Polymorphisms associated with fentanyl pharmacokinetics, pharmacodynamics and adverse effects

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Running title: Pharmacogenetics of fentanyl
Fentanyl is an agonist of the µ-opioid receptor commonly used in the treatment of moderate-severe pain. In order to study whether pharmacogenetics explain some of the variability in the response to fentanyl, several genes related to fentanyl receptors, transporters and metabolic enzymes have been analysed.

Thirty-five healthy volunteers (19 men and 16 women) receiving a single 300 µg oral dose of fentanyl, were genotyped for 9 polymorphisms in cytochrome P450 (CYP) enzymes (CYP3A4 and CYP3A5), ATP binding cassette subfamily B member 1 (ABCB1), opioid receptor mu 1 (OPRM1), catechol-O-methyltransferase (COMT) and adrenoceptor beta 2 (ADRB2) by real-time PCR. Fentanyl concentrations were measured by ultra-performance liquid chromatography combined with tandem mass spectrometry (UPLC-MS/MS).

Fentanyl pharmacokinetics is affected by sex. Carriers of CYP3A4*22 allele, which is known to reduce the mRNA expression, showed higher area under the concentration-time curve (AUC) and lower clearance (Cl) values. Although this finding might be of importance, its validity needs to be confirmed in other similar settings. Furthermore carriers of the ABCB1 C1236T T/T genotype presented a lower AUC and higher Cl, as well as lower half-life (T1/2). As subjects were blocked with naltrexone, the effect of fentanyl on pharmacodynamics might be biased; however, we could observe that fentanyl had a hypotensive effect. Moreover, ADRB2 C523A A allele carriers showed a tendency towards reducing systolic blood pressure. Likewise, OPRM1 and COMT minor allele variants were risk factors for development of somnolence. CYP3A5*3, ABCB1 C3435T and ABCB1 G2677T/A were not associated with fentanyl’s pharmacokinetics, pharmacodynamics and safety profile.
Fentanyl is an opioid drug used as analgesic and anaesthetic. It is used for the treatment of acute and severe chronic pain produced by cancer, injuries or surgery (1) as well as in patients with renal failure due to its primarily hepatic elimination (2). Fentanyl evokes its sedative effect mainly through activation of \( \mu \)-opioid receptors, which are abundant in the central nervous system and the peripheral nervous system. Due to its high lipophilicity, fentanyl is able to easily cross the blood-brain barrier (BBB) (3,4), thus having great potency (between 75-200 times more potent than morphine) (3,5). Considered as one of the most frequent analgesics (3,4), it produces adverse effects such as vomiting, nausea, gastrointestinal constipation, respiratory depression, dependence and tolerance (6). However, it produces less adverse effects (AEs) than morphine (7) as lower doses are required to obtain similar analgesic effects (8).

When administered in sublingual doses, fentanyl’s calculated bioavailability is 54%. Peak plasma concentrations vary between 0.2 and 1.3 ng/mL (after administration of 100 to 800 \( \mu \)g of the drug) and the time to reach the maximum concentration (\( T_{\text{max}} \)) ranges from 22.5 to 240 minutes. Volume of distribution (Vd) varies between 3 to 6 L/kg and it exhibits a half-life (\( T_{1/2} \)) of around 7 hours and a clearance (Cl) of 0.5 L/h/kg. Approximately, 80% of fentanyl binds to plasma proteins, being its main target the \( \alpha_1 \)-glycoprotein (9). In addition, fentanyl presents a log P of 4.05, which explains its high lipophilicity (10).

After sublingual administration, fentanyl is absorbed and distributed throughout the body reaching its target tissues, where it can be transported by the P-glycoprotein (P-gp), encoded by \( ABCB1 \), an adenosine triphosphate (ATP)-dependent efflux transporter. In the brain, it plays an important role as an outward transporter for opioids across the BBB. Fentanyl is metabolized in the liver by two isoforms of the cytochrome P450 (CYP) complex, CYP3A4 and CYP3A5, which are responsible for its N-dealkylation forming the nontoxic and inactive metabolite norfentanyl. Only a small amount of drug (less than 1%) can be metabolized by N-dealkylation, amide hydrolysis or alkyl hydroxylation leading to the inactive metabolites hydroxynorfentanyl and...
despropionylfentanyl (11). Although it is mostly excreted in urine (12), it can be eliminated in faeces and by exhalation (13).

Since CYP3A4 is the main enzyme responsible for the metabolism of fentanyl, variants in its coding gene have been studied in association with fentanyl’s disposition. Carriers of CYP3A4*20 and CYP3A4*22, two rare polymorphisms, could show higher levels of the parent drug. This can be explained by CYP3A4*20 resulting in a premature stop codon and therefore, a truncated protein with complete loss of function (14). Similarly, CYP3A4*22 leads to low hepatic CYP3A4 activity (14,15). CYP3A5 is a member of the CYP3A family (15) whose most common non-functional allele is CYP3A5*3. This allele causes alternative splicing, thus producing a truncated protein and higher levels of metabolites (11,16). Regarding their effect on fentanyl, CYP3A5*3 is known to cause an increase in systemic exposure to the drug whereas there is no evidence of any relationship between this effect and CYP3A4 polymorphisms (11).

The ABCB1 gene is highly polymorphic, with at least 38 variants described (17). Although some of these polymorphisms have shown an effect on fentanyl pharmacokinetics (18) and the incidence of adverse effects (7,19), there is great controversy about their influence (20). Therefore, further investigation needs to be performed.

The µ-opioid receptor is encoded by the OPRM1 gene, whose most prevalent variation is A118G (4). It has shown reduced signal transduction and OPRM1 expression (19). Therefore, patients with the A/A genotype required lower morphine doses for effective analgesia (17,19).

Moreover, the most studied polymorphism in the cathechol-O-methyltransferase, encoded by COMT, known as G472A or Val158Met, has been associated with opioid response (21,22) and adverse events (AEs) (11,19). Several studies show that when adenosine replaces guanosine, the enzyme reduces its activity three to four times, which causes increased pain sensitivity and production of pro-inflammatory cytokines (17).
Furthermore, variations in the ADRB2 gene, which encodes for a β2-adrenergic receptor, might affect the cardiovascular response to anaesthesia. For the C523A substitution, it has been proved that the A/A genotype is associated with an increase in the severity of hypotension caused by opioids as compared to the C/C genotype (23).

Our hypothesis is that several polymorphisms present in genes involved in fentanyl’s pharmacokinetics and mechanism of action might have an impact on the disposition and effect of the drug. Therefore, our aim was to study the role of polymorphisms of metabolizing enzymes, transporters and receptors on the pharmacokinetics, pharmacodynamics and tolerability of fentanyl in healthy volunteers. This approach avoids confounding factors, such as the high number of concomitant treatments prescribed in chronic pain.

METHODS

Study population

Our study population comprised 35 healthy volunteers who were enrolled in a single-dose bioequivalence clinical trial of fentanyl performed at the Clinical Trials Unit of Hospital Universitario de la Princesa (Madrid, Spain). All subjects included in the study signed a written informed consent that allowed both clinical trial and pharmacogenetic studies. They were free to withdraw from the study at any time. The protocol fulfilled the Spanish law on biomedical research and was approved by the Research Ethics Committee, duly authorised by the Spanish Drug Agency.

Inclusion criteria were as follows: age 18-55 years, volunteers free from any psychiatric or organic conditions, normal vital signs and electrocardiogram (ECG), normal medical records and physical examination and no clinically significant abnormalities in serology, haematology, biochemistry, and urine test. Exclusion criteria were: subjects who had received pharmacological treatment in the last 15 days or any kind of medication in the 48 hours prior to receiving the study medication, body mass index (BMI) outside the 18.5-30 kg/m² range, having donated blood in the previous month, history of sensitivity to any drug, suspected consumption of controlled substances, smokers, daily consumers of alcohol and/or acute alcohol poisoning in the previous week,
pregnant or breastfeeding women or subjects with abnormal blood pressure (BP) and pulse.

Study design and procedures

We analysed the data of two formulations of fentanyl 300 µg sublingual tablets after single dose administration to healthy volunteers under fasting conditions. The clinical trial was a phase I, randomised, open-label, crossover, two-period, two-sequence, single-centre study, separated by a 7-day washout period, with blind determination of plasma concentrations of fentanyl.

To avoid respiratory depression, during each treatment period, subjects received a total of three oral doses of naltrexone 50 mg at the following time points: approximately 12 h before fentanyl administration and 30 min and 12 h after fentanyl administration, according to FDA Draft Guidance on fentanyl citrate (24). Naltrexone hydrochloride is an opioid antagonist with no opioid agonist properties which blocks the effect of opioids by competitive binding at opioid receptors, decreasing the adverse events of fentanyl.

Fentanyl was administered by sublingual oral route. Subjects fasted from 10 hours before until 5 hours after administration. Each individual was given a standardized meal that was the same during the two admission phases. Five hours after the administration they received a meal consisting of pasta, meat with potatoes and one yogurt. Nine hours after the drug administration they received a snack consisting of two glasses of milk or fruit juice and a cake. Finally, dinner (salad, omelette and fruit) was served 12 hours after dosing. For the pharmacokinetic analysis, 22 blood samples were obtained in EDTA K2 tubes at baseline (before administration), 0.17 h, 0.33 h, 0.5 h, 0.67 h, 0.83 h, 1 h, 1.25 h, 1.50 h, 1.75 h, 2 h, 2.5 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h, 12 h, 16 h, 24 h and 32 h postdose. Samples were centrifuged at 3,500 revolutions per minute (rpm) (1900 x g) and then, plasma was collected and stored at -20º C until its shipment to the external analytical laboratory.

Plasma concentrations of fentanyl were quantified by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) in an external laboratory.
The method was validated according to EMA guidelines (25) and had a lower limit of quantification of 10 pg/mL. First of all, 150 µL of internal standard working solution and 500 µL of ammonia 0.625% (0.33M) were transferred into tubes containing 100 µL of plasma from each sample (study sample, calibration standard or quality control) and then it was extracted with tert-butyl methyl ether. Chromatographic separations were performed on a reverse-phase column (Acquity UPLC BEH C18, 1.7 µm, 50 x 2.1 mm, from Waters Corp., MA, USA). The mobile phase A was ammonium formate 2 mM prepared in water, formic acid 0.1%. The mobile phase B consisted of formic acid 0.1% prepared in acetonitrile. The chromatographic separation was isocratically performed at room temperature at a flow-rate of 0.6 mL/min.

Pharmacodynamic analysis

Systolic and diastolic blood pressure (SBP/DBP) and 12-lead ECG were measured in supine position before the drug was administered, 0.5 and 2 hours after dosing in each period. QT and HR values were automatically calculated by the ECG device. To correct the QT interval (QTc), the Bazett correction formula was used (26). According to the International Council for Harmonisation E14 clinical guidance (27) we considered QTc interval prolongation an absolute QTc interval greater than 450 milliseconds or a change from baseline in QTc interval greater than 30 milliseconds. This interval is studied as an indicator of potential AEs on the heart such as fatal cardiac arrhythmias (28).

Pharmacokinetic analysis

Pharmacokinetic parameters were estimated by non-compartmental methods using WinNonlin Professional Edition (version 7.0, Scientific Consulting, Inc, Cary USA). The maximum plasma concentration (Cmax) and Tmax were obtained directly from raw data. The area under the curve (AUC) from administration to last measurement (AUCt) was calculated according to the linear trapezoidal rule. The total AUC from administration to infinity (AUC∞) was calculated as the sum of AUCt and the residual area (Ct divided by ke, with Ct as the last measured concentration and ke as the apparent terminal elimination rate constant, which was estimated by log-linear regression from the
terminal portion of the log-transformed concentration–time plots. $T_{1/2}$ was calculated as $-\ln2/k_e$. Values of AUC correspond to $AUC_t$ unless otherwise indicated. The total drug clearance adjusted for bioavailability (Cl/F) was calculated by dividing the dose by the $AUC_{\infty}$. Volume of distribution adjusted for bioavailability (Vd/F) was calculated as Cl/F divided by $k_e$.

All parameters were logarithmically transformed for statistical analysis to obtain a normal distribution, which was confirmed with a Shapiro-Wilks normality test. $AUC_t$ and Cmax were adjusted for dose and weight (dW) whereas Cl/F and Vd/F were adjusted for weight (W). Since the study showed bioequivalence (figure 1), we analysed both formulations for each subject.

Safety and tolerability assessment

The safety and tolerability of fentanyl were assessed by clinical evaluation of AEs and other parameters including vital signs, physical examination and ECG. Throughout the study, volunteers were asked about any experienced AE. Additionally, those AEs that were spontaneously notified by the volunteers were documented. Causality was determined using the Karch and Lasagna criteria (29), according to five types of AE: definite, probable, possible, unlikely and unrelated. Only definite, probable or possible AEs were considered as adverse drug reactions (ADRs) and included in the statistical analysis. Time sequence, intensity and outcome of AEs were also recorded.

Sedation was assessed applying the Ramsay Score, which uses a 6-point scale to determine the state of volunteers (1: subjects agitated, restless or both, 2: subjects cooperative and orientated, 3: drowsy subjects that respond to simple commands, 4: asleep with brisk response to stimulus, 5: asleep with slow response to stimulus, 6: asleep without reply to stimulation) (30). As volunteers only presented the conditions 2 and 3, we assigned the name “somnolence” to the number 3 of the scale and we studied it as a separate ADR.

Genotyping

DNA was extracted from 1 mL of peripheral blood samples using an automatic DNA extractor (MagNa Pure® System, Roche Applied Science, IN, USA). DNA was quantified
spectrophotometrically in a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Inc, DEL, USA). In addition, the purity of the samples was measured by 260/280 absorbance ratio.

The 9 variants studied were CYP3A4*20 (rs67666821), CYP3A4*22 (rs35599367), CYP3A5*3 (rs776746), ABCB1 C3435T (rs1045642), ABCB1 C1236T (rs1128503), ABCB1 G2677T/A (rs2032582), OPRM1 A118G (rs1799971), COMT G472A (rs4680) and ADRB2 C523A (rs1042718), which were all genotyped by real-time PCR using a StepOne® PCR Instrument (Applied Biosystems, CA, USA) and TaqMan assays following the manufacturer recommendations (Applied Biosystems, CA, USA), except for CYP3A4*20, which was genotyped using KASPar SNP Genotyping System (LGC Genomics, Herts, UK). The Sequence Detection System ABI PRISM 7900HT (Applied Biosystems, Darmstadt, Germany) was used for fluorescence detection and allele assignment (31).

**Statistical analysis**

Statistical analysis was performed with the SPSS 24.0 software (SPSS Inc., IL, USA). Statistical significance was set at p-values (p) lower or equal than 0.05. Hardy-Weinberg equilibrium was estimated for all analysed variants. Deviations from the equilibrium were detected by comparing the observed and expected frequencies using a Fisher exact test based on the De Finetti program (available at https://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Differences in the genotypic frequencies according to sex were determined using a corrected Pearson $\chi^2$ test. This test was also used to compare the relationship between ADRs and genotypes. To study the influence of genotypes and sex on pharmacokinetics and pharmacodynamics, a parametric univariate analysis was conducted (t-test or ANOVA). Bonferroni’s post hoc test was used to compare means between groups.

In order to simplify the analysis, G2677T/A genotypes were divided into three groups depending on the activity of the alleles: C/C, C/A + C/T and A/A + A/T. In addition, due to their low number, ADRs were classified into four groups: nausea/vomiting, headache/dizziness, abdominal alterations (constipation, abdominal discomfort and abdominal pain) and somnolence.
Multiple linear regression models were used to study factors related to all the pharmacokinetic and pharmacodynamic dependent variables. Logistic regression models with dichotomous outcomes were used to study factors related to ADRs. Categorical variables with more than two categories, such as polymorphisms, were transformed to dummy variables.

RESULTS

Demographic characteristics and genotypic frequencies

Our study population comprised 35 healthy volunteers (19 men and 16 women) who participated in the bioequivalence trial and were enrolled in the pharmacogenetic analysis. Average age was similar between men and women (26.37 ± 4.95 years versus 25.69 ± 6.14 years respectively; p = 0.719). Nevertheless, men were taller (1.75 ± 0.07 m versus 1.63 ± 0.05 m; p < 0.001), weighed more (75.95 ± 8.61 kg versus 57.63 ± 7.68 kg; p < 0.001) and exhibited higher BMI values (28.29 ± 2.84 kg/m² versus 25.93 ± 2.43 kg/m²; p < 0.05). Twenty-six volunteers were Caucasians and 9 were Latin-Americans.

Genotype frequencies of the genes evaluated are shown in Table 1. All genetic variants were in Hardy-Weinberg equilibrium except for CYP3A4*20, which was monomorphic as all subjects carried the wild-type genotype. No differences were observed in genotype frequencies between men and women. Since 25% of our study population were Latin and 75% Caucasians, we compared the genotype frequencies with the one of reference listed on 1000 Genomes Project (32), finding no significant differences in none of the studied genes.

Pharmacokinetic analysis

Mean and standard deviation (SD) of pharmacokinetic parameters are shown in table 2. Women showed significantly higher $C_{\text{max}}$, but this difference disappeared after correction for dose and weight. Moreover, $V_d$ was higher in women compared to men, significant after correction for weight and other covariates (30.6 L/kg versus 20.7 L/kg; non-standardised $\beta$ coefficient = 0.423, $p = 0.002$). However, in the multivariate analysis, sex appeared to be a factor that affects $\text{AUC/dW}$, as women had lower values (non-standardised $\beta$ coefficient = -0.312, $p = 0.024$).
As shown in Table 3, we found significant association between some pharmacokinetic parameters and polymorphisms in CYP3A4 and ABCB1. Carriers of CYP3A4 *1/*22 genotype showed a higher AUC (non-standardised β coefficient = 0.982, p = 0.002) and a lower Cl (non-standardised β coefficient = -0.86, p = 0.015). On the contrary, we did not find any correlation between fentanyl pharmacokinetic parameters and CYP3A5*3 variant.

Regarding ABCB1 C1236T variant, individuals with the C/T genotype showed a lower AUC/dW (non-standardised β coefficient = -0.548, p < 0.001) and T_{1/2} (non-standardised β coefficient = -0.587, p = 0.001) and a higher Cl/W (non-standardised β coefficient = 0.566, p = 0.001) than subjects carrying C/C and T/T genotypes. However, we did not observe any difference regarding ABCB1 C3435T and G2677T/A variants.

Pharmacodynamic analysis

Fentanyl had an hypotensive effect, since it reduced the SBP by 5.7 mmHg and 7.3 mmHg after 30 min and 2 h post-dose, respectively (p=0.001). However, we did not find any relationship between the pharmacokinetic parameters of fentanyl and BP, HR and QTc. Regarding differences between sexes, women showed lower BP and higher QTc.

Regarding the effect of polymorphisms on pharmacodynamics parameters, we observed a tendency towards a reduced SBP 30 minutes after drug administration (4.2 ± 6.6 mmHg vs. 8.3 ± 4.59 mmHg; p = 0.055) in subjects heterozygous for ADRB2 C523A variant. No other gene was related to changes in BP, HR or QTc.

Adverse drug reactions

During the study, no severe, serious or life-threatening AEs were registered. A total of 8 volunteers (22.9%) suffered from at least one ADR. From those, the most common were somnolence (37.1%), nausea/vomiting (11.4%) and abdominal discomfort (8.3%). The incidence of ADRs was higher in women than in men (31.3% and 15.8%, respectively), but it was not statistically significant (P=0.424).
OPRM1 A118G polymorphism was associated with somnolence as carriers of the G allele showed a higher risk of suffering from this ADR (OR = 8.352, OR 95% CI [1.691-54.788], p = 0.021). Specifically 84.6% of volunteers with the mutant allele presented this ADR, whereas only the 34.6% of volunteers with the wild-type genotype suffered from somnolence. Similarly, regarding the COMT G472A polymorphism we found that 59.1% of heterozygous G/A individuals had a higher risk of suffering from somnolence than wild-type homozygotes (OR = 8.788, OR 95% CI [1.158-66.659] p = 0.036), who suffer from this ADR in 50% of cases.

There was no other significant association between the studied polymorphisms nor the pharmacokinetic parameters and the incidence of ADRs.

**DISCUSSION**

**Fentanyl pharmacokinetics**

In regard to pharmacokinetic parameters, our results were comparable to those found in the literature (33,34). Moreover, these values varied according to sex. This circumstance is consistent with the well-known influence of sex on drug pharmacokinetics. Due to body size differences, men usually show higher Vd than women. However, in this case, Vd was higher in women after correction for weight, since fentanyl is a very lipophilic drug and women have a greater fat percentage than men (35,36). Nevertheless, these differences are small and probably not sufficient to justify a dose adjustment according to sex.

Concerning the influence of genotypes on fentanyl pharmacokinetics, we found higher AUC and lower Cl in subjects carrying the CYP3A4*22 allele. This variant plays an important role since it results in a reduced mRNA expression, thus, in a low CYP3A4 enzyme activity (15,16). Therefore, our results are consistent with the expected since a lower activity would produce a lower metabolism of fentanyl. Other variants in CYP3A4 were investigated but not related with exposure to fentanyl (37). To the best of our knowledge, this is the first study to associate the CYP3A4*22 variant with the pharmacokinetics of fentanyl. Additionally, regarding CYP3A5, previous studies showed a two-fold increase in systemic exposure to fentanyl linked to the presence of
CYP3A5*3 allele. However, Barrat et al. conducted a study in 620 cancer pain patients finding that both CYP3A4*22 and CYP3A5*3 account for only a small proportion of variability (<2%) of the metabolic ratio norfentanyl/fentanyl (38). Moreover, since fentanyl is mainly metabolized by CYP3A4, although CYP3A5 contributes to CYP3A-dependent drug clearance, it is expected that a reduced-function allele in CYP3A4 could have a greater implication in a diminished fentanyl’s metabolism. Moreover, as subjects carrying the CYP3A4*22 allele showed a 47% reduction in clearance, it would be expected an even higher reduction in *22/*22 subjects. However, further research is required to confirm our results.

After multivariate analysis including sex and genotypes, women showed significantly lower AUC values. After performing a collinearity test, we found that both sex and CYP3A4*22 were independent and influenced pharmacokinetics. Thus, it could be explained by the higher activity of CYP3A4 in women, as previously reported (39).

Regarding ABCB1, there is, to date, some controversy over the clinical implications of its three most common variants (7,18,40). ABCB1 C1236T and ABCB1 C3435T are synonymous mutations, which means they do not produce a change in the amino acid sequence (18). Even though these polymorphisms do not change the protein structure, they may induce a change in the substrate joining site and inhibitor interaction site (20). Interestingly, in our study, mutant homozygous have similar values to those present in wild-type homozygous. A plausible justification could be the fact that the volunteers with the T/T genotype are men, who tend to have higher AUC/dW values, or the size of the population studied, that does not allow us to obtain conclusive results.

Fentanyl pharmacodynamics and adverse drug reactions

Fentanyl had a hypotensive effect, which is described as a non-frequent adverse event in fentanyl’s drug label (9).

Regarding the ADRB2 C523A variant, our results are similar to those found in the literature, which state that A/A homozygotes present a lower arterial BP (23). Although none of the subjects carried the A/A genotype to compare our results with the
mentioned, we observed a tendency in the heterozygous individuals towards a reduced BP.

However, almost 40% of the volunteers experienced somnolence, which was significantly associated with the carriage of the OPRM1 A118G G allele. The OPRM1 gene encodes for the µ-opioid receptor (4), thus having a direct implication on the analgesic effect that fentanyl produces. Specifically, the A118G substitution is considered to underlie discrepancies in the analgesic requirements and sensitivity to opioids as it has been related with a reduced signal transduction, reduced expression of OPRM1 and reduced binding affinity for opioids such as morphine (17,19). This explains the higher dose requirements in people carrying the G allele. Additionally, literature describes this variant as a protective polymorphism against AEs (19), which, considering the high dose requirements of carriers of this allele, was unexpected. On the other hand, other studies have proven a tendency towards reduced opioid requirements when carrying the G allele (41), which is concordant with our results as volunteers with lower requirements received the same dose than those with higher requirements, having thus more side effects even after plasma levels correction. However, as a single dose to naltrexone-treated healthy volunteers designed study, we were not able to evaluate the differences in efficacy or dose requirements.

Similarly, the polymorphism G472A in COMT is known to be involved in dose adjustment, as carriers of the minor A allele require lower doses (19). Our results confirm those previously described as we found a higher risk of somnolence in carriers of the A allele. Contrary to what was expected, the mutant homozygotes did not appear in our analysis as a significant variable. A possible explanation could be that only 5 volunteers presented the A/A genotype, which complicates the analysis of the impact of this genotype. However, the fact that two of these five volunteers (40%) suffered from somnolence indicates a tendency for enduring this ADR when carrying the mutant allele. Further studies with preselected subjects with wild-type homozygous versus mutant homozygous genotypes could be useful to elucidate whether those with the presence of the polymorphism show higher risk of suffering from somnolence.
Nevertheless, the small sample size of the studied population, the low number of volunteers who suffered from ADRs due to naltrexone treatment, or the fact that the drug was orally administered, which is known to produce less AEs in comparison to intravenous or subcutaneous administration (42) makes it difficult to give them consistency. These reasons also allow us to explain the absence of any association between the AEs and other genes previously described in the literature such as ABCB1 and ADRB2. The most commonly studied variants of ABCB1 (C3435T, C1236T and G2677T/A) have generally been associated with the accumulation of opioids in the brain, leading to an increased risk of AEs (19). Regarding ADRB2, although a mild association was found in our analysis, the C523A variation has been described as a factor related to hypotension (23), which was not observed in the study.

Study limitations

The study was performed after single-dose administration to healthy subjects, which prevents us from assessing long-term effectiveness and safety. Moreover, they were treated with naltrexone because of safety reasons, which limited the evaluation of adverse reactions and pharmacodynamics. Pharmacokinetics, pharmacodynamics, and tolerability might vary in patients receiving chronic treatment. It is of importance that these results are interpreted with caution given the small sample size. However, a single-dose design in healthy subjects can assess the effect of genetic polymorphisms over fentanyl without other confounding factors such as smoking or concomitant treatment. Larger studies are needed to increase the statistical power of these results. The validity of these results undoubtedly needs to be confirmed in other similar settings and by studies in patients receiving chronic fentanyl treatment.

CONCLUSIONS

Fentanyl pharmacokinetics is affected by sex, having women higher values of Vd/W probably due to their higher percentage of adipose tissue and the lipophilic properties of fentanyl. Moreover, women showed lower AUC values. CYP3A4 influences pharmacokinetic parameters as carriers of the *22 allele, which is known to reduce the mRNA expression, showed higher AUC and lower Cl values. However, given the small
sample size, this finding needs to be confirmed. In addition, carriers of the \( \text{ABCB1} \) C1236T variant presented a lower AUC and higher Cl, as well as lower \( T_{1/2} \). As subjects were blocked with naltrexone, the effect of fentanyl on pharmacodynamics might be biased; however, we could observe that fentanyl had a hypotensive effect. In addition, \( \text{ADRB2} \) C523A polymorphism showed a tendency towards reducing SBP. The minor allele variants of polymorphisms in \( \text{OPRM1} \) and \( \text{COMT} \) are risk factors for development of somnolence. \( \text{CYP3A5}, \text{ABCB1} \) C3435T and \( \text{ABCB1} \) G2677T/A were not associated with fentanyl’s pharmacokinetics, pharmacodynamics and safety profile.
REFERENCES


function allele in the Spanish population classifies CYP3A4 as a polymorphic enzyme.


Table 1. Genotype frequencies of enzymes, transporters and receptors in the study subjects.

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<tr>
<td><strong>ABCB1 / C3435T</strong></td>
<td>C/C</td>
<td>12 (34.3)</td>
<td>6 (31.6)</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>18 (51.4)</td>
<td>9 (47.4)</td>
<td>9 (56.3)</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>5 (14.3)</td>
<td>4 (21.2)</td>
<td>1 (6.3)</td>
</tr>
<tr>
<td><strong>ABCB1 / C1236T</strong></td>
<td>C/C</td>
<td>17 (48.6)</td>
<td>7 (36.8)</td>
<td>10 (62.5)</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>15 (42.9)</td>
<td>9 (47.4)</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>3 (8.6)</td>
<td>3 (15.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>ABCB1/C2677AT</strong></td>
<td>C/C</td>
<td>15 (42.9)</td>
<td>7 (36.8)</td>
<td>8 (50)</td>
</tr>
<tr>
<td></td>
<td>C/T+C/A</td>
<td>16 (45.7)</td>
<td>8 (42.1)</td>
<td>8 (50)</td>
</tr>
<tr>
<td></td>
<td>A/A+A/T</td>
<td>4 (11.4)</td>
<td>4 (21.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>OPRM1 / A118G</strong></td>
<td>A/A</td>
<td>22 (62.9)</td>
<td>11 (57.9)</td>
<td>11 (68.8)</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>12 (34.3)</td>
<td>8 (42.1)</td>
<td>4 (25)</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>1 (2.9)</td>
<td>0 (0)</td>
<td>1 (6.3)</td>
</tr>
<tr>
<td><strong>COMT / G472A</strong></td>
<td>G/G</td>
<td>8 (22.9)</td>
<td>5 (26.3)</td>
<td>3 (18.8)</td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>22 (62.9)</td>
<td>12 (63.2)</td>
<td>10 (62.5)</td>
</tr>
<tr>
<td></td>
<td>A/A</td>
<td>5 (14.3)</td>
<td>2 (10.5)</td>
<td>3 (18.8)</td>
</tr>
<tr>
<td><strong>ADRB2 / C523A</strong></td>
<td>C/C</td>
<td>22 (62.9)</td>
<td>10 (52.6)</td>
<td>12 (75)</td>
</tr>
<tr>
<td></td>
<td>C/A</td>
<td>13 (37.1)</td>
<td>9 (47.4)</td>
<td>4 (25)</td>
</tr>
</tbody>
</table>

Values are expressed as number of subjects (%). Abbreviations: ABCB1, ATP binding cassette subfamily B member 1; ADRB2, adrenoceptor beta 2; COMT, catechol-O-methyltransferase; CYP, cytochrome p450 oxidase; OPRM1, opioid receptor mu 1.
Table 2. Pharmacokinetic parameters of fentanyl after a single oral dose of 300 µg

<table>
<thead>
<tr>
<th>Parameters</th>
<th>All (n=35)</th>
<th>Men (n=19)</th>
<th>Women (n=16)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (pg·h/mL)</td>
<td>3388.5 (1647.5)</td>
<td>3139.6 (1410.6)</td>
<td>3684 (1895.5)</td>
<td>0.366</td>
</tr>
<tr>
<td>AUC/dW (pg·h·mg/mL·kg)</td>
<td>752.4 (362.2)</td>
<td>805.4 (399.5)</td>
<td>689.3 (313.0)</td>
<td>0.396</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (pg/mL)</td>
<td>644.9 (191.9)</td>
<td>576 (138.7)</td>
<td>726.8 (217.3)</td>
<td>0.033</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;/dW (pg·mg/mL·kg)</td>
<td>142.3 (38.7)</td>
<td>145.6 (38.7)</td>
<td>138.3 (39.5)</td>
<td>0.526</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>14.4 (7.0)</td>
<td>13.4 (8.3)</td>
<td>15.7 (5.0)</td>
<td>0.116</td>
</tr>
<tr>
<td>Vd (L)</td>
<td>1656 (590.8)</td>
<td>1554.7 (595.6)</td>
<td>1776.2 (580.6)</td>
<td>0.265</td>
</tr>
<tr>
<td>Vd/W (L/kg)</td>
<td>25.2 (9.6)</td>
<td>20.7 (8.1)</td>
<td>30.6 (8.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>Cl (L/h)</td>
<td>975.1 (541.1)</td>
<td>1053.1 (617.3)</td>
<td>882.4 (435.4)</td>
<td>0.349</td>
</tr>
<tr>
<td>Cl/W (L/h·kg)</td>
<td>14.6 (7.5)</td>
<td>14.1 (80.0)</td>
<td>15.2 (71.4)</td>
<td>0.539</td>
</tr>
</tbody>
</table>

Values are shown as mean (standard deviation). Abbreviations: dW, corrected for dose/weight; W, corrected for weight.
Table 3. Association between the studied polymorphisms and the pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>n</th>
<th>AUC/dW (pg·h·mg/mL·kg)</th>
<th>C_{max}/dW (pg·mg/mL·kg)</th>
<th>T_{1/2} (h)</th>
<th>Vd/W (L/kg)</th>
<th>Cl/W (L/h·kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A4*22</td>
<td>*1/*1</td>
<td>33</td>
<td>733.8 (364.5)</td>
<td>140.6 (39.2)</td>
<td>14.3 (7.2)</td>
<td>25.6 (9.7)</td>
<td>15.0 (7.6)</td>
</tr>
<tr>
<td></td>
<td>*1/*22</td>
<td>2</td>
<td>1058.6 (96.3)*</td>
<td>169.4 (6.8)</td>
<td>16.3 (3.0)</td>
<td>18.5 (0.6)</td>
<td>8.0 (1.2)*</td>
</tr>
<tr>
<td>CYP3A5*3</td>
<td>*1/*3</td>
<td>7</td>
<td>791.2 (422.4)</td>
<td>145.0 (45.1)</td>
<td>16.0 (7.5)</td>
<td>25.8 (10.4)</td>
<td>14.3 (8.9)</td>
</tr>
<tr>
<td></td>
<td>*3/*3</td>
<td>28</td>
<td>742.6 (353.6)</td>
<td>141.6 (37.8)</td>
<td>14.0 (7.0)</td>
<td>25.1 (9.5)</td>
<td>14.7 (7.3)</td>
</tr>
<tr>
<td>ABCB1</td>
<td>C/C</td>
<td>17</td>
<td>872.9 (416.4)</td>
<td>703.2 (180.2)</td>
<td>17.8 (7.3)</td>
<td>27.6 (10.4)</td>
<td>11.9 (5.3)</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>15</td>
<td>595.0 (258.5)*</td>
<td>606.0 (196.9)</td>
<td>10.7 (5.4)*</td>
<td>23.2 (8.8)</td>
<td>18.5 (8.7)*</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>3</td>
<td>855.9 (222.7)</td>
<td>509.3 (164.7)</td>
<td>14.0 (2.5)</td>
<td>21.5 (7.0)</td>
<td>10.6 (2.7)</td>
</tr>
<tr>
<td>ABCB1</td>
<td>C/C</td>
<td>12</td>
<td>755.5 (325.3)</td>
<td>148.3 (39.0)</td>
<td>17.8 (7.8)</td>
<td>30.4 (9.6)</td>
<td>13.1 (4.7)</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>18</td>
<td>714.3 (380.7)</td>
<td>139.4 (38.8)</td>
<td>12.6 (6.3)</td>
<td>23.5 (9.0)</td>
<td>16.2 (8.8)</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>5</td>
<td>881.6 (425.0)</td>
<td>138.4 (44.2)</td>
<td>12.8 (5.4)</td>
<td>18.9 (6.2)</td>
<td>12.7 (8.2)</td>
</tr>
<tr>
<td>ABCB1</td>
<td>C/C</td>
<td>15</td>
<td>908.8 (431.2)</td>
<td>158.2 (38.3)</td>
<td>18.0 (7.7)</td>
<td>26.8 (10.8)</td>
<td>11.5 (5.5)</td>
</tr>
<tr>
<td></td>
<td>C/T+C/A</td>
<td>16</td>
<td>602.2 (249.3)</td>
<td>128.9 (35.9)</td>
<td>11.8 (5.4)</td>
<td>25.3 (8.7)</td>
<td>17.9 (8.5)</td>
</tr>
<tr>
<td></td>
<td>A/A+A/T</td>
<td>4</td>
<td>766.0 (255.7)</td>
<td>135.8 (37.0)</td>
<td>11.6 (5.3)</td>
<td>19.1 (7.5)</td>
<td>12.8 (5.0)</td>
</tr>
</tbody>
</table>

* p < 0.05; Values are shown as mean (standard deviation). Abbreviations: ABCB1, ATP binding cassette subfamily B member 1; CYP, cytochrome p450 oxidase; dW, corrected for dose/weight; W, corrected for weight.
Figure 1. Mean time curve of fentanyl plasma concentrations.