorphone. Therefore, these two enzymes are inextricably linked. We have shown in a pharmacokinetic analysis (Samer *et al*., 2010) that when the activity of one enzyme is reduced by drug inhibition, the metabolic rate of the other enzyme increases. Indeed blocking CYP3A4 (with ketoconazole) tripled oxymorphone AUC_∞ ($P = 0.0004$) and reduced noroxycodone and noroxymorphone AUCs by 80% ($P < 0.0005$) as compared with oxycodone without inhibitions. Conversely, blocking CYP2D6 (with quinidine) reduced oxymorphone and noroxymorphone C_{max} by 40% ($P = 0.01$) and 80% ($P = 0.0009$), and increased noroxycodone AUC_∞ by 70% ($P = 0.0004$). These observations are in agreement with our pharmacodynamic findings.

Oxycodone needs therefore to be used with extreme caution in those patients who are UM for CYP2D6 and/or when CYP3A inhibitors are given concomitantly. These precautions should be added to drug information sheets, especially in the light of the high incidence of dependence and misuse with oxycodone.

The differences observed in oxycodone pharmacodynamic effects after the combination of ketoconazole + quinidine + oxycodone, compared with quinidine + oxycodone, but not in comparison with ketoconazole + oxycodone, need further investigations. In terms of pharmacokinetics, ketoconazole + quinidine was as efficient as quinidine in reducing oxymorphone exposure, and more efficient than quinidine in reducing noroxymorphone exposure (Samer *et al*., 2010). The combination of ketoconazole + quinidine was more efficient than quinidine or ketoconazole alone in increasing oxycodone exposure. Ketoconazole and ketoconazole + quinidine both reduced the exposure to noroxycodone, compared with quinidine. The possibility of μ-opioid receptor antagonist action for noroxycodone should therefore be clarified. Another hypothesis to consider is a drug–drug interaction via ABCB1. Oxycodone was recently shown to be a ABCB1 substrate, inducing overexpression of ABCB1 and affecting the

tissue distribution of paclitaxel in rats (Hassan *et al*., 2007). Ketoconazole is a well-known ABCB1 (Yasuda *et al*., 2002) and UGT2B7 inhibitor (Takeda *et al*., 2006) and quinidine is another ABCB1 inhibitor. Ketoconazole might have interfered with ABCB1-mediated oxycodone transport in the CNS or have blocked oxycodone glucuronidation, inducing higher pharmacodynamic effects. Quinidine might have reinforced the effects of ketoconazole, explaining the lack of clear differences in terms of pharmacokinetics. A direct drug–drug interaction between ketoconazole and quinidine, via CYP3A or ABCB1, by inhibition or competition for the substrate, is also not excluded.

Naloxone effects

The reversibility of the antinociceptive effects of oxycodone by the μ-opioid receptor antagonist naloxone was for the first time assessed in the experimental pain tests. Pain, pupil size and toxicity were reversed by naloxone within a few minutes, although not completely. Surprisingly, all but the PM experienced withdrawal symptoms after oral oxycodone. Whether oxycodone is a pure μ or mixed μ- and k-opoid agonist is still debated in animal studies (Chen *et al*., 1991; Ross and Smith, 1997; Beardsley *et al*., 2004; Thompson *et al*., 2004; Lalovic *et al*., 2006; Lemberg *et al*., 2006b; Smith *et al*., 2007), Radio-ligand binding studies with rat brain membranes have shown that oxycodone may act as a k-2b-opioid agonist, and in streptozotocin-induced diabetic mice, oxycodone antinociception may be mediated by μ-1 opioid receptors, inducing the release of dynorphin A and indirectly activating k-opioid receptors (Nozaki and Kamei, 2007). In humans, no systematic studies have been performed. Our data demonstrate that the antinociceptive, pupillary and respiratory effects of oxycodone were reversed by naloxone in healthy volunteers indicating that the effects are mediated, at least in part, by the μ-opioid receptor.

In conclusion, both CYP2D6 genetic polymorphism, and CYP2D6 and CYP3A drug–drug interactions had a major effect on the analgesic efficacy and toxicity of oxycodone. CYP2D6 UM experienced higher analgesic effect and toxicity, whereas CYP2D6-deficient metabolizers had reduced pharmacodynamic effects. Correlations between CYP2D6 activity, CYP2D6 O-demethylated metabolites oxymorphone and noroxymorphone and pharmacodynamic parameters were demonstrated (but not pupil size). In addition, drug–drug interactions had a major effect on the efficacy and safety of oxycodone. The results of our exploratory study predict that drug–drug interaction via CYP2D6 and CYP3A would have clinical consequences, dependent on CYP2D6 genotype. Indeed, CYP3A blockade increased oxycodone analgesic efficacy and toxicity, and CYP2D6 genetic polymorphism influenced the magnitude of the drug–drug interaction which was more important in CYP2D6 UM. The importance of active metabolites such as oxymorphone mainly responsible for oxycodone analgesic activity was also demonstrated. In conclusion, oxycodone has to be used with extreme caution in UM for CYP2D6 especially when a CYP3A inhibitor is co-prescribed. In deficient metabolizers for CYP2D6, the analgesic activity might be reduced and alternative treatments should be considered.

Acknowledgements

Conflict of interest

None.

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