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Impact of polymorphisms in transporter and metabolizing enzyme genes on olanzapine pharmacokinetics and safety in healthy volunteers

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ABSTRACT

Olanzapine is an atypical antipsychotic widely used for the treatment of schizophrenia, which often causes serious adverse drug reactions. Currently, there are no clinical guidelines implementing pharmacogenetic information on olanzapine. Moreover, the Dutch Pharmacogenomics Working Group (DPWG) states that CYP2D6 phenotype is not related to olanzapine response or side effects. Thus, the objective of this candidate-gene study was to investigate the effect of 72 polymorphisms in 21 genes on olanzapine pharmacokinetics and safety, including transporters (e.g. *ABCB1*, *ABCC2, SLC22A1*), receptors (e.g. *DRD2*, *HTR2C*), and enzymes (e.g. *UGT*, *CYP* and *COMT*), in a cohort of healthy volunteers. Polymorphisms in *CYP2C9*, *SLC22A1*, *ABCB1*, *ABCC2*, and *APOC3* were related to olanzapine pharmacokinetic variability. The incidence of adverse reactions was related to several genes: palpitations to *ABCB1* and *SLC22A1*, asthenia to *ABCB1*, somnolence to *DRD2* and *ABCB1*, and dizziness to CYP2C9. However, further studies in patients are warranted to confirm the influence of these genetic polymorphisms on olanzapine pharmacokinetics and tolerability.

1. Introduction

Schizophrenia is a serious mental disorder that significantly reduces patients' quality of life. Currently, 1% of the population suffers from this disease, being more frequent and appearing earlier in men than in women [1,2]. The management of the disease requires pharmacological treatment, i.e. antipsychotics [3]. Olanzapine is a first-line atypical antipsychotic, more effective in reducing positive symptoms than other atypical antipsychotics, but associated with greater metabolic effects [4]. It is a thienobenzodiazepine antagonist to dopamine DRD1, DRD2, DRD3 and DRD4, serotonin 5-HT2A, 5-HT2C, 5-HT3, 5-HT6, α1 adrenergic, histamine H1 and M1-M5 muscarinic receptors [5].

Olanzapine is extensively absorbed and shows linear pharmacokinetics. The time to reach the maximum concentration (C_{max}) is 5–8 h (t_{max}). It binds extensively to plasma proteins (93 %), predominantly albumin and α1-acid-glycoprotein, and shows a volume of distribution of 1150 L [5]. Olanzapine is metabolized by glucuronidation to its main metabolite, the 10-*N*-glucuronide, apparently by UDP-glucuronosyltransferase (UGT) isoforms UGT1A4 and UGT2B10 [6]. The cytochrome P450 (CYP) isoforms CYP1A2 and CYP2D6 transform the parent drug to the N-desmethyl and 2-hydroxymethyl metabolites. Depending on health status, age and gender, the elimination half-life ($t_{1/2}$) ranges between 32.3–51.8 h and the clearance between 17.5–27.7 l/h [5]. Nicotine is a strong CYP1A2 inducer, therefore, smoking is related to an increased olanzapine metabolism [7].

Olanzapine can cause moderate to severe adverse drug reactions (ADRs). Among them, the most frequent (>1%) are: metabolic disorders (e.g. weight gain, elevated cholesterol, glucose or triglyceride levels);

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nervous systems disorders (e.g. somnolence, dizziness, akathisia, parkinsonism or dyskinesia); blood and lymphatic system disorders (e.g. eosinophilia or leukopenia); gastrointestinal disorders (i.e. anticholinergic effects including constipation and dry mouth); hepatobiliary disorders (i.e. transient asymptomatic elevations of hepatic aminotransferases); reproductive system disorders (e.g. erectile dysfunction or decreased libido); other ADRs (e.g. hyperprolactinemia, high uric acid, asthenia, fatigue, pyrexia, arthralgia). The possibility of developing one or more of these ADRs justifies prescribing olanzapine at the lowest safe and effective dose [5]. To date, no current dosing recommendations for olanzapine are available based on UGT or CYP1A2/CYP2D6 phenotype. Regarding *UGT1A4* and *UGT2B10*, to our knowledge, there are no polymorphisms described yet with sufficient prevalence and relevant functional impact. Moreover, the Dutch Pharmacogenomics Working Group (DPWG) states that CYP2D6 intermediate (IM) and poor metabolizers (PM) are not related to olanzapine response or side effects, however, no data are available for ultrarapid metabolizers (UM) [8]. Thus, the objective of this candidate-gene study was to investigate the effect CYP2D6 UM phenotype and of 72 polymorphisms in 21 genes on olanzapine pharmacokinetics and safety, including transporters, receptors and enzymes.

2. Materials and methods

2.1. Study population

This work is a continuation of other studies with antipsychotics conducted at the Hospital Universitario de La Princesa. Regarding this work, all healthy volunteers participating in the present pharmacogenetic study were previously enrolled in three clinical trials performed at the Clinical Trials Unit of Hospital Universitario de La Princesa (UECHUP), Madrid (Spain) in 2018 and 2019. One of them was a multiple-dose phase-1 clinical trial (EUDRA CT: 2018−000744-26) and two of them were bioequivalence clinical trials (EUDRA CT: 2018−000994-58 and 2018−002875-16). The safety and pupillometry effects of aripiprazole and olanzapine in the multiple-dose clinical trial were previously published [8,9]. All of them were duly authorised by the Spanish Drugs Agency (AEMPS) and the Research Ethics Committee of Hospital Universitario de La Princesa. Every healthy volunteer (n = 80) signed the informed consent for participation in the clinical trial, of which 67 consented to participate in the pharmacogenetic study. Both the development of the trials and the handling of data were carried out in accordance with Spanish legislation, the International Council on Harmonisation (ICH) guidelines on Good Clinical Practice [11] and the Revised Declaration of Helsinki [12].

As criteria for inclusion, volunteers with the following characteristics were recruited: males or females aged from 18 to 55, free from organic or psychiatric conditions, with normal medical records and physical examination, without significant abnormalities in laboratory tests (haematological, biochemical and other parameters that present values outside of the normal range defined in the protocol of the clinical trials) with normal vital signs and electrocardiogram (ECG), without allergies to any drug and capable of following the instructions during the study. The following exclusion criteria were applied: use of prescribed pharmacological treatment with CYP1A2 inhibitors or inducers in the last 2–4 weeks (depending on the clinical trial) or any kind of medication in the 48 h prior to enrolment; elevated blood pressure (>140/90 mmHg), more than 100 bpm or fever (>37.7 °C at screening examination); undergoing any surgical procedure in the previous month; vegetarian or any special diet; not agreeing to avoid products containing alcohol, grapefruit or caffeine 48 h prior to drug intake; body mass index outside of the 18.5–30.0 range; positive drug screening; smoking and alcoholism; blood donation in the last month before enrolment; pregnancy or breastfeeding women; participation in a similar clinical trial in the previous 3 months or not surpassing five half-lives; lactose intolerance; risk of narrow angle glaucoma.

2.2. Study design and procedures

The two bioequivalence trials compared 2.5 or 5 mg olanzapine test capsule formulations with Zyprexa® 2.5 or 5 mg (Eli Lilly Nederland B. V.), respectively. Since the test formulations demonstrated to be bioequivalent to Zyprexa®, the arithmetic mean of the pharmacokinetic parameters was calculated for each volunteer. They were phase 1, open, two sequence, two period, randomised, crossover, single-dose fasting, one-centre clinical trials. In the first period, half of the volunteers received the test formulation and the other half received Zyprexa®. In the second period, after a 14-day washout period, the groups were exchanged, resulting in two different sequences. In both periods, safety was evaluated, and blood samples were obtained for the determination of plasma levels and genotyping. On the previous night of drug intake, subjects were hospitalized. The drug was administered at 9 a.m. in the morning and the volunteers remained hospitalized until 9:30 pm. The volunteers returned 24 h, 48 h and 72 h after drug intake for additional blood extractions and safety visits.

Concerning the multiple-dose clinical trial [9,10], it aimed to compare the metabolic effects and tolerability of multiple administration of two atypical antipsychotics: olanzapine 5 mg and aripiprazole 10 mg tablets (Alter Laboratories, SA) in healthy volunteers. It was a phase-1, multiple-dose, open, randomized, crossover, two-period, two-sequence clinical trial: in the first period, half of the volunteers received olanzapine and the other half received aripiprazole. In the second period, after a 28-day washout period, the groups were exchanged, resulting in two different sequences. In both periods the volunteers were hospitalized for 5 days during which they were given the corresponding drug once daily under fasting conditions. Subjects were hospitalized from 1 h before the first dose until 24 h after the last drug administration. The volunteers returned 48 h, 96 h, 144 h and 240 h after the last drug administration for additional blood extraction and safety visits.

2.3. Pharmacokinetic analysis

For bioequivalence clinical trials, 21 olanzapine plasma determinations were performed between pre-dose and 72 h after drug intake. As for the multiple-dose clinical trial, only the first 24 h of the concentration-time curve were considered, since the second dose was administered after 24 h. During this period, 8 olanzapine plasma determinations were performed.

Pharmacokinetic parameters were calculated by non-compartmental analysis. For this purpose, the WinNonLin Professional Software was used (version 8.1, Pharsight Corporation, Palo Alto, California, USA). The area under the curve (AUC) between the pre-dose and the last observed time point was calculated according to the linear trapezoidal standard (AUCt). Several parameters were calculated as derived from the AUC. First, total drug clearance adjusted for bioavailability (Cl/F) was calculated as the dose divided by AUC_{∞} and weight. Second, the volume of distribution was calculated dividing Cl/F by the terminal rate constant (k_e). The k_e was calculated as the slope of the line traced over the log-linear part of the concentration-time curve, calculated by linear regression. Other parameters were observed directly in the time-concentration graph: C_{max} and t_{max}. Finally, $t_{1/2}$ was calculated as –ln 2/ke.

2.4. Safety

In the three clinical trials, safety was assessed through measurements of vital signs and blood, biochemical, urine and serological tests. Physical examination was established at pre-dose and follow-ups. Measurements of blood pressure (BP), heart rate (HR) and 12-lead electrocardiograms (ECG) were scheduled at pre-dose and at several occasions in the following 24 h, depending on the clinical trial design (e.g., 4 h, 6 h, 8 h, 12 h post-dose). Adverse events (AEs) spontaneously

reported by volunteers or reported after open question were also collected. Causality assessment was performed following the algorithm of Spanish pharmacovigilance system [13]. Only those AEs with a definite, probable or possible relationship with olanzapine intake were considered adverse drug reactions (ADRs).

2.5. Genotyping

DNA was extracted from blood samples collected from volunteers in EDTA-K2 tubes using a MagNA Pure instrument (Roche Applied Science, USA). Genotyping was performed with a QuantStudio 12 K Flex qPCR instrument with an OpenArray thermal block (Applied Biosystems, Thermofisher, USA). A custom array was used to genotype the following SNPs: *CYP1A2**1C (rs2069514), *1 F (rs762551), *1B (rs2470890), *CYP2A6**9 (rs28399433), *CYP4F2* rs2108622, *CYP2B6**9 (rs3745274), *5 (rs3211371), rs4803419, rs2279345, *4 (rs2279343), *CYP2C8**2 (rs11572103), *3 (rs10509681), *4 (rs1058930), *CYP2C9**2 (rs1799853), *3 (rs1057910), *CYP2C19**2 (rs4244285), *3 (rs4986893), *4 (rs28399504), *17 (rs12248560), *CYP2D6**3 (rs35742686), *4 (rs3892097), *6 (rs5030655), *7 (rs5030867), *8 (rs5030865), *9 (rs5030656), *10 (rs1065852), *14 (rs5030865), *17 (rs28371706), *41 (rs28371725), *CYP3A4**22 (rs35599367), *2 (rs55785340), *6 (rs46464389), *CYP3A5**3 (rs776746), *6 (rs10264272), *ABCB1* C3435 T (rs1045642), G2677 T/A (rs2032582), C1236 T (rs1128503), rs3842, 1000−44G > T (rs10276036), 2895 + 3559C > T (rs7787082), 330–3208 C > T (rs4728709), 2481 + 788T > C (rs10248420), 2686–3393 T > G (rs10280101), 2320−695G > A (rs12720067), 2482−707A > G (rs11983225), 2212−372A > G (rs4148737), *ABCC2* c.1247 G > A (rs2273697), rs717620, *SLCO1B1* *5 (rs4149056), *1B c.388A > G (rs2306283), *17 c.−910G > A (rs4149015), rs11045879, *SLC22A1**2 (rs72552763), *3 (rs12208357), *5 (rs34059508), *UGT1A1**80 (rs887829), *DRD2* rs1799732, rs1800497, rs6277, *DRD3* rs6280, *HTR2A* rs6313, rs6314, rs7997012, *HTR2C* rs1414334, rs3813929, rs518147, *LEP* rs7799039, *LEPR* rs1137101, *APOC3* rs4520, rs5128 and *COMT* rs13306278, rs4680. A copy number variation (CNV) assay was performed to determine CYP2D6 number of copies and the *5 allele (deletion of the gene), as described in a previously published work [14].

2.6. Phenotyping and haplotyping

The alleles described in the previous section were used to infer enzyme or transporter phenotype following the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines: *CYP2C19* [15], *CYP2B6* [16], *CYP2C9* [17], *CYP2D6* [18], *CYP3A5* [19] and *SLCO1B1* [20]. For CYP1A2 and CYP3A4/5 combined phenotype, the methodology published in previous articles was followed [21–23].

For genes without a validated phenotype, variants were grouped according to their impact on protein activity. Three haplotypes were established: wild-type (WT), heterozygous (HT) or mutated (MUT) depending on the number of mutated alleles or SNPs present. Two haplotypes were established for *ABCB1*, the first included the polymorphisms C1236 T, C3435 T and G2677A/T, where WT was considered to be individuals carrying 0 mutated alleles, HT to those carrying 1 or 2 mutated alleles and MUT to individuals with 3 or more mutated alleles. For the second haplotype, all 12 genotyped *ABCB1* variants were considered, and individuals carrying up to 6 mutated polymorphisms were considered WT, HT were those carrying 7–12 mutated polymorphisms and MUT were those carrying 13 or more mutated polymorphisms. *DRD2* variants were merged in two haplotypes following the same fashion as for *ABCB1* first haplotype. For *5-HTR2A*, those volunteers carrying 0 or 1 mutated allele were considered WT, those carrying 2 mutated alleles were considered HT and those carrying 3 or more mutated alleles were considered MUT. For *COMT*, *SLC22A1*, 5-*HTR2C* and *CYP2C8*, individuals carrying no mutated allele were classified as WT, those carrying one mutated allele as HT and those with two or more mutated alleles as MUT. Otherwise, variants were analyzed individually.

2.7. Statistical analysis

The statistical analysis was performed with SPSS software (version 23, SPSS Inc, Chicago, USA). The pharmacokinetic parameters AUC_t and C_{max} were divided by the Dose/Weight (DW) ratio (AUC/DW and C_{max}/DW) to eliminate the effect of weight and dose. For statistical analysis, a logarithmic transformation was applied to all pharmacokinetic variables to normalize distributions.

First, a univariate analysis was performed. Mean pharmacokinetic variables were compared according to sex, race, study design, genotypes, haplotypes and phenotypes. The statistical tests used were a *t*-test (variables with two categories) or an ANOVA test (variables with three or more categories). In addition, a Bonferroni post-hoc analysis was performed when ANOVA was applied. For the univariate analysis of the incidence of ADRs according to sex, race, genotype, haplotype or phenotype, a Chi^2 test was used.

Although two different AUC variables were calculated (i.e. $AUC_{t=72\ h}$ and $AUC_{t=24h}$) they were merged into a generic AUC_t variable. Accordingly, the decision was made to perform a multivariate analysis of pharmacokinetics, in order to correct the effect of sampling time on the AUC and the other variables derived from it. This analysis evaluated each log-transformed pharmacokinetic parameter by means of linear regression. All factors with significant differences in the univariate analysis were included as independent variables, as well as race, study design and sex. For the multivariate analysis of the incidence of ADRs, a similar approach was applied, using logistic regression. Each ADR was analyzed and, as independent variables, all factors with significant differences in the univariate analysis were included, as well as race, study design, sex, and pharmacokinetic variables. For univariate and multivariate analysis, the significance level was established at $p < 0.05$. Throughout the text, significant results are shown using the p-value for the *t*-test or ANOVA. For multivariate analysis, significant results ($p < 0.05$) are shown using the non-standardized β coefficient (linear regression) or odds ratio (OR, logistic regression) and the R^2 value.

3. Results

3.1. Demographic characteristics

In the three studies, 38 women and 42 men were enrolled. There were 36 Caucasians, 42 Latin-Americans and 2 Blacks. Women had lower weight and height than men ($p < 0.001$, non-standardized β coefficient = 14.051 and 0.138, R^2 = 0.288 and 0.517, respectively), however, no significant differences in age or body mass index (BMI) were observed (Table 1). Latin-Americans were older than Caucasians ($p = 0.015$, non-standardized β coefficient = 4.614, R^2 = 0.075) and showed a lower height ($p = 0.019$, non-standardized β coefficient = -0.360, R^2 = 0.517). No significant differences were found in weight, however differences in BMI were observed (non-standardized β coefficient = 1,754, R^2 = 0088) due to the differences in height. No significant differences were observed in any of the parameters depending on the clinical trial design (Table 1).

3.2. Pharmacokinetics

Before DW correction, the AUC of women was significantly higher than that of men (165.67 ± 66.38 vs. 137.97 ± 52.94 ng*h/mL, $p = 0.049$). After DW correction, no significant differences were observed in pharmacokinetic parameters between sexes, except for Vd/F, which was lower in men than women ($p = 0.042$) (Table 2). No differences were observed among Caucasians, Latin-Americans and Blacks, except for C_{max}/DW, which was lower in Latin-Americans (non-standardized coefficient β -0.127, R^2 = 0.773). Regarding the study design, as expected, C_{max}/DW was the only parameter that did not show significant

Table 1
Volunteer demographic characteristics by gender, race, and study design.

Sex	N	Age (years)	Weight (kg)	Height (m)	BMI (kg/m2)
Women	38	30.7 (9.6)	63.2 (9.8)	1.61 (0.06)	24.30 (3.4)
Men	42	29.8 (7.7)	**77.2 (11.9)**[#]	**1.75 (0.08)**[#]	25.10 (2.6)
Race					
Caucasian	36	27.3 (7.0)	71.00 (15.5)	1.72 (0.10)	**23.80 (3.2)**
Latin-American	42	**32.7 (9.0)**[#]	69.60 (10.6)	**1.66 (0.10)**[#]	25.40 (2.7)
Black	2	31.5 (13.4)	82.30 (3.2)	1.71 (0.05)	28.30 (0.6)
Design					
Bioequivalence	56	29.7 (7.0)	70.19 (13.37)	1.69 (0.10)	24.53 (3.20)
Multiple-dose	24	31.5 (11.6)	71.35 (12.21)	1.68 (0.11)	25.24 (2.55)
Total	80	30.2 (8.6)	70.5 (13.0)	1.68 (0.10)	24.70 (3.00)

Data are shown as mean (standard deviation). **Bold:** statistically significant.
[#] $p < 0.05$ versus women or Caucasians. Underlined: $p < 0.05$ in multivariate analysis. BMI: body mass index.

differences between the two groups. The AUC/DW ($p = 0.025$, non-standardized β coefficient = -0.593, $R^2 = 0.763$), the $t_{1/2}$ ($p = 0.010$, non-standardized β coefficient -0.259, $R^2 = 0.187$) and the Vd/F ($p < 0.001$, non-standardized β coefficient = -0.247, $R^2 = 0.491$) were lower in the multiple-dose clinical trial compared to bioequivalence clinical trials. The t_{max} ($p = 0.013$) and Cl/F ($p = 0.001$, non-standardized β coefficient = -1.190, $R^2 = 0.300$) were higher in the multiple-dose clinical trial compared to the bioequivalence clinical trials (Table 2).

The number of genotypes available for each polymorphism, haplotype or phenotype differed due to the variable genotyping success rate of the genotyping platform. Among all genotypes, haplotypes or phenotypes, those significantly related to any pharmacokinetic parameter are shown in Table 3. Individual polymorphisms are only shown when no significant relationships with haplotypes were found. In addition, pharmacokinetic data for the remaining variables, i.e. those without significant relationships, are shown in Supplementary Table 1.

Some significant differences in pharmacokinetic parameters were associated with the polymorphism of CYP enzymes. CYP2C9 PMs were related to higher $t_{1/2}$ ($p = 0.015$) and Vd/F (non-standardized β coefficient = -0.247, $R^2 = 0.491$) compared to IMs and NMs. The *CYP2A6**9 allele was related to higher Vd/F ($p = 0.008$, non-standardized β coefficient 0.249, $R^2 = 0.491$) and to lower C_{max}/DW (non-standardized β coefficient = -0.157, $R^2 = 0.773$) compared to the *1 allele. The *CYP2C8**4 allele was related to higher t_{max} ($p = 0.041$, non-standardized β coefficient 0.440, $R^2 = 0.283$) and lower C_{max}/DW (non-standardized β coefficient = -0.234, $R^2 = 0.773$) compared to the *1 allele.

In addition, pharmacokinetic variability was observed depending on the polymorphism of transporters. The *SLC22A1* MUT haplotype was related to: higher AUC/DW and C_{max}/DW ($p = 0.011$ and 0.003; non-standardized β coefficients = 0.111 and 0.183 and $R^2 = 0.763$ and 0.773, respectively); to lower t_{max} ($p = 0.034$, non-standardized β coefficient = -0.203, R2 = 0283) and to lower Cl/F ($p < 0.004$) compared to WT and HT subjects. These differences were mainly due to the presence of the *5 allele. The AUC/DW of *1/*1 individuals was lower than that of *1/*5 individuals (2520.48 ± 796.72 versus 3356.43 ± 408.43 kg*ng*h/mL*mg, $p = 0.004$, non-standardized β coefficient = 0.335, $R^2 = 0.763$), the C_{max}/DW followed the same fashion (109.58 ± 24.73 and 177.59 ± 70.35 kg*ng/mL*mg, respectively, $p = 0.007$), while the Cl/F (295.89 ± 105.08 versus 175.04 ± 66.53 mL/h*kg, $p = 0.001$, non-standardized β coefficient = -4.248, $R^2 = 0.300$) and Vd/F (11.78 ± 2.88 and 7.49 ± 2.67 L/kg, $p = 0.018$, non-standardized β coefficient = -0.322, $R^2 = 0.491$) were higher in *1/*1 subjects in comparison to *1/*5 carriers. Moreover, *ABCB1* C1236 T C/C individuals exhibited a higher $t_{1/2}$ than C/T and T/T individuals ($p = 0.040$). *ABCB1* rs10276036 T/T carriers showed a higher $t_{1/2}$ than that of T/C and C/C individuals ($p = 0.030$). *ABCB1* rs3842 T allele was related to a lower C_{max}/DW compared to the C allele ($p = 0.023$), to lower AUC/DW (non-standardized β coefficient = -0.112, R2 = 0.763) and to higher Cl/F ($p = 0.016$). Finally, *ABCC2* rs717620, C/C individuals presented a higher t_{max} ($p = 0,007$, non-standardized β coefficient = -0,416, $R^2 = 0283$) and a lower C_{max}/DW (non-standardized β coefficient = -0,157, R2 = 0773) than C/T and T/T carriers.

For *APOC3*, rs5128 G/G individuals presented a lower $t_{1/2}$ compared to C/C individuals ($p = 0.026$), and to C/G individuals ($p = 0.082$). A trend was also observed in the t_{max}, which was higher in C/C individuals than in G/G carriers ($p = 0.090$); and in the Vd/F, which was higher in C/G individuals than G/G carriers ($p = 0.068$) (Table 3).

3.3. Safety

No significant differences were observed in the incidence of ADRs according to sex, dose or design. Conversely, differences were observed according to race: 12 of 36 Caucasians suffered one or more adverse effects (33.3 %), 29 of 42 Latin-Americans (69.0 %) and 1 of 2 Blacks (50 %) ($p = 0.004$). 61.9 % Latin-Americans (26 out of 42) presented somnolence, compared to 30.6 % of Caucasians (11 out of 36) and 50 % of Blacks (1 out of 2) ($p = 0.008$).

ABCB1 rs10248420 G allele was associated with the appearance of somnolence, which was also more frequent in *CYP3A5* *1/*1 individuals, compared to *1/*3 and *3/*3 carriers (Table 4). Somnolence was additionally associated with *DRD2* MUT and HT individuals, compared to the WT haplotype (Table 4). Dizziness was more frequent in

Table 2
Pharmacokinetic parameters according to sex, race and study design.

		AUC/DW (kg*ng*h/mL*mg)	C_{max}/DW kg*ng/mL*mg	t_{max} (h)	$t_{1/2}$ (h)	V_d/F (L/kg)	Cl/F (mL/h*kg)
Sex:	**N**						
Women	38	2533.24 (846.09)	103.31 (30.48)	4.51 (1.68)	32.23 (11.42)	12.44 (3.11)	295.40 (110.27)
Men	42	2646.57 (663.89)	116.75 (23.66)	4.53 (2.11)	29.01 (12.58)	**10.98 (2.19)**[#]	289.47 (86.28)
Race:							
Caucasian	36	2584.21 (662.74)	115.82 (32.97)	4.63 (1.99)	27.84 (5.25)	11.85 (3.26)	303.15 (91.48)
Latin-American	42	2552.74 (814.50)	**105.33 (22.42)**	4.45 (1.86)	32.57 (15.54)	11.59 (2.32)	287.37 (104.28)
Black	2	3586.26 (444.55)	118.02 (4.38)	4.00 (2.48)	34.58 (4.85)	10.69 (2.20)	215.25 (15.92)
Design:							
Bioequivalence	56	2929.37 (551.53)	110.14 (24.43)	4.17 (1.63)	31.25 (8.13)	12.36 (2.40)	288.44 (79.86)
Múltiple-dose	24	**1807.28(548.96)**[#]	110.89 (34.95)	**5.34(2.28)**[#]	**28.28(20.51)**[#]	**9.46 (2.70)**[#]	**305.06 (144.73)**[#]
Total	80	2592.74 (753.17)	110.37 (27.77)	4.52 (1.90)	30.55 (12.07)	11.68 (2.75)	292.31 (97.86)

Data are shown as mean (standard deviation). **Bold:** statistically significant.
[#] $p < 0.05$ versus women or bioequivalence clinical trials. Underlined: $p < 0.05$ in multivariate analysis.

Table 3
Pharmacokinetic parameters according genotypes, haplotypes and phenotypes.

Genotype/Phenotype/Haplotype		N	AUC / DW kg*ng*h/mL*mg	C_{max} / DW kg*ng/mL*mg	t_{max} h	$t_{1/2}$ h	V_d/F L/kg	Cl/F mL/h*kg
***ABCB1* C1236 T rs1128503**	**C/C**	26	2716.96 (818.36)	109.72 (24.66)	5.08 (2.40)	**36.65(16.33)**#	11.68(2.55)	247.08 (75.29)
	C/T	28	2467.87 (709.78)	116.10 (33.25)	4.16 (1.52)	26.87 (8.73)	11.20 (3.20)	311.56 (101.21)
	T/T	12	2445.61 (932.32)	108.72 (22.40)	4.71 (1.85)	29.44 (11.14)	11.77 (2.01)	310.92 (112.62)
***ABCB1* rs10276036**	**T/T**	26	2716.96 (818.36)	109.72 (24.66)	5.08 (2.40)	**36.65(16.33)**#	11.68 (2.55)	247.08 (75.29)
	T/C	28	2385.39 (687.31)	112.74 (32.97)	4.24 (1.49)	26.54 (8.71)	11.62 (3.65)	327.23 (115.67)
	C/C	12	2445.61 (932.32)	108.72 (22.40)	4.71 (1.85)	29.44 (11.14)	11.77 (2.01)	310.92 (112.62)
***ABCB1* 193T > C rs3842**	**T/T**	44	2512.86 (813.28)	111.36 (22.86)	4.52 (2.08)	30.68 (11.41)	11.39 (2.31)	282.95 (88.80)
	T/C	18	2526.49 (831.03)	104.20(27.63)	4.94 (1.76)	32.49 (17.66)	12.48 (3.91)	316.29 (137.60)
	C/C	2	**2669.07 (563.65)**	**166.89 (85.50)**	4.75 (2.48)	27.11 (4.56)	9.41 (5.39)	**255.98 (181.00)**#
***ABCC2* -24C > T rs717620**	**C/C**	49	2484.63 (830.65)	108.93 (29.27)	4.96 (2.00)	31.90 (14.49)	11.44 (2.87)	285.15 (107.78)
	C/T T/T	18	2710.96 (701.04)	**118.93 (24.98)**	**3.69(1.55)**#	28.66 (8.71)	12.13 (3.21)	308.84 (102.24)
***APOC3* 40C > G rs5128**	**C/C**	38	2689.27 (726.86)	115.77 (32.50)	4.80 (1.96)	32.48 (13.42)	11.57 (2.65)	269.49 (81.98)
	C/G	25	2302.71 (860.27)	102.56 (20.40)	4.62 (2.00)	30.68 (12.69)	12.19 (3.21)	310.96 (121.17)
	G/G	4	2695.99 (910.83)	128.78(6.13)	3.00 (1.37)	**20.3 (10.33)**###	8.97 (3.16)	366.72 (159.76)
CYP2A6 *9	***1/*1**	57	2562.39 (810.94)	114.85 (28.92)	4.48 (1.87)	29.73 (12.87)	**11.2 (2.88)**#	292.79 (109.15)
	***1/*9**	8	2393.92 (851.35)	96.15 (15.63)	4.84 (2.13)	39.87 (14.39)	14.36 (2.42)	284.24 (104.32)
	***9/*9**	2	2668.18 (131.04)	**81.40 (13.34)**	7.75 (2.48)	31.71 (0.84)	13.27 (0.34)	295.034 (21.84)
***CYP2C8* *4**	***1/*1**	62	2554.74 (807.99)	112.47 (28.54)	4.45 (1.73)	30.90 (13.30)	11.56 (2.92)	292.23 (108.10)
	***1/*4**	5	2429.99 (750.39)	**101.04 (26.31)**	**6.75 (3.5)**#	32.50 (11.31)	13.05 (4.08)	284.98 (61.10)
CYP2C9 Phenotype	**NM**	45	2502.05 (771.54)	110.93 (21.86)	4.62 (1.95)	29.17 (11.13)	11.58 (3.01)	306.05 (109.43)
	IM	17	2677.94 (830.73)	118.33 (40.91)	4.44 (2.12)	30.35 (8.38)	11.33 (2.70)	270.64 (79.26)
	PM	5	2485.4 (1062.15)	94.93 (27.71)	5.25 (1.84)	**51.61(28.51)**#	**13.35 (3.63)**	234.95 (155.48)
***SLC22A1* Haplotype**	***WT***	39	2409.15 (777.73)	102.20 (22.61)	5.22 (2.22)	32.27 (15.00)	12.48 (3.22)	308.54 (119.36)
	HT	17	2691.72 (807.37)	122.90 (24.85)	4.02 (1.05)	26.26 (5.75)	10.70 (1.79)	294.54 (81.5)
	MUT	6	**2626.84(946.7)**###	**131.07(49.2)**###	**3.25 (1.6)**###	37.49 (13.72)	10.73 (3.11)	**215.20 (90.19)**#

Data are shown as mean (standard deviation). **Bold:** statistically significant.
# $p < 0.05$ against other genotypes.
$p < 0.05$ against WT individuals; Underlined: $p < 0.05$ in multivariate analysis.

CYP2C8 MUT individuals and in CYP2C9 PM individuals. Moreover, the only subject suffering from asthenia was an *ABCB1* rs4728709 C/C subject. Similarly, the only volunteer who suffered palpitations was an *ABCB1* rs3842 C/C individual, who also exhibited the *SLC22A1**1/*5 genotype (Table 4).

4. Discussion

There are no pharmacogenetic guidelines for olanzapine treatment. Noteworthy, the DPWG group claims that it is not necessary to adjust the dose of olanzapine in CYP2D6 PMs and IMs since these groups are not associated, at the usual doses, with differences in olanzapine toxicity or effectiveness. Nevertheless, they state that there is currently no evidence to apply the same for UMs. Our interest was to explore the impact of CYP2D6 phenotypes on the pharmacokinetics and safety of olanzapine, especially the impact of UM [8]. Neither of which had an effect in this work. In addition, we investigated many other polymorphisms in transporters and metabolizing enzymes.

In line with previous studies, women presented a lower weight and height than men [9,24], which is often associated with greater exposure to drugs and therefore with a greater risk for ADRs. In addition, the Vd/F was higher in women, which is likely explained by women habitually presenting a higher body fat percentage, favoring a greater distribution and accumulation of lipophilic drugs like olanzapine. However, there seems to be no consensus on the impact of sex on olanzapine pharmacokinetics [24,25]. Likewise, there is no clear consensus on the effect of race on olanzapine bioavailability. A study related African Americans to higher olanzapine clearance; however, these differences were caused by *CYP3A43* rs472660; the A allele was related to higher clearance, which was significantly more prevalent in African Americans than in Caucasians [26]. Here, we were unable to replicate these findings as we did not explore this variant as it is extremely infrequent in our population.

The multivariate analysis accurately corrected the effect of the study design on AUC and its derived parameters. In our dataset, pharmacokinetic parameters showed great similarity to those reported in the drug label [5]. The t_{max} and $t_{1/2}$ observed here (around 4.5 h and 31 h, respectively) were slightly lower but consistent with the intervals proposed on the drug label (5–8 h and around 34 h, respectively). Interestingly, the drug label indicates a higher $t_{1/2}$ for women compared to men (around 37 h versus 32 h, respectively); here, although the difference was not statistically significant, a similar trend was observed (around 32 h versus 29 h, respectively). These differences could be explained by the sex-dependent regulation of *CYP1A2* expression, higher in cell cultures obtained from women than from men [27].

In this study, several novel associations were identified among polymorphisms located in genes that code for metabolizing enzymes, in contrast to the absence of associations with *CYP1A2* or *CYP2D6*, which is consistent with previous works [28]. Some tendency towards a higher AUC and lower Cl/F was observed for CYP2D6 PMs, however this association did not reach the significance level. One study related *CYP2D6**3 and *4 alleles to a larger percent of BMI gain in olanzapine treated schizophrenic men [29]. Since weight gain is a common adverse effect of olanzapine, these subjects may accumulate olanzapine, which is consistent with the trend of CYP2D6 PMs towards greater bioavailability described here.

Our results regarding *CYP2C9* are consistent with previous studies, where CYP2C9 was proposed to metabolize olanzapine and its polymorphism was related to the appearance of ADRs [24,30]. Here, CYP2C9 PMs congruently accumulated olanzapine to a wider extent than other phenotypes. In addition, clozapine, an antipsychotic structurally very similar to olanzapine, is likely metabolized by this cytochrome [31]. Our findings regarding *CYP2A6* and *CYP2C8* are

Table 4
Significant relationships between genotypes, phenotypes, haplotypes and adverse drug reactions.

ADR	Genotype. haplotype. phenotype		Number of volunteers affected	Sig.
Somnolence	*ABCB1* rs10248420	A/A	0 out of 4 (0%)	p = 0.047 log OR = 42.406
		A/G	16 out of 25 (64 %)	
		G/G	18 out of 35 (51 %)	
	CYP3A5 Phenotype	*1/*1	4 out of 4 (100 %)	p = 0.049
		*1/*3	9 out of 14 (64.3 %)	
		*3/*3	21 out of 49 (42.9 %)	
	DRD2 Haplotype	WT	0 out of 4 (0%)	p = 0.025
		HT	16 out of 24 (66.7 %)	
		MUT	17 out of 38 (44.7 %)	
Dizziness	*CYP2C8* Haplotype	WT	2 out of 43 (4.7 %)	p = 0.048
		HT	0 out of 18 (0%)	
		MUT	2 out of 6 (33.3 %)	
	CYP2C9 Phenotype	PM	2 out of 5 (40 %)	p = 0.020
		IM	1 out of 17 (5.9 %)	
		NM	1 out of 45 (2.2 %)	
Asthenia	*ABCB1* rs4728709	T/T	0 out of 56 (0%)	p = 0.045
		T/C	0 out of 8 (0%)	
		C/C	1 out of 3 (33.3 %)	
Palpitations	*ABCB1* rs3842	T/T	0 out of 44 (0%)	p = 0.031
		T/C	0 out of 18 (0%)	
		C/C	1 out of 2 (50 %)	
	SLC22A1 *5	*1/*1	0 out of 65 (0%)	p = 0.030
		*1/*5	1 out of 2 (50 %)	

ADR: adverse drug reaction; Sig.: statistical significance. p value: univariate analysis. log OR: multivariate analysis.

inconsistent and probably due to the presence of outliers and the low sample size.

Furthermore, *SLC22A1* encodes for the liver organic cation transporter 1 (OCT1), which transports drugs into the hepatocyte, where they are metabolized. This gene presents a great genetic variability: alleles *3 and *5 are associated with a decrease in the function of the transporter, while the effect of the allele *2 seems to be specific to the substrate [32]. To the best of our knowledge, no studies were conducted to test whether olanzapine is a substrate for SLC22A1, however, clozapine likely is [31]. Here, we can suggest that olanzapine pharmacokinetics is influenced by *SLC22A1* genotype or haplotype, where the *5 allele has a predominant effect. Carriers of *SLC22A1* defective alleles may be linked to reduced hepatic drug uptake, reduced metabolism and enhanced exposure. To the best of our knowledge, this work is the first to document such a result.

Polymorphism of *ABC* transporters was similarly related to drug exposure variability. First, to the best of our knowledge, this is the first study to address the impact of *ABCC2* variants on olanzapine exposure. This transporter is expressed in the apical membrane of different cell types, such as hepatocytes, renal tubule cells or enterocytes, which exports substrates from the interior of the cell to the bile, urine or intestinal lumen [33]. To date, no consensus has been reached on the effect of rs717620 on the function of the transporter [34,35]. Here, the T allele has been related to higher olanzapine exposure. This suggests it is a loss of function variant, which reduces drug excretion and increases drug plasma concentrations.

Despite our efforts – and those of other researchers – dedicated to clarifying the effect of polymorphisms in *ABCB1* on the pharmacokinetics, safety and metabolic effects of olanzapine, aripiprazole and antidepressant drugs, we are still unable to draw any conclusions on the matter [9,23,36]. With every new article, new associations are found, some consistent with previous ones, and others contradictory. However, there is no consensus, and the possible general explanation is that P-glycoprotein activity plays a role in favor of or against bioavailability, depending on the location of the body where it is expressed [37]. The C1236 T, C3435 T and G2677A/T SNPs are the three most studied *ABCB1* polymorphisms related to olanzapine pharmacokinetics [38]. In this work, significant differences were found for C1236 T, where C/C individuals presented higher exposure and reduced clearance. This is consistent with the results of another study, where C/C and C/T individuals had a better response to antipsychotics and required a lower dose [39]. However, these findings contradict another study, where the C1236 T T allele was related to higher olanzapine bioavailability [38]. Furthermore, in this study it was observed that the rs10276036 polymorphism was in linkage disequilibrium with C1236 T (D':1.0, R2:1.0). Significant differences were additionally observed for *ABCB1* rs3842, where C/C individuals showed a greater olanzapine exposure. To our knowledge, there is no previous work in which a relationship with olanzapine is established, but its involvement in the pharmacokinetics of other drugs was previously studied [40], showing the same trend. Although more studies are needed to examine the impact of *ABCB1* polymorphisms on the pharmacokinetics of olanzapine and other drugs, it does not seem that these will be key factors.

Allelic variation in *APOC3* gene was related to effects on lipid metabolism [41]. Here, *APOC3* rs5128 G/G carriers showed lower $t_{1/2}$ than C allele carriers. In a previous study, C/C individuals showed a higher increase in triglycerides, a frequent ADR of olanzapine [42], what would be caused by an overexposure to the drug. Hence, the rs5128 G allele seems to be related to an underexposure of the drug. A possible explanation for this is that the alteration of triglyceride particle transport, which is mediated by APOC3, subsequently affects the distribution of olanzapine. This could occur if, given olanzapine lipophilicity, it was distributed through the triglyceride particles whose transport is modulated by APOC3. However, this is only a hypothesis, and should be further addressed in future studies.

In terms of safety, there were no severe ADRs, probably due to the administration of a single dose (or only five doses in one of the studies), which was lower than that used in clinical practice [25]. The results obtained for CYP2C9 in safety are consistent with pharmacokinetics and other studies [24]. In summary, PMs accumulate the drug and suffer more ADRs, in this case dizziness. Additionally, our results for *SLC22A1* are congruent with our pharmacokinetic results, as *5 carriers were related to the development of palpitations. However, since palpitations were only reported by one volunteer, this result should be considered carefully and evaluated in further studies. *DRD2* MUT individuals suffered more somnolence than WT individuals. This is consistent with that expected, since *DRD2* is one of olanzapine's targets, in which rs1800497 and rs1799732 mutant alleles are associated with an increased risk of ADRs [43]. The association between the *ABCB1* rs10248420 G allele and somnolence is consistent with a previous study, in which carriers of the A allele were associated with non-response to clozapine treatment [44]. Somnolence was inconsistently related to *CYP3A5**1, since this allele encodes for a CYP3A5 active enzyme compared to non-expressors (carriers of the *3 allele); it is therefore inconsistent to relate a higher prevalence of an ADR to an enhanced drug metabolism. Nevertheless, these pharmacokinetic effects were not observed in our study. *ABCB1* rs4728709 T allele was associated with a lower incidence of asthenia, which is consistent with previous work with vincristine [45]. Finally, *ABCB1* rs3842 T allele was related to the development of palpitations, also consistent with pharmacokinetic results.

4.1. Limitations

This study has two main limitations. Firstly, the administration of a single dose and the inclusion of healthy subjects do not allow us to conclude on olanzapine effectiveness or long-term safety. Secondly, the sample size available is limited and arbitrary, since this is candidate-gene study was conducted based on the available pharmacokinetic

and safety data from two bioequivalence trials and a multiple dose clinical trial. Furthermore, the p value <0.05 was established as the threshold of significance, since this is an observational study in which no sample size was calculated to demonstrate a particular effect. Therefore, the results presented here should be considered carefully. For this reason, it would be convenient to increase the sample size to enrich our population in carriers of low prevalence genotypes, what would improve the statistical power of the study. For this reason, it would be convenient to increase the sample size to enrich our population in carriers of low prevalence genotypes, what would improve the statistical power of the study. In contrast, this study presents several strengths: controlled diet conditions, avoidance of drug interactions, and the avoidance of psychiatric diseases.

5. Conclusions

Currently, there is not enough evidence to implement genotyping before the prescription of olanzapine. Due to its metabolic characteristics, the polymorphism of *CYP1A2* and *CYP2D6* was proposed as a possible biomarker for predicting the pharmacokinetics and safety of olanzapine. However, in this study, no significant differences were found in any of the parameters analyzed. For *CYP1A2*, this is probably due to the lack of functional impact of the polymorphisms usually studied. For *CYP2D6*, it seems to play a secondary role in olanzapine metabolism, which is in line with the clinical recommendations of the DPWG. Nonetheless, a tendency towards increased exposure was observed for PMs, therefore this biomarker should be further studied. As novel results, in this work we propose that CYP2C9 seems to participate in olanzapine metabolism and its variants seem to have an impact on pharmacokinetic variability. Likewise, SLC22A1 seems to participate in olanzapine pharmacokinetics and its polymorphism, the *5 allele, may be linked to higher drug exposure. Other transporters, namely ABCB1 and ABCC2 could also influence olanzapine pharmacokinetics. Finally, polymorphisms in *APOC3* may produce alterations in olanzapine distribution. More pharmacogenetic studies are warranted, covering important pharmacogenes such as *CYP1A2* or *CYP2D6* and other genes related to olanzapine pharmacokinetics, namely other CYP, FMO and UGT enzymes, ABC and SLC transporters or APO genes.

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Declaration of Competing Interest

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Appendix A. Supplementary data

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