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ORIGINAL ARTICLE

The effects of aripiprazole and olanzapine on pupillary light reflex and its relationship with pharmacogenetics in a randomized multiple-dose trial

Dora Koller[1] | Miriam Saiz-Rodríguez[1] | Pablo Zubiaur[1] | Dolores Ochoa[1,2] | Susana Almenara[1] | Manuel Román[1,2] | Daniel Romero-Palacián[1] | Alejandro de Miguel-Cáceres[1] | Samuel Martín[1,2] | Marcos Navares-Gómez[1] | Gina Mejía[1,2] | Aneta Wojnicz[1] | Francisco Abad-Santos[1,2]

[1]Clinical Pharmacology Department, Hospital Universitario de La Princesa, Instituto Teófilo Hernando, Pharmacology Department, Faculty of Medicine, Autonomous University of Madrid, Madrid, Spain

[2]Plataforma SCReN (Spanish Clinical Research Network), Instituto de Investigación Sanitaria La Princesa, UICEC Hospital Universitario de La Princesa, Madrid, Spain

Correspondence
Francisco Abad-Santos, Clinical Pharmacology Department, La Princesa University Hospital, Diego de León 62, 28006, Madrid, Spain.
Email: francisco.abad@salud.madrid.org

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Aims: Pupillography is a noninvasive and cost-effective method to determine autonomic nerve activity. Genetic variants in cytochrome P450 (*CYP*), dopamine receptor (*DRD2*, *DRD3*), serotonin receptor (*HTR2A*, *HTR2C*) and ATP-binding cassette subfamily B (*ABCB1*) genes, among others, were previously associated with the pharmacokinetics and pharmacodynamics of antipsychotic drugs. Our aim was to evaluate the effects of **aripiprazole** and **olanzapine** on pupillary light reflex related to pharmacogenetics.

Methods: Twenty-four healthy volunteers receiving 5 oral doses of 10 mg aripiprazole and 5 mg olanzapine tablets were genotyped for 46 polymorphisms by quantitative polymerase chain reaction. Pupil examination was performed by automated pupillometry. Aripiprazole, dehydro-aripiprazole and olanzapine plasma concentrations were measured by high-performance liquid chromatography–tandem mass spectrometry.

Results: Aripiprazole affected pupil contraction: it caused dilatation after the administration of the first dose, then caused constriction after each dosing. It induced changes in all pupillometric parameters ($P < .05$). Olanzapine only altered minimum pupil size ($P = .046$). Polymorphisms in *CYP3A*, *HTR2A*, *UGT1A1*, *DRD2* and *ABCB1* affected pupil size, the time of onset of constriction, pupil recovery and constriction velocity. Aripiprazole, dehydro-aripiprazole and olanzapine pharmacokinetics were significantly affected by polymorphisms in *CYP2D6*, *CYP3A*, *CYP1A2*, *ABCB1* and *UGT1A1* genes.

Conclusions: In conclusion, aripiprazole and its main metabolite, dehydro-aripiprazole altered pupil contraction, but olanzapine did not have such an effect. Many polymorphisms may influence pupillometric parameters and several polymorphisms had an effect on aripiprazole, dehydro-aripiprazole and olanzapine pharmacokinetics.

The authors confirm that the Principal Investigator for this paper is Francisco Abad-Santos and that he had direct clinical responsibility for patients.

Pupillography could be a useful tool for the determination of autonomic nerve activity during antipsychotic treatment.

KEYWORDS
antipsychotics, genetics and pharmacogenetics, pharmacodynamics, pharmacokinetics, schizophrenia

1 | INTRODUCTION

Aripiprazole (ARI) and olanzapine (OLA) are atypical (second generation) antipsychotics commonly prescribed for patients with schizophrenia or schizoaffective disorders.[1] ARI has partial agonistic activity at dopamine D2, D3, D4 and serotonin 5-HT1A, 5-HT2C as well as α1-adrenergic receptors and also exhibits 5-HT2A and 5-HT7 receptor antagonism.[2] OLA has higher antagonistic affinity for 5-HT2A serotonin receptors than for D2 dopamine receptors. Additionally, it has antagonistic activity at dopamine D3 and D4, serotonin 5-HT3 and 5-HT6, histamine H1, α1-adrenergic, and muscarinic M1–5 receptors.[3,4]

ARI is extensively metabolized by cytochrome P450 (CYP) enzymes CYP3A4 and CYP2D6. Dehydro-aripiprazole (DARI), its main active metabolite, accounts for 40% of the parent compound in plasma. Moreover, the pharmacological activity of DARI is similar to ARI.[5] Olanzapine is predominantly metabolized by direct glucuronidation via the UDP-glucuronosyltransferase (UGT) enzyme family, principally by UGT1A4,[6] CYP1A2 and to a lesser extent by CYP2D6 and CYP3A4.[7]

Pupillography is a noninvasive and cost-effective method to determine autonomic nerve activity,[8] which was developed in 1958.[9] It was thoroughly described that opioid drugs cause pupil constriction (miosis).[10–13]

Several atypical antipsychotics caused pupil miosis in overdose patients. It can be due to inducing unopposed parasympathetic stimulation of the pupil with significant α1-adrenergic receptor blockade.[14] By contrast, these drugs could affect the pupil diameter due to their affinity for dopamine and serotonin receptors[15,16] as serotonin and dopamine are effectors on various types of muscles including the sphincter pupillae and the dilator pupillae.[17] Accordingly, genetic polymorphisms present in these genes can affect pupil response.[18]

The aim of the current study was to evaluate if ARI and OLA affect pupillometric parameters in healthy subjects after multiple dose administration. Furthermore, their relationship with pharmacokinetics and pharmacogenetics was also evaluated.

What is already known about this subject

- Atypical antipsychotics can provoke pupil contraction due to blocking α1-adrenergic receptors. However, these drugs could affect pupil diameter due to their affinity for dopamine and serotonin receptors. Accordingly, polymorphisms present in these genes can affect pupil response.

What this study adds

- This study is the first to reveal that aripiprazole has a significant influence on pupillary light reflex compared to olanzapine. Furthermore, several genetic polymorphisms affect these changes. Therefore, pupillography could be an important and useful tool to assess autonomic nervous system activity during antipsychotic drug treatment.

2 | MATERIALS AND METHODS

2.1 | Study population

A multiple-dose clinical trial including 24 healthy volunteers (12 males and 12 females) was performed at the Clinical Trials Unit of Hospital Universitario de La Princesa (Madrid, Spain). The protocol was approved by the Research Ethics Committee duly authorized by the Spanish Drugs Agency and under the guidelines of Good Clinical Practice (EUDRA-CT: 2018–000744-26). All subjects were adequately informed about the study and, if agreeing to participate, signed an informed consent form. The trial complied with the international standards and with the Declaration of Helsinki.

The inclusion criteria were the following: male and female volunteers between 18 and 65 years old; free from any known organic or psychiatric conditions; normal vital signs and electrocardiogram (ECG); normal medical records and physical examination; no clinically significant abnormalities in haematology, biochemistry, serology and urine tests.

2.2 | Study design

The clinical trial was phase I with multiple oral dose design, open-label, randomized, crossover, 2-periods, 2-sequences, single-centre and comparative study performed between June 2018–April 2019. ARI 10 mg/day tablets or OLA 5 mg/day film-coated tablets were administered during 5 consecutive days. Block randomization was used to

assign the treatment to each volunteer on the first day.[19] The drug was administered at 09:00 each day under fasting conditions. The subjects were hospitalized from 1 hour before the first dose until 24 hours after the last dose. In the second period, after a washout period of 28 days, each volunteer received the opposite drug they received in the first period. The random allocation sequence, the recruitment of participants and their assignment to interventions were performed by investigators of the Clinical Trials Unit.

Twenty-two blood samples were collected from each participant for pharmacokinetic assessments during each period, thus 44 samples in total: 7 samples on day 1 (predose and 1, 2, 3, 5, 8 and 12 hours after dosing); 1 (predose) sample on days 2, 3 and 4; 7 samples on day 5 (predose and 1, 2, 3, 5, 8 and 12 hours after dosing) and 1 sample on days 6, 7, 9, 11 and 15 (corresponding to 24, 48, 96, 144 and 240 hours after the last dose, respectively). Each blood sample was labelled with the protocol code, volunteer number, treatment period and day and extraction time without specifying the administered drug. Subsequently, the samples were centrifuged at 1900 *g* for 10 minutes and then the plasma was collected and stored at −20°C until the determination of drug concentrations.

2.3 | Pharmacokinetic analysis

Plasma concentrations of ARI, DARI and OLA were quantified by a high-performance liquid chromatography–tandem mass spectrometry method developed in our laboratory.[20]

The pharmacokinetic parameters were calculated by noncompartmental analysis by Phoenix WinNonlin (version 8, Pharsight, Mountain View, CA, USA) as *single dose* (i.e. for the first day) and *multiple dose* (i.e. considering all time points). Peak plasma concentration (C_{max}) and time to reach maximum concentration (T_{max}) were obtained directly from the original data. The area under the plasma concentration–time curve from time zero to the last observed time point (AUC_{last}) was calculated using the trapezoidal rule. The AUC from time zero to infinity (AUC_{inf}) was determined as the sum of the AUC_{last} and the extrapolated area calculated as the last plasma concentration (C_{last}) divided by the terminal rate constant (k_e) that was determined by regression analysis of the log-linear part of the concentration–time curve. Elimination half-life ($T_{1/2}$) was determined by $0.693/k_e$. The total apparent clearance adjusted for bioavailability (Cl/F) was calculated using the formula: $Cl/F = dose/AUC_{inf}$. The volume of distribution adjusted for bioavailability (Vd/F) was calculated as Cl/F divided by k_e. AUC and C_{max} were adjusted for dose/weight ratio (AUC/dW and C_{max}/dW, respectively) and were logarithmically transformed for statistical analysis.

2.4 | Pupillary light reflex measurements

Pupillometric measurements were performed right before and 4 hours after drug administration on each day of hospitalization. The data were recorded with a PRL-200 automated monocular infrared pupillometer (NeurOptics, Irvine, CA, USA). Each measurement was performed in a hospital room with artificial illumination. In order to adjust for differences in luminosity, light intensity (in lux) was measured at the moment of the pupillometric determination.

Before starting the measurement, the subject was instructed to focus on a small target object with the eye that was not being tested open (left eye). Stimuli were single light pulses with a fixed intensity of 180 μW during 154 ms. Once the device was focused on the target pupil (right eye), a white light stimulus was flashed. The measurements were sampled at a frequency of 32-frames/s and lasted up to 5 seconds, allowing a full or partial recovery of the pupil size after light constriction.

Eight different pupillometric parameters were measured based on the user guide.[21] Maximum pupil diameter (MAX) and minimum pupil diameter (MIN) represent the pupil diameter before constriction and just at the peak of constriction, respectively. The percentage of constriction (CON) was calculated by (MAX − MIN)/MAX. Latency (LAT) is time of the onset of constriction. ACV and MCV are average and maximum constriction velocity, respectively. The negative sign differentiates constriction from dilation being the opposite movement. After reaching its constriction, the pupil tends to recover and dilate back to its initial resting size, which is measured as average dilation velocity (ADV). T75 is the total time taken by the pupil to recover 75% of the initial resting pupil size after it reached the peak of constriction.

2.5 | Genotyping

DNA was extracted from 1 mL of peripheral blood using a MagNA Pure LC DNA Isolation Kit in an automatic DNA extractor (MagNa Pure System, Roche Applied Science, Indianapolis, Indiana, USA). Subsequently, it was quantified spectrophotometrically using a NanoDrop ND-1000 Spectrophotometer (Nanodrop Technologies, Wilmington, Delaware, USA) and the purity of the samples was determined by the $A_{260/280}$ absorbance ratio.

Samples were genotyped with TaqMan assays on an OpenArray platform on a QuantStudio 12 K Flex instrument. Results were analysed with the QuantStudio 12 K Flex and the TaqMan Genotyper softwares (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

The assay included 120 single nucleotide polymorphisms, of which the following 46 were analysed based on their importance in the metabolism and mechanism of action of ARI and OLA: *CYP1A2* *1C (rs2069514); *1F (rs762551); *1B 5347 T > C (rs2470890); *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 (rs4646438); *CYP3A5* *3 (rs776746); *6 (rs10264272); *ABCB1* C3435T (rs1045642); G2677T/A (rs2032582); C1236T (rs1128503); rs3842; 1000-44G > T (rs10276036); 2895 + 3559C > T (rs7787082); 330-3208C > T (rs4728709); 2481 + 788 T > C (rs10248420);

2686-3393 T > G (rs10280101); 2320-695G > A (rs12720067); 2482-707A > G (rs11983225); 2212-372A > G (rs4148737); *ADRA2A* rs1800544; *BDNF* Val66Met (rs6265); *COMT* rs4680; rs13306278; *DRD2* TaqIA (rs1800497); 957C > T (rs6277); −141 Ins/Del (rs1799732); *DRD3* Ser9Gly (rs6280); *HTR2A* T102C (rs6313); C1354T (rs6314); rs7997012; *HTR2C* −759C/T (rs3813929); −697G/C (rs518147); rs1414334; *OPRM1* rs1799971; and *UGT1A1* rs887829.

Copy number variations in the *CYP2D6* gene were determined with the TaqMan Copy Number Assay (Assay ID: Hs00010001_cn; Thermo Fisher Scientific, Waltham, MA, USA) which detects a specific sequence in exon 9.[22] Samples were run in the same instrument.

Since the *CYP2D6* *29 (rs16947) polymorphism was not included in the array, it was genotyped with the same instrument using individual TaqMan probes. Additionally, the *CYP3A4* *20 (rs67666821) polymorphism was genotyped by the KASPar SNP Genotyping System (LGC Genomics, Herts, UK). The ABI PRISM 7900HT Sequence Detection System (Thermo Fisher Scientific) was used for fluorescence detection and allele assignment.[23]

2.6 | Statistical analysis

Statistical analyses were performed with the SPSS 24.0 software (SPSS Inc., Chicago, IL, USA). *P* values ≤.05 were considered significant. 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 http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

ANOVA was used to compare mean pharmacokinetic values according to different categories, e.g. genotype, sex and race. Changes in pupillometric parameters (MAX, MIN, T75, MCV, CON, ACV, ADV, LAT) were analysed by repeated measures ANOVA. The values were adjusted for differences in light intensity (in lux) before analysis. Repeated measures ANOVA was used to associate pupillometric parameters to pharmacokinetic parameters and polymorphisms. A Bonferroni correction was applied for each analysis. Multiple linear regression models were used to study factors related to all pupillometric and pharmacokinetic dependent variables.

CYP2D6 genotypes were classified in 4 phenotypes (poor metabolizer: PM; intermediate metabolizer: IM; normal/rapid metabolizer: NM and ultra-rapid metabolizer: UM), which is based on the functionality of alleles[24] and according to the standardizing pharmacogenetic terms consensus.[25] *CYP3A4* *2, *20, *22 and *CYP3A5* *3 and *6 genotypes were merged into a CYP3A phenotype as follows: subjects with at least 1 CYP3A4 reduced activity allele (i.e. CYP3A4 *1/*22 or *22/*22) and no CYP3A5 activity (CYP3A5 *3/*3) were considered PM; subjects with normal CYP3A4 activity (CYP3A4 *1/*1) and no CYP3A5 activity (CYP3A5 *3/*3) were considered IM and subjects with normal CYP3A4 activity (CYP3A4 *1/*1) and at least 1 CYP3A5 functional allele (CYP3A5 *1/*1 or *1/*3) were categorized as extensive metabolizers (EM).[26] Furthermore, a value was assigned to *CYP1A2* *1B, *1C and *1F alleles based on their functionality: 0.5 to *1C, 1 to *1, 1.5 to *1F and 1.25 to *1B. An activity score was calculated as the sum of the values assigned to each allele and finally was translated into phenotypes: NMs and UMs.[27]

3 | RESULTS

3.1 | Demographic and genotypic characteristics

Ten subjects were Caucasian and 14 were Latin American. The average age was similar between males and females. Males had greater weight and height than females; however, the body mass index values did not differ significantly (Table 1).

Genotype and phenotype frequencies of the analysed variants are shown in Table S1. *HTR2C* rs3813929, rs518147, *ABCB1* rs4728709, *COMT* rs13306278, *CYP2D6* *14 (rs5030865), *17 (rs28371706), *3 (rs35742686), *6 (rs5030655), *7 (rs5030867), *8 (rs5030865), *CYP3A4* *2 (rs55785340) and *6 (rs4646438) were not in Hardy–Weinberg equilibrium ($P \leq .05$). The rest of the polymorphisms were in Hardy–Weinberg equilibrium ($P \geq .05$).

Genotype frequencies of *ABCB1* rs1128503, rs2032582, 10276036 and rs4148737 and *HTR2C* rs518147 polymorphisms were significantly different between males and females (Table S1).

3.2 | Pharmacokinetic analysis

Mean and standard deviation of ARI, DARI and OLA pharmacokinetic parameters are shown in Table 2. Females had higher ARI $T_{1/2}$

TABLE 1 Demographic characteristics

	n (%)	Age (y)	Weight (kg)	Height (m)	BMI (kg/m^2)
All	24 (100)	31.5 ± 11.6	71.4 ± 12.2	1.68 ± 0.11	25.3 ± 2.6
Males	12 (50)	28.5 ± 7.4	78.4 ± 12.2	1.76 ± 0.09	25.4 ± 2.8
Females	12 (50)	34.6 ± 14.3	64.3 ± 7.4	1.60 ± 0.07	25.1 ± 2.5
P		.104	.003	<.0001	.798

Values are shown as mean ± standard deviation unless otherwise indicated.
BMI, body mass index

TABLE 2 Pharmacokinetic parameters of aripiprazole, dehydro-aripiprazole and olanzapine after administration of a single dose and 5 multiple doses

Aripiprazole	All SINGLE DOSE	Males	Females	All MULTIPLE DOSE	Males	Females
AUC (ng·h/mL)[b]	724.9 ± 236.5	647.0 ± 197.2	802.7 ± 254.6	11 102.4 ± 8234.0	7790.0 ± 4071.5	14 415.7 ± 10061.4
C_{max} (ng/mL)	50.6 ± 15.5	47.7 ± 14.4	53.5 ± 16.6	138.0 ± 45.9	129.6 ± 47.4	146.3 ± 44.9
T_{max} (h)	5.2 ± 2.4	4.8 ± 2.8	5.6 ± 2.0	3.2 ± 1.4	3.3 ± 1.4	3.2 ± 1.5
$T_{½}$ (h)	NA	NA	NA	66.1 ± 24.6	56.1 ± 19.9	76.1 ± 25.5[a]
Vd/F (L/kg)	NA	NA	NA	6.0 ± 1.6	5.1 ± 1.0	7.0 ± 1.4[a]
Cl/F (mL/h/kg)	NA	NA	NA	68.1 ± 21.4	69.1 ± 25.6	67.1 ± 17.5
DEHYDRO-aripiprazole	**All SINGLE DOSE**	**Males**	**Females**	**All MULTIPLE DOSE**	**Males**	**Females**
AUC_{24h} (ng·h/mL)	77.4 ± 43.9	90.3 ± 56.9	64.5 ± 20.6[a]	5149.8 ± 1628.6	4721.3 ± 1670.3	5578.3 ± 1534.8
C_{max} (ng/mL)	5.4 ± 8.5	6.5 ± 5.3	4.3 ± 1.2	34.9 ± 8.5	35.6 ± 9.6	34.1 ± 7.4
T_{max} (h)	21.9 ± 4.5	20.9 ± 5.4	22.9 ± 3.4	6.1 ± 4.4	7.1 ± 3.9	5.1 ± 4.8
$T_{½}$ (h)	NA	NA	NA	107.3 ± 62.5	89.4 ± 45.4	126.9 ± 74.2
Vd/F (L/kg)	NA	NA	NA	40.0 ± 44.0	22.4 ± 10.3	57.6 ± 57.1[a]
Cl/F (mL/h/kg)	NA	NA	NA	203.5 ± 51.0	181.3 ± 47.3	230.7 ± 44.2[a]
OLANZAPINE	**All SINGLE DOSE**	**Males**	**Females**	**All MULTIPLE DOSE**	**Males**	**Females**
AUC (ng·h/mL)[b]	127.6 ± 33.1	127.8 ± 38.6	127.4 ± 28.4	1289.5 ± 370.1	1142.7 ± 291.2	1436.2 ± 393.1
C_{max} (ng/mL)	7.9 ± 2.2	7.5 ± 2.0	8.2 ± 2.5	19.1 ± 4.8	18.4 ± 4.0	19.9 ± 5.5
T_{max} (h)	5.3 ± 2.3	5.4 ± 2.7	5.3 ± 1.9	4.4 ± 1.7	4.6 ± 1.6	4.3 ± 1.9
$T_{½}$ (h)	NA	NA	NA	77.1 ± 28.2	79.5 ± 33.4	74.8 ± 23.1
Vd/F (L/kg)	NA	NA	NA	26.6 ± 15.9	26.1 ± 17.2	27.0 ± 15.3
Cl/F (mL/h/kg)	NA	NA	NA	229.7 ± 54.7	218.9 ± 51.9	240.6 ± 57.5

[a]$P \leq 0.05$ vs. males after adjusting for weight. NA: not available.

[b]for single dose administration the 24 h area under the concentration–time curve (AUC_{24h}), while for multiple dose administration area under the curve from zero to infinity (AUC_{inf}) are shown.

Abbreviations: C_{max}: maximum plasma concentration; T_{max}: time to reach the maximum plasma concentration; AUC: area under the curve; $T_{1/2}$: half-life; Cl/F: total drug clearance adjusted for bioavailability; Vd/F: volume of distribution adjusted for bioavailability.

(*P* = .044) and Vd/F (*P* = .001) and DARI Vd/F (*P* = .048) and Cl/F (*P* = .015) after multiple dose administration. Moreover, males had higher DARI AUC_{24h} (*P* = .035) after single dose administration. No differences were found in OLA pharmacokinetic parameters between males and females.

3.3 | The effects of aripiprazole and olanzapine on pupillary light reflex

Following the first oral administration of ARI, the pupil was significantly dilated. Subsequently, on the next 4 drug administration days ARI caused minor constriction. All pupillometric parameters changed significantly (MAX: *P* = .008; MIN: *P* = .009; CON: *P* = .013; LAT: *P* = .009; ACV: *P* = .012; MCV: *P* = .006; ADV: *P* = .024; T75: *P* = .015; Figure 1). OLA showed the same tendency, but only MIN reached the statistically significant level (*P* = .046; Figure 1). No differences were found between males and females regarding any pupillometric parameters.

3.3.1 | Single dose administration

ARI AUC_{last} and T_{max} had an influence on CON (*P* = .029 and *P* = .043, respectively). Moreover, AUC_{last} had an impact on MCV and ADV (*P* = .004 and *P* = .034, respectively; Table 3).

Furthermore, DARI AUC_{24h} had an impact on MAX (*P* = .042), MIN (*P* = .050), CON (*P* = .047), ACV (*P* = .049) and MCV (*P* = .046; Table 3).

These associations were not confirmed in the multivariate analysis (*P* > .05). Nonetheless, OLA pharmacokinetics did not have an effect on any of the pupillometric parameters.

3.3.2 | Multiple dose administration

DARI C_{max} had an impact on several pupillometric parameters: MAX (*P* = .029), MIN (*P* = .049), CON (*P* = .015), ACV (*P* = .041), MCV (*P* = .027), ADV (*P* = .033) and T75 (*P* = .045; Table 3). These associations were not confirmed in the multivariate analysis (*P* > .05).

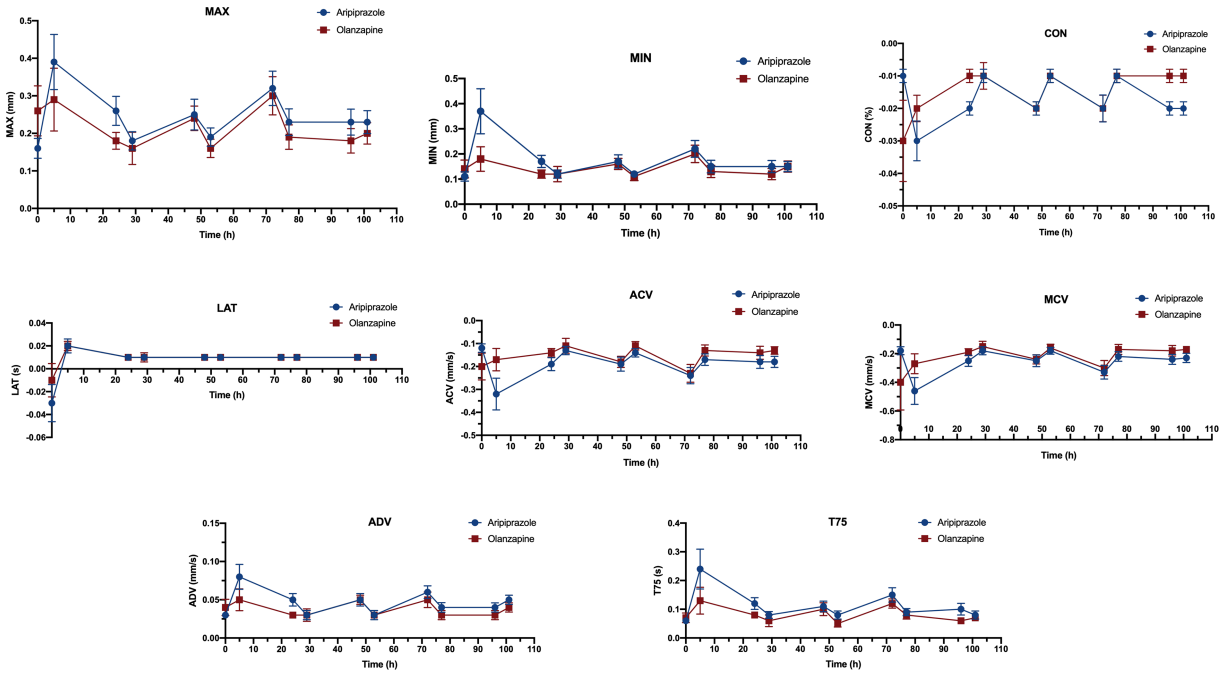

FIGURE 1 Changes in pupillometric parameters after aripiprazole and olanzapine administration. MAX, maximum pupil diameter, MIN, minimum pupil diameter; CON, percentage of constriction; LAT, latency; ACV, average constriction velocity; MCV, maximum constriction velocity; ADV, average dilation velocity; T75, total time taken by the pupil to recover 75% of the initial resting pupil size

TABLE 3 Influence of the pharmacokinetic parameters of aripiprazole and dehydro-aripiprazole on pupillometric parameters

	Aripiprazole		Dehydro-aripiprazole	
	Single dose		Single dose	Multiple dose
Variable	AUC_{last} (ng·h/mL)	T_{max} (h)	AUC_{24h} (ng·h/mL)	C_{max} (ng/mL)
MAX	---	---	P = .042 ↓↓	P = .029 ↓↓
MIN	---	---	P = .050 ↓↓	P = .049 ↓↓
CON	P = .029 ↓↑	P = .043 ↓↓	P = .047 ↓↓	P = .015 ↓↓
ACV	---	---	P = .049 ↓↓	P = .041 ↓↓
MCV	P = .004 ↓↑	---	P = .046 ↓↑	P = .027 ↓↑
ADV	P = .034 ↓↓	---	---	P = .033 ↓↓
T75	---	---	---	P = .045 ↓↓

The arrows show the relationship between pharmacokinetic and pupillometric parameters. The first arrow refers to the pharmacokinetic parameter, while the second arrow refers to the pupillometric parameter. ↓↑ is indirectly proportional, while ↓↓ is directly proportional with the changes.
Abbreviations: MAX: maximum pupil diameter; MIN: minimum pupil diameter; CON: percent of constriction; LAT: latency; ACV: average constriction velocity; MCV: maximum constriction velocity; ADV: average dilation velocity; T75: total time taken by the pupil to recover 75% of the initial resting pupil size. C_{max}: maximum plasma concentration; T_{max}: time to reach the maximum plasma concentration; AUC_{24h}: 24 h area under the concentration–time curve; AUC_{last}: area under the curve from time zero to the last observed time point.

Nonetheless, ARI and OLA pharmacokinetics did not have any association with any of the pupillometric parameters.

3.4 | The influence of polymorphisms on pupillometry

3.4.1 | Aripiprazole

Subjects with the CYP3A IM phenotype had significantly higher MAX levels than PMs (P = .019). Moreover, *HTR2A* rs6314 T carriers and *UGT1A1* rs8877829 T/T homozygotes had higher MIN levels than C/C subjects (P = .025 and .039, respectively). Additionally, subjects with the CYP3A PM phenotype and *DRD2* rs1800487 A2 carriers had higher CON values than with IM phenotype and A1 carriers, respectively; however, only CYP3A reached the significant level (P = .008 and .058, respectively).

Likewise, CYP3A IM and EM subjects, *DRD2* rs1800487 A2 carriers, *ABCB1* rs10280101 A/A, rs12720067 C/C and rs11983225 T/T subjects had higher LAT values than CYP3A PM subjects, *DRD2* rs1800487 A1 carriers and *ABCB1* 10280101 C, *ABCB1* rs12720067 T and *ABCB1* rs19983225 C carriers, respectively (P = .020, .039

and .034, respectively). Moreover, CYP3A PM subjects had lower ACV and MCV values than IM subjects (P = .028 and .022, respectively). Finally, *HTR2A* rs6314 T allele carriers had higher T75 levels than C/C homozygotes, although it did not reach the statistically significant level (P = .058).

After performing the multivariate tests, the influence of *HTR2A* rs6314 on MIN and T75 remained significant (P = .001 and .020, respectively; Figure 2).

3.4.2 | Olanzapine

DRD2 rs1800497 A2 allele carriers had higher MAX, ACV and MCV values than A1/A1 homozygotes (P = 0.025, .043 and .038).

After performing the multivariate tests, the influence of *DRD2* rs1800497 on MAX remained significant (P = .039; Figure 2).

3.5 | The influence of polymorphisms on pharmacokinetics

The univariate and multivariate analyses revealed associations between ARI, DARI and OLA pharmacokinetic parameters and several polymorphisms (Tables S2, S3 and S4). Additionally, the results of the multivariate analysis are shown in Table 4.

3.5.1 | Aripiprazole

Vd/F and Cl/F were notably higher in CYP2D6 UMs than in NMs and IMs (Vd/F: p = 0.001 and P = .016; Cl/F: P = .016 and .016, respectively). Additionally, AUC_{inf} and C_{max} on days 1 and 5 were significantly higher in CYP1A2 UMs than in NMs and RMs (P = .034, .040 and .012, respectively). Moreover, Cl/F was lower in CYP1A2 UMs compared to the other phenotypes (P = .033). Additionally, T_{max} was higher in *ABCB1* rs1045642 TT compared to CC subjects (P = .033). *ABCB1* rs4148737 C/C subjects had 2 times higher $T_{1/2}$ and T_{max} than T carriers (P = .024 and .004). The results are shown in Table S2. Several of these associations were confirmed in the multivariate analysis (Table 4).

3.5.2 | Dehydro-aripiprazole

C_{max} was significantly higher in CYP2D6 IM subjects than in NMs and UMs (P = .006). CYP3A4 PMs had higher AUC_{last} and C_{max} than IMs and EMs (P = .001 and .001, respectively). Additionally, Vd/F was higher in CYP1A2 UMs than in NMs and RMs (P = .046). Additionally, T_{max} was higher in *ABCB1* rs1045642 T/T and lower in *ABCB1* rs4148737 C/C subjects compared to the other genotypes (P = .019 and P = .045, respectively). The results are shown in Table S3. Several of these associations were confirmed in the multivariate analysis (Table 4).

3.5.3 | Olanzapine

$T_{1/2}$ was 2 times higher in CYP3A EM subjects compared to IMs and PMs (P = .025). Additionally, *ABCB1* rs10280101 A/A, rs12720067 C/C and rs11983225 T/T subjects had significantly higher $T_{1/2}$ compared to the other genotypes (P = .046, .046 and .046, respectively). Finally, *UGT1A1* rs887829 T/T homozygotes had higher T_{max} than

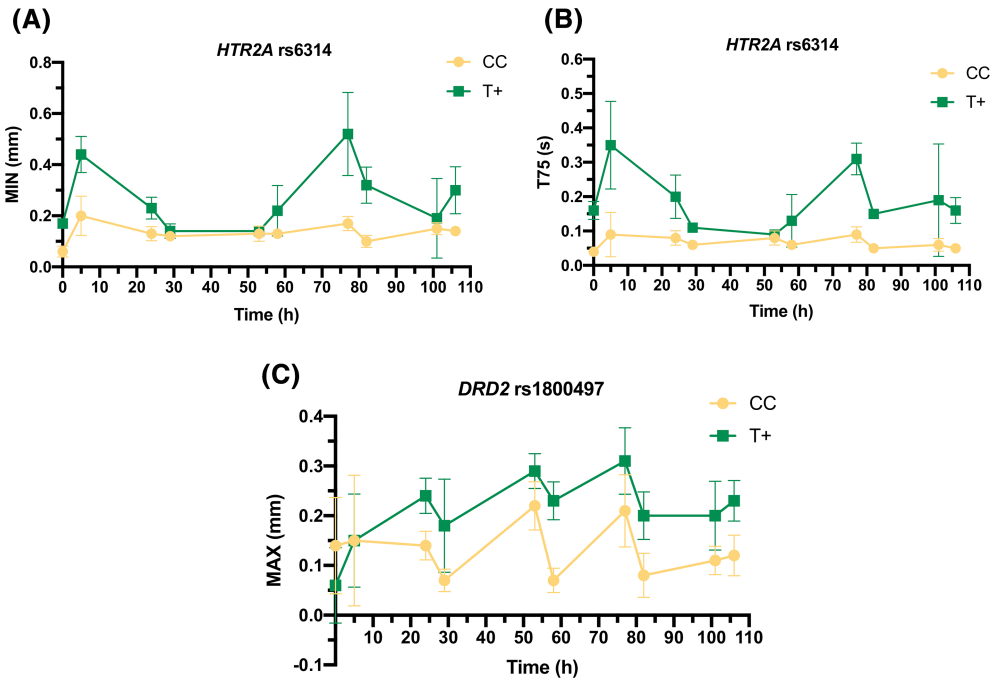

FIGURE 2 The influence of HTR2A rs6314 and DRD2 rs1800497 polymorphisms on pupillometric parameters. (A) The influence of HTR2A rs6314 on minimum pupil diameter (MIN) during aripiprazole treatment. (B) The influence of HTR2A rs6314 on total time taken by the pupil to recover 75% of the initial resting pupil size (T75) during aripiprazole treatment. (C) The influence of DRD2 rs1800497 on maximum pupil diameter (MAX) during olanzapine treatment

TABLE 4 Influence of genetic polymorphisms on aripiprazole, dehydro-aripiprazole and olanzapine pharmacokinetic parameters in the multivariate analysis. Results with $P \leq .05$ are highlighted in bold

Aripiprazole						
Variable	**AUC (ng·h/mL)**	**C_{max} (ng/mL)**	**T_{max} (h)**	**$T_{1/2}$ (h)**	**Cl/F (L/h·kg)**	**Vd/F (L/kg)**
CYP2D6 phenotype	**β = −1063.9; *P* = .049**	---	---	**β = 16.2; *P* = .005**	β = 15.1; *P* = .055	**β = 1.9; *P* = .001**
CYP1A2 phenotype	**β = 64555; *P* = .035**	**β = 421; *P* = .044**	---	**β = 29.3; *P* = .041**	β = −22.1; *P* = .054	---
ABCB1 rs1045642	---	---	β = 0.183; *P* = .738	---	---	---
ABCB1 rs4148737	**β = 58941; *P* = .026**	---	β = .183; *P* = .545	**β = −17.5; *P* = .027**	---	---
Dehydro-aripiprazole						
Variable	**AUC (ng·h/mL)**	**C_{max} (ng/mL)**	**T_{max} (h)**	**$T_{1/2}$ (h)**	**Cl/F (L/h·kg)**	**Vd/F (L/kg)**
CYP2D6 phenotype	---	**β = 17.3; *P* = .043**	---	---	---	---
CYP3A4 phenotype	β = 4257; *P* = .273	**β = 55.2; *P* = .005**	**β = 3.14; *P* = .015**	---	---	---
CYP1A2 phenotype	---	**β = 70.5; *P* = .039**	---	---	---	β = 10.2; *P* = .387
ABCB1 rs1045642	---	---	**β = 2.93; *P* = .021**	---	---	---
ABCB1 rs4148737	---	---	β = −1.99; *P* = .240	---	---	**β = 17.1; *P* = .041**
Olanzapine						
Variable	**AUC (ng·h/mL)**	**C_{max} (ng/mL)**	**T_{max} (h)**	**$T_{1/2}$ (h)**	**Cl/F (L/h·kg)**	**Vd/F (L/kg)**
CYP3A4 phenotype	---	---	---	**β = −17; *P* = .037**	---	---
ABCB1 rs10280101	---	---	---	β = −26.9; *P* = .145	---	---
ABCB1 rs12720067	---	---	---	β = −26.9; *P* = .145	---	---
ABCB1 rs11983225	---	---	---	β = −26.9; *P* = .145	---	---
UGT1A1 rs887829	---	---	**β = 1.58; *P* = .006**	---	---	---

Abbreviations: CYP: cytochrome p450 oxidase; *ABCB1*: ATP binding cassette subfamily B member 1; *UGT1A1*: UDP glucuronosyltransferase family 1 member A1; C_{max}: maximum plasma concentration; T_{max}: time to reach the maximum plasma concentration; AUC: area under the curve; $T_{1/2}$: half-life; Cl/F: total drug clearance adjusted for bioavailability; Vd/F: volume of distribution adjusted for bioavailability.

C/T heterozygotes and C/C homozygotes ($P = .016$). The results are shown in Table S4. Several of these associations were confirmed in the multivariate analysis (Table 4).

4 | DISCUSSION

4.1 | The influence of sex on pharmacokinetics, pharmacogenetics and pupillometry

In the current study, ARI and DARI pharmacokinetics were affected by sex. Females had higher ARI $T_{1/2}$ and Vd/F than males, which is consistent with our previous studies.[18,28] However, in the present study females had higher DARI Vd/F and Cl/F and lower AUC_{last}, which may be explained by the low sample size. Based on previous studies, Cl/F should be lower in males compared to females.[29,30] Nonetheless, no dosage adjustment is recommended for ARI or OLA despite sex differences because they are predominantly explained by the differences in body weight.[30,31]

The differences observed in the prevalence of *ABCB1* rs1128503, rs2032582, rs10276036 and rs4148737 polymorphisms regarding sex may be explained by the reduced sample size. Regarding *HTR2C*, it is explained by the location of the gene on chromosome X.

Some polymorphisms were not in Hardy–Weinberg equilibrium. Regarding *HTR2C*, it is due to the location of the gene on chromosome X. The disequilibrium for *ABCB1* rs4728709 and *COMT* rs13306278 could be explained by the small sample size. Regarding the *CYP2D6* and *CYP3A4* polymorphisms it is explained by the low frequency of mutated alleles.

No differences were found between males and females in any of the pupillometric parameters, which is consistent with previous studies.[18,32]

4.2 | Effects of aripiprazole, dehydro-aripiprazole and olanzapine on pupillometry

The mechanism of action of ARI and OLA is still not perfectly understood.[33] ARI and DARI achieve their pharmacological effect possibly by partial agonistic activity at dopamine D2 and 5-HT1A receptors and antagonistic activity at 5-HT2A receptors. Pharmacodynamic effects on receptors other than dopamine D2, 5-HT1A and 5-HT2A may explain other clinical effects: changes in pupillary light reflex could be caused by partial agonistic activity at α1-adrenergic receptors.[31] Pupil dilatation is primarily an α1-adrenergic receptor-mediated effect,[34] while it is mediated to a lesser extent by dopamine and serotonin receptors.[35,36]

The dilatation observed after the first ARI administration could be explained by its partial agonism at these receptors. On the contrary, OLA is an antagonist at these receptors,[3] therefore, it could explain the lack of pupil dilatation. Both ARI and DARI have higher affinity for dopamine D2 and 5-HT1A than for α1-adrenergic receptors.[37] When DARI was present, neither ARI nor DARI bound to α1-adrenergic receptors due to competitive inhibition caused by the higher affinity for dopamine D2 and 5-HT1A receptors. Hence, a constriction was observed after drug administration. Our results could confirm the fact that pupillary changes may rather be caused by the metabolite than the parent compound.[34] This could be the reason why DARI C_{max} and AUC_{last} had an influence on several pupillometric parameters, while the pharmacokinetic parameters of ARI influenced only a few of them.

In conclusion, ARI and DARI caused changes in pupillary light reflex due to their unique pharmacological profile. Measuring dynamic pupillary light reflex is already a valid test for the pharmacodynamic effects of opioid- and some noradrenergic drugs.[38,39] Both drugs caused pupil constriction in 2 previous studies[18,40]; however, in another study, neither ARI nor OLA affected pupil contraction.[41] Hence, more studies are needed to alleviate the ambiguity and they should be repeated in patients. Afterwards, pupillometry could be introduced in the practice to assess autonomic nerve activity.

4.3 | Polymorphisms and pupillometry

In previous studies with opioids, CYP2D6 UMs experienced increased and PMs experienced decreased pupil size compared to EMs.[38,39] We could not replicate these findings with ARI and OLA, which may be due to their different mechanism of action. Additionally, we did not find any PM and only 2 UMs were present in our population. Our results confirm those in our previous study with healthy volunteers that no associations can be found between CYP2D6 phenotypes and pupillometric parameters.[18]

CYP3A phenotypes are unrelated to opioid pharmacokinetics.[42] Notwithstanding, in the present study, CYP3A IM pupil size was increased compared to that of PMs after ARI administration. This was expected as ARI caused pupil constriction after multiple dose administration; the pupil was under prolonged ARI exposure in PM subjects.

The effects of dopamine and serotonin on the pupillary light reflex are well known. High serotonin levels cause pupil dilatation[36] and dopamine may cause pupil dilatation or constriction through sympathetic and parasympathetic nerves, respectively.[35] Based on our results, pupil constriction could be due to the antagonist activity of ARI at 5-HT2A and 5-HT7 receptors, while its dilatation could be explained by its partial agonism at dopamine D2, D3, D4 and serotonin 5-HT1A, 5-HT2C receptors.[37] This theory was confirmed by our study: both *HTR2A* rs6314 and *DRD2* rs1800487 had an influence on the pupil size, the proportion of its change, the time of onset of constriction and pupil recovery. Additionally, *DRD2* rs1800487 also affected the pupil size and its constriction velocity after OLA treatment. In our previous study some *HTR2A*, *HTR2C*, *DRD2* and *DRD3* polymorphisms were also related to pupillometric parameters.[18] The lack of associations with *HTR2C* and *DRD3* polymorphisms in the present study could be due to the low sample size.

Both ARI and OLA are transported by the P-glycoprotein (P-gp, ABCB1, MDR1).[43] However, to our knowledge, no previous study could associate the genetic differences in *ABCB1* to pupil contraction. We previously analysed 3 polymorphisms: rs1045642 (C3435T), rs1128503 (C1236T) and rs2032582 (G2677T/A), but no associations were found.[18] In the current study we analysed 12 *ABCB1* polymorphisms. No association was found with the 3 previously mentioned polymorphisms; however, 3 others had an influence on pupil recovery after ARI treatment. It may suggest that, even though the 3 most common polymorphisms do not affect the pupillary light reflex, others could have an influence. Further research including other less studied *ABCB1* polymorphisms should be performed.

UGT1A1 rs8877829 subjects with the mutated T/T genotype had greater pupil size after ARI treatment. Based on in vitro studies, ARI does not undergo direct glucuronidation by UGT enzymes[31]; however, no sufficient evidence is available thus far. Considering our results, *UGT1A1* may be involved in ARI metabolism.

4.4 | Pharmacokinetics and polymorphisms

Our study confirms the impact of CYP2D6 phenotypes on ARI and DARI pharmacokinetics.[18,44,45] All pharmacokinetic parameters, except for T_{max}, were different in UMs compared to NMs and IMs.

CYP3A only had an impact on DARI and OLA pharmacokinetics, confirming its involvement in ARI and OLA metabolism.[7] The lack of association with the parent drug, ARI, may be due to the low sample size, because CYP2D6 and CYP3A4 contribute about equally to the metabolism of aripiprazole.[7] Moreover, CYP3A activity varies predominantly by sex and inhibition or induction of a wide range of substrates, rather than by polymorphisms.[46]

Based on the literature, ARI is not a substrate of CYP1A2.[31] Unexpectedly, in our study, the CYP1A2 phenotype influenced ARI and DARI pharmacokinetics as UMs showed a lower disposition compared to the other phenotypes. To the best of our knowledge, this is the first study to report a similar result. Based on our findings, more studies should be performed to confirm the role of CYP1A2 in ARI pharmacokinetics.

To date, there are no consistent findings about the role of polymorphisms in ABCB1. In our previous studies the C1236T (rs1128503) polymorphism had an influence on ARI pharmacokinetics.[18,47] In the current study, the C3435T (rs1045642) and rs4148737 polymorphisms were related to ARI and DARI $T_{1/2}$ and C_{max}—and the rs10280101, rs12720067 and rs11983225 polymorphisms had an influence on OLA $T_{1/2}$ levels. Presumably *ABCB1* has an effect on the pharmacokinetics of these antipsychotics being substrates of P-gp.[43] According to our knowledge, no other study analysed polymorphisms in *ABCB1* other than C3435T (rs1045642), G2677TA (rs2032582), C1236T (rs1128503). More studies are needed including more polymorphisms in *ABCB1* to provide a wider insight of its role in ARI and OLA pharmacokinetics. Additionally, as stated previously, the lack of consensus on P-gp pharmacogenetics

is partially explained by the lack of studies and the guidelines describing phenotype interference from variants.

Finally, the *UGT1A1* rs887829 polymorphism affected OLA pharmacokinetics. In a previous study, this polymorphism was related to some adverse effects but not pharmacokinetics.[48] OLA is metabolized predominantly by direct glucuronidation via the UGT enzyme family,[7] but clear evidence was found only for UGT1A4.[6] Additionally, *UGT1A4* and *UGT2B10* polymorphisms significantly contributed to the interindividual variability in OLA metabolism.[49,50] Our study is the first reporting an association between an *UGT1A1* polymorphism and OLA pharmacokinetics.

4.5 | Study limitations

Only 24 subjects were included in the study, which we consider its main limitation. Therefore, it is important to interpret these results with caution: studies including more subjects are necessary to increase the statistical reliability of the results. Moreover, the present study should be repeated in schizophrenic patients, whose brain structure and genetics may differ from healthy volunteers. Moreover, neither ARI, nor OLA reached steady state during 5 days of treatment. Both could have had a greater effect on autonomic nerve activity if they had reached steady state. However, the Ethics Committee does not authorize a treatment longer than 5 days with antipsychotics in healthy volunteers. Accordingly, we cannot apply pupillometry to assess autonomic disfunction in the clinical practice yet. Additionally, the Cl/F and Vd/F values were calculated without knowing the bioavailability, which can yield questionable results, especially for DARI.

5 | CONCLUSIONS

ARI administration produced pupil contraction: it affected all pupillometric parameters. After the first dosing it caused dilatation, which was followed by constriction after each day of treatment. OLA did not cause any changes in any of the pupillometric parameters. Additionally, the effects of ARI on the pupil size, the time of onset of constriction, pupil recovery and constriction velocity were associated with polymorphisms in *CYP3A*, *HTR2A*, *UGT1A1*, *DRD2* and *ABCB1* genes. ARI, DARI and OLA pharmacokinetics were significantly affected by polymorphisms in *CYP2D6*, *CYP3A*, *CYP1A2*, *ABCB1* and *UGT1A1* genes. In conclusion, pupillography could be a noninvasive tool to assess autonomic nervous system activity during antipsychotic drug treatment.

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CONTRIBUTORS

Wrote manuscript: Dora Koller; designed research: Dora Koller, Francisco Abad-Santos, Miriam Saiz-Rodríguez, Dolores Ochoa; performed clinical trial: Manuel Román, Gina Mejía, Francisco Abad-Santos, Daniel Romero-Palacián, Alejandro de Miguel-Cáceres, Samuel Martín, Dolores Ochoa; analysed data: Dora Koller, Susana Almenara, Francisco Abad-Santos; determination of drug concentrations: Dora Koller, Pablo Zubiaur, Aneta Wojnicz; pharmacogenetics: Dora Koller, Pablo Zubiaur, Marcos Navares, Miriam Saiz-Rodríguez.

DATA AVAILABILITY STATEMENT

Clinical Trial registry name, URL and registration number: TREATMENT-HV, EUDRA-CT: 2018–000744-26, https://eudract.ema.europa.eu/.

ORCID

Dora Koller https://orcid.org/0000-0002-0415-0466

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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