DE GRUYTER OPEN

Acta Medica Marisiensis 2017;63(2):73-79 DOI: 10.1515/amma-2017-0023

RESEARCH ARTICLE

The Influence of CYP2D6 Phenotype on the Pharmacokinetic Profile of Atomoxetine in Caucasian Healthy Subjects

Ioana Todor[1], Dana Muntean[1], Maria Neag[2], Corina Bocsan[2], Anca Buzoianu[2], Laurian Vlase[1*], Daniel Leucuta[3], Ana-Maria Gheldiu[1], Adina Popa[4], Corina Briciu[4]

1 University of Medicine and Pharmacy "Iuliu Hatieganu", Faculty of Pharmacy, Department of Pharmaceutical Technology and Biopharmaceutics, Cluj-Napoca, Romania
2 University of Medicine and Pharmacy "Iuliu Hatieganu", Faculty of Medicine, Department of Pharmacology, Toxicology and Clinical Pharmacology, Cluj- Napoca, Romania
3 University of Medicine and Pharmacy "Iuliu Hatieganu", Department of Medical Informatics and Biostatistics, Cluj-Napoca, Romania
4 University of Medicine and Pharmacy "Iuliu Hatieganu", Faculty of Pharmacy, Department of Clinical Pharmacy, Cluj-Napoca, Romania

Objective: To analyze a potential phenotypic variation within the studied group based on the pharmacokinetic profile of atomoxetine and its active metabolite, and to further investigate the impact of CYP2D6 phenotype on atomoxetine pharmacokinetics. **Methods**: The study was conducted as an open-label, non-randomized clinical trial which included 43 Caucasian healthy volunteers. Each subject received a single oral dose of atomoxetine 25 mg. Subsequently, atomoxetine and 4-hydroxyatomoxetine-O-glucuronide (glucuronidated active metabolite) plasma concentrations were determined and a noncompartmental method was used to calculate the pharmacokinetic parameters of both compounds. Further on, the CYP2D6 metabolic phenotype was assessed using the area under the curve (AUC) metabolic ratio (atomoxetine/ 4-hydroxyatomoxetine-O-glucuronide) and specific statistical tests (Lilliefors (Kolgomorov-Smirnov) and Anderson-Darling test). The phenotypic differences in atomoxetine disposition were identified based on the pharmacokinetic profile of the parent drug and its metabolite. **Results**: The statistical analysis revealed that the AUC metabolic ratio data set did not follow a normal distribution. As a result, two different phenotypes were identified, respectively the poor metabolizer (PM) group which included 3 individuals and the extensive metabolizer (EM) group which comprised the remaining 40 subjects. Also, it was demonstrated that the metabolic phenotype significantly influenced atomoxetine pharmacokinetics, as PMs presented a 4.5-fold higher exposure to the parent drug and a 3.2-fold lower exposure to its metabolite in comparison to EMs. **Conclusions**: The pharmacokinetic and statistical analysis emphasized the existence of 2 metabolic phenotypes: EMs and PMs. Furthermore, it was proved that the interphenotype variability had a marked influence on atomoxetine pharmacokinetic profile.

Keywords: atomoxetine, 4-hydroxyatomoxetine-O-glucuronide, phenotype, metabolic ratio, pharmacokinetics

Received 28 May 2017 / Accepted 20 June 2017

Introduction

Atomoxetine is the first nonstimulant medication approved by the United States Food and Drug Administration (FDA) for the treatment of attention deficit hyperactivity disorder (ADHD) in children, adolescents and adults [1–3]. The agent is a potent and selective norepinephrine reuptake inhibitor with minimal affinity for the other monoamine transporters or receptors. A second-line approach for ADHD, atomoxetine can be considered as first treatment option when anxiety disorders, major depression, tics or substance abuse problems are diagnosed alongside ADHD and in specific cases in which stimulant medication is not effective or is poorly tolerated [1,4].

Atomoxetine is rapidly absorbed after oral intake and peak plasma concentrations (C_{max}) are reached in approximately 1 to 2 hours [3,5]. The pharmacokinetic studies revealed that three oxidative pathways are involved in the biotransformation of this compound, respectively aromatic-ring hydroxylation, benzylic hydroxylation and N-demethylation. Aromatic ring-hydroxylation is CYP2D6-dependent and results in the formation of the main metabolite, 4-hydroxyatomoxetine, which exhibits similar pharmacological activity to that of the parent compound. Following hydroxylation, the metabolite is subsequently glucuronidated and excreted through urine [3,5,6]. Due to the fact that CYP2D6 is a polymorphic enzyme, differences were noted between phenotypic groups. For example, the absolute bioavailability of atomoxetine ranges from 63% for extensive metabolizers (EMs) to 94% for poor metabolizers (PMs) (3,5). In addition, the mean steady-state plasma concentrations are about 10-fold higher in PMs compared with EMs. Regardless of the metabolic status, the largest fraction of atomoxetine is eliminated into urine, principally as 4-hydroxyatomoxetine-O-glucuronide, while less than 3 % of the initial dose is eliminated unchanged [3,5,7].

Previous studies have revealed that CYP2D6 genotype/phenotype may influence atomoxetine pharmacokinetics in various races and ethnic groups [8–10]. Taking into consideration the complexity of this matter, the aim of the present study was to evaluate a potential phenotypic variation within a certain group based on the pharmacokinetic profile of atomoxetine and to further investigate and

* Correspondence to: Laurian Vlase
E-mail: laurian.vlase@umfcluj.ro

confirm the impact of CYP2D6 metabolic phenotype on atomoxetine pharmacokinetics in Caucasian healthy volunteers.

Material and methods

Subjects

The study was conducted in accordance with Good Clinical Practice guidelines and the principles of Helsinki (1964) and its amendments (Tokyo 1975, Venice 1983, Hong Kong 1989). Moreover, the protocol was approved by the Ethics Committee of the University of Medicine and Pharmacy "Iuliu Hatieganu", from Cluj-Napoca (Romania) and written informed consent was obtained from each volunteer prior to performing any study-related procedures.

Forty-three Caucasian males and females were enrolled in the clinical study. The inclusion criteria required for the study population to comprise healthy subjects between 18 and 55 years of age and to have a body mass index (BMI) between 19 and 25 kg/m^2. Their health status was assessed based on their medical history, physical examination and routine laboratory investigations (hematology, biochemistry and serological tests). Subjects were excluded if they were smokers, they had a history of substance or alcohol abuse, a history of documented allergy or if they took regular medication, except for oral contraceptives. Any medical condition or lifestyle factors that may influence drug response were also considered exclusion criteria.

Study design

The study was designed as an open-label, non-randomized clinical trial during which every subject received a single oral dose of atomoxetine (25 mg). The study drug was administered in the morning, after an overnight fast of at least 12 h and with at least 150 mL of water. Alcohol, beverages or food containing methylxanthines (coffee, tea, cola, etc.) were forbidden starting with 48 h prior to drug intake and until the last blood sample collection. Also, subjects were allowed to drink water only starting 2 h post-dosing and during their 24-h confinement they were provided with standardized meals. The pharmaceutical product used was Strattera® (atomoxetine hydrochloride, 25 mg hard capsules; manufactured by Lilly SA, Hampshire, Great Britain).

Blood plasma samples collection and bioanalytical methods

Venous blood samples (5 ml) were drawn before dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 36 and 48 hours after drug administration and stored in heparin vacutainer tubes. After centrifugation, the separated plasma was stored frozen (-20°C) until analysis.

A validated high-throughput liquid chromatography-mass spectrometry (LC/MS) method was used to determine the plasma concentrations of atomoxetine and 4-hydroxyatomoxetine-*O*-glucuronide. The chromatographic system was an Agilent 1100 series (binary pump, autosampler, thermostat; Agilent Technologies, Santa Clara, CA, USA) coupled with a Brucker Ion Trap SL (BruckerDaltonics GmbH, Bremen, Germany). In addition, the chromatographic column used was a Zobrax SB-C18 (100 mm x 3.0 mm i.d, 3.5 µl; Agilent Technologies) and the mobile phase consisted of 2 mM ammonium formate solution and acetonitrile mixture, elution in gradient: 11 % acetonitrile at start, 41% at 2 minutes. The flow rate was 1 mL/min and the thermostat temperature was set at 48°C. The mass spectrometry detection was in single ion monitoring mode, positive ions, using an electro-spray ionization source. The ions monitored were m/z 256 for the parent dug and m/z 448 for 4-hydroxyatomoxetine-*O*-glucuronide. The retention times for atomoxetine and its glucuronidated metabolite were 4.1 min and 2.2 min, respectively. The analytical method was validated in terms of specificity, linearity, intra- and inter-day precision, accuracy and analyte recovery. The calibration curves for both compounds were linear between 8-600 ng/mL, with correlation coefficients (r) 0.9951 ± 0.0016 (mean ± standard deviation (S.D.), n = 5) for atomoxetine and 0.9982 ± 0.0018 for its glucuronidated metabolite, respectively. For atomoxetine, intra- and inter-day precision was less than 8.2%, the accuracy (bias) less than -11.5% and the recovery ranged between 89-103%, respectively. As for 4-hydroxyatomoxetine-*O*-glucuronide, intra- and inter-day precision was less than 10.7%, the accuracy less than 9.3% and the recovery ranged between 91-105%, respectively.

Pharmacokinetic analysis

The pharmacokinetic parameters of atomoxetine and 4-hydroxyatomoxetine (glucuronidated form) were estimated using noncompartmental methods. The analysis was performed using Phoenix WinNonlin software, version 6.3 (Pharsight Co., Mountain View, Calif., USA). The maximum plasma concentration (C_{max}, ng/mL) and the time to reach C_{max} (t_{max}, h) were obtained directly by visual inspecting the plasma concentration-time profiles. The area under the plasma concentration–time curve from time zero to the last measurable concentration (AUC_{0-t}) was calculated by using the linear trapezoidal rule. Furthermore, the area was extrapolated to infinity (AUC) by dividing last measurable concentration by the elimination rate constant (C_t/ k_{el}) and adding this value to AUC_{0-t}. kel was determined by log-linear regression analysis of the terminal portion of the plasma concentration-time curve and the half-life ($t_{½}$) was calculated using the following formula $t_{½} = 0.693/k_{el}$.

After defining the CYP2D6 metabolizer status, the analysis of variance (ANOVA) was employed to compare the pharmacokinetic profile of atomoxetine and its main metabolite, corresponding to each phenotypic group (EMs versus PMs), with the purpose of identifying any potential differences. t_{max} was an exception in this case, as the values corresponding to this parameter were compared by using a

non-parametric test (Friedman). A *p value <0.05* was considered to be statistically significant.

Statistical analysis

The AUC metabolic ratio (MR_ AUC = AUC atomoxetine/AUC 4-hydroxyatomoxetine-*O*-glucuronide) was used as a tool in order to identify the CYP2D6 metabolic phenotype. After calculating the MR_ AUC for each subject, the lower values were associated with the EM phenotype, while the higher values were attributed to the PM phenotype.

Lilliefors (Kolgomorov-Smirnov) and Anderson-Darling tests were applied to evaluate whether the MR_ AUC data are normally distributed or not. A significance level of 0.05 and a two tailed *p* value was set for all the tests. Moreover, quantile-quantile plot (Q-Q plot) technique was used to provide additional insight regarding intersubject variability. The statistical analysis was performed in R software environment for statistical computing and graphics version 3.2.3.

Results

Assessment of CYP2D6 metabolizer status

The results of the phenotypic analysis are summarized in a series of tables and graphics presented below.

Table I includes the AUC values of atomoxetine and its active metabolite (glucuronidated form, 4-hydroxyatomoxetine-*O*-glucuronide), as well as the MR_ AUC corresponding to each volunteer. Subject no. 5, 15 and 38 presented considerably higher AUC values for the parent drug and much lower values for the glucuronidated metabolite when compared to the remaining 40 individuals. Consequently, the MR_AUC was significantly increased for the aforementioned volunteers, which hinted to the existence of two different phenotypic groups in the study population.

The Q-Q plot (*Figure 1*) offers a visual perspective of the differences that existed between the calculated AUC, emphasizing that the data set was not normally distributed.

Furthermore, the results of the statistical evaluation are included in *Table II* and confirm the intersubject variability, as p value was < 0.05 for both tests.

The impact of phenotype variability on atomoxetine pharmacokinetics

The mean plasma drug concentration-time profiles of atomoxetine and 4-hydroxyatomoxetine-*O*-glucuronide, for each phenotype (EMs and PMs), are illustrated in *Figure 2* (parent drug (A) and active metabolite – glucuronidated form (B)). The plasmatic concentrations of both analytes suffered notable changes in the PM group, compared to EMs. More precisely, atomoxetine plasma levels increased, while the plasma concentrations of 4-hydroxyatomoxetine-*O*-glucuronide were reduced as a consequence of the slow metabolic process. Additionally, the slope of the terminal linear segment from the semi-logarithmic plot was clearly different for the two phenotypic groups, which suggested that in PMs the elimination process of both analytes was altered.

Further on, the mean pharmacokinetic parameters related to each metabolizer status, as well as the statistical interpretation of the results are presented in *Table III*. If considering the parent drug, atomoxetine, most of the calculated parameters, namely C_{max}, AUC_{0-t}, AUC, k_{el} and $t_{½}$ presented marked differences between the two phenotypic groups. As for the glucuronidated form of the active metabolite, statistically significant differences were reported for all pharmacokinetic parameters.

Discussion

The impact of CYP2D6 polymorphism represents a great interest for clinical practice as this isoenzyme is involved in the metabolism of approximately 25% of the drugs available on the market [11]. This genetic feature is responsible for interindividual variability in CYP2D6 enzyme activity which subsequently serves as a criteria for the assignment of individuals in phenotypic groups as follows: ultrarapid metabolizers (UMs – functional copy number duplications), extensive metabolizers (EMs - individuals with two 'wild-type' alleles), intermediate metabolizers (IMs - individuals with one reduced and one loss of function allele) and poor metabolizers (PMs - those with two loss of function alleles) [12–14]. In the Caucasian population, these phenotypes account for approximately 3-5 % (UMs), 70-80 % (EMs), 10-17 % (IMs) and 5-10 % (PMs) [12]. While the majority of people possess a normal CYP2D6 activity and are designated as CYP2D6 EMs [3], a study that aimed to analyze the CYP2D6 diversity in the world found that Europe has the highest prevalence of PM phenotypes [11]. Drugs thought to be primarily affected by the CYP2D6 polymorphism are those for which this isoenzyme represents the major metabolic pathway and include antidepressants (tricyclic antidepressants, selective serotonin reuptake inhibitors), antipsychotics, opioids, antiemetics, antiarrhythmics, beta-blockers, tamoxifen and atomoxetine [12].

The metabolic status can be evaluated either by genotyping, a technique by which the metabolic capacity is predicted after analyzing the functional status of each allele or by phenotyping, a procedure which allows for the phenotype to be established after measuring the metabolic ratio, respectively the substrate test drug/metabolite's concentrations in urine or plasma [15]. The downside of genetic testing is that not all mutations and different variants of alleles are known, which hinders in a certain degree its capacity to predict *in vivo* phenotype. Therefore, there are situations in which the genotype will not accurately predict the phenotype. In some cases, predicted EMs (phenotypes predicted from genotypes) can be phenotypically considered PMs as a result of drug interaction [15,16]. A phenomenon named "phenocopying" can be responsible for such a situation. This process describes the conversion of an EM to a PM as a result of inhibition of the enzyme by another drug or by itself [17]. For example, it was concluded that inhibition

Table I. The individual values of AUC and MR_ AUC corresponding to atomoxetine (ATX) and its glucuronidated metabolite, 4-hydroxyatomoxetine-O-glucuronide (HATX-gluc) (n = 43)

Subject no.	AUC _ATX (hr*ng/ml)	AUC _HATX-gluc (hr*ng/ml)	MR_ AUC (ATX/HATX-gluc)
1	2015.26	5887.55	0.34
2	4132.22	5437.91	0.76
3	1519.93	7321.71	0.21
4	566.48	6318.92	0.09
5	7673.04	2591.11	2.96
6	1000.01	5312.52	0.19
7	3098.07	7994.51	0.39
8	1059.93	7990.74	0.13
9	718.89	4745.92	0.15
10	1161.89	7476.28	0.16
11	955.27	4628.99	0.21
12	2558.22	5478.19	0.47
13	1078.82	5103.49	0.21
14	1615.89	6321.87	0.26
15	7688.97	1167.81	6.58
16	890.76	4156.67	0.21
17	745.38	4413.7	0.17
18	2045.92	4970.07	0.41
19	3050.25	4418.4	0.69
20	281.61	5607.42	0.05
21	1799.88	5268.02	0.34
22	3317.53	5167.65	0.64
23	982.13	5770.4	0.17
24	362.52	4120.32	0.09
25	953.85	6187.12	0.15
26	2118.73	4533.94	0.47
27	861.76	4643.69	0.19
28	1698.9	6342.91	0.27
29	719.85	4596.24	0.16
30	2020.11	5004.72	0.40
31	1715.43	3689.51	0.46
32	2051.61	3285.21	0.62
33	374.17	5670.81	0.07
34	398.28	4408.64	0.09
35	458.87	5623.28	0.08
36	1206.36	6373.5	0.19
37	936.4	4842.81	0.19
38	8473.9	3215.38	2.64
39	1748.95	5093.13	0.34
40	1263.36	4672.21	0.27
41	485.17	5233.89	0.09
42	822.01	3748.75	0.22
43	745.5	4151.36	0.18

AUC_ATX - area under the plasma concentration–time curve from time 0 to infinity for atomoxetine; AUC_HATX-gluc - area under the plasma concentration–time curve from time 0 to infinity for 4-hydroxyatomoxetine-O-glucuronide; MR_ AUC- metabolic ratio calculated as AUC_ATX / AUC_HATX-gluc

of CYP2D6 by paroxetine markedly affected atomoxetine disposition, resulting in a pharmacokinetic profile of this drug similar to PMs of CYP2D6 substrates [18]. According to Shah RR *et al.*, the genotype-phenotype mismatch is viewed as an obstacle in achieving personalized medicine and one approach to avoid this situation is to combine genotype studies with routine phenotyping of subjects [19].

Currently, phenotyping is usually preferred for routine in developing countries as genotyping is a more expensive procedure and is not available in most hospitals [20]. Defining the phenotype status of a population is especially important in drug-drug interaction (DDI) studies considering that DDIs involving enzymatic inhibition can occur in EMs, but not in PMs as they do not have CYP2D6 enzymes to compete for [21]. According to Frank *et al.*, debrisoquine, sparteine, metoprolol or dextrometorphan are acknowledged as well-established probe drugs. If the clearance of a drug depends exclusively on CYP2D6, this can be viewed as appropriate to evaluate an individual's enzymatic activity [22]. In the present study, atomoxetine was used as a probe drug considering that it is primarily metabolized by CYP2D6 [6]. In 1985, a study conducted by Farid *et al.* investigated the pharmacokinetic profile of atomoxetine and hinted to the potential influence of CYP2D6 polymorphism on the metabolism of this agent as a bimodal data distribution was reported for its clearance in healthy volunteers [23].

As depicted in *Table I*, a comparison of the MR_ AUC values corresponding to each volunteer showed that subjects 5, 15 and 38 presented higher values in comparison to the rest of the group, which suggested a decreased metabolism of atomoxetine in these particular cases. For this reason, the study population was considered to comprise 2 phenotypes: PMs (3 subjects) and EMs (40 subjects). This hypothesis was also sustained by statistics. The 3 extreme values and the right skewed asymmetry of the Q-Q plot (*Figure 1*) emphasized the presence of a heterogeneous group. In addition, the results of the two statistical tests described in *Table II* ($p<0.05$) showed that the analyzed data (MR_ AUC values) did not follow a normal distribution. Hence, both the MR_ AUC data set and the statistical analysis provided enough evidence to support the existence of two groups within the study population, each corresponding to a different phenotype. More precisely, the PM group included 3 individuals (subject 5, 15 and 38), while the remaining 40 subjects were characterized as EMs. Female subjects using oral contraceptives can be considered a potential interfering factor for this analysis due to the fact that hormones such as progesterone, testosterone, pregnanolone, pregnenolone, 17β-estradiol, and 17β- hydroxyprogesterone competitively inhibit CYP2D6 activity, whereas epiallopregnanolone and alfaxalone noncompetitively inhibit the same isoenzyme [24]. In addition, pharmacological studies revealed that estrogen-induced cholestasis can repress CYP2D6 expression and activity [25]. However, although the study protocol did permit the use of oral contraceptives during the clinical trial, an enquiry revealed that 50 % of the female subjects were using bar-

Table II. Statistical tests for normality considering the MR_ AUC data set

	Anderson-Darling test		Lilliefors (Kolmogorov-Smirnov) test	
MR_AUC (ATX/HATX-gluc)	A = 9.5941*	p<0.001**	D = 0.36037*	p<0.001**

AUC - area under the plasma concentration–time curve from time zero to infinity;
MR_ AUC– metabolic ratio (MR_ AUC = AUC atomoxetine (ATX)/AUC 4-hydroxyatomoxetine-O-glucuronide (HATX-gluc)), *A - test result ; D - test result; **p<0.05 – statistically significant

rier contraceptive methods, while the rest did not report the use of any birth control method. Therefore, there was no need of exclusion of any subject and data related to all subjects was deemed relevant for the present investigation.

Once the metabolizer status of each subject was known, the present research wanted to reveal potential differences in plasmatic profiles and pharmacokinetic parameters between the two phenotypic groups (PMs versus EMs). For this reason, *Figure 2* depicts the plasma concentration-time profiles of atomoxetine (A) and 4-hydroxyatomoxetine-*O*-glucuronide (B) for each CYP2D6 metabolic status. This graphical representation showed that the parent drug presented higher plasma concentrations for PMs than for EMs. On the other hand, the plasmatic profile of 4-hydroxyatomoxetine-*O*-glucuronide displayed lower mean plasma levels in the PM group in comparison with the ones attributed to the EM group. These results demonstrated that, due to the reduced enzymatic activity of CYP2D6, the biotransformation of atomoxetine to its active metabolite (4-hydroxyatomoxetine) was clearly impaired.

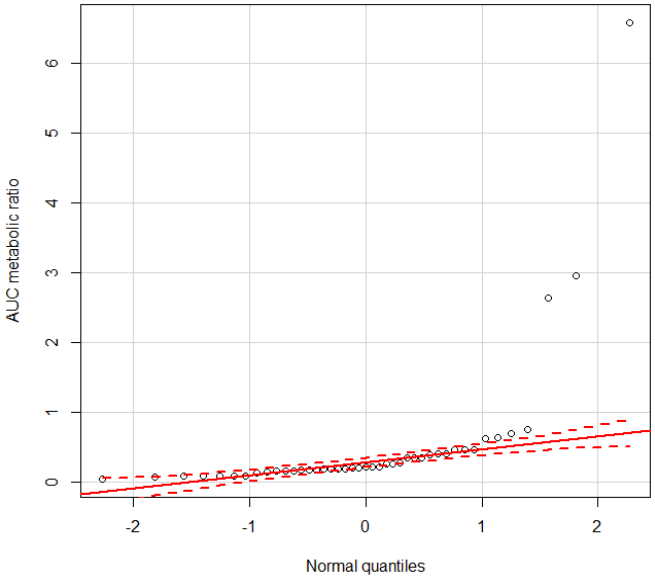

Figure 1. AUC metabolic ratio (MR_AUC) presented as a quantile-quantile plot

Table III. The pharmacokinetic (Pk) parameters of atomoxetine (ATX) and 4-hydroxyatomoxetine-O-glucuronide (HATX-gluc) coresponding to each phenotype (PMs and EMs) and the statistical analysis used to detect potential differences between the groups

Analyte	Pk parameters (mean±SD)	PMs (n=3)	EMs (n=40)	*p value (ANOVAa)
ATX	Cmax (ng/mL)	344.47±36.22	223.58±94.24	0.043,S
	tmax (h)	2.83±2.75	1.46±1.21	Friedman,NS
	AUC0-t (ng*h/mL)	5736.14±1076.97	1291.07±812.28	0.000,S
	AUC (ng*h/mL)	6235.64±641.4	1373±860.48	0.000,S
	kel (1/h)	0.04±0.01	0.22±0.09	0.000,S
	t½ (h)	16.08±2.28	3.95±2.11	0.000,S
HATX-gluc	Cmax (ng/mL)	49.6±15.64	697.39±266.53	0.000,S
	tmax (h)	9.33±1.15	2.36±1.09	Friedman,S
	AUC0-t (ng*h/mL)	1639.43±561.82	5177.35±1101.06	0.000,S
	AUC (ng*h/mL)	1639.43±561.82	5264.73±1113.54	0.000,S
	kel (1/h)	0.04±0.01	0.13±0.03	0.000,S
	t½ (h)	21.46±8.71	5.69±1.52	0.000,S

SD - standard deviation; PMs – poor metabolizers; EMs – extensive metabolizers
*p<0.05 – statistically significant (S); NS - non-significant
aANOVA except where stated otherwise

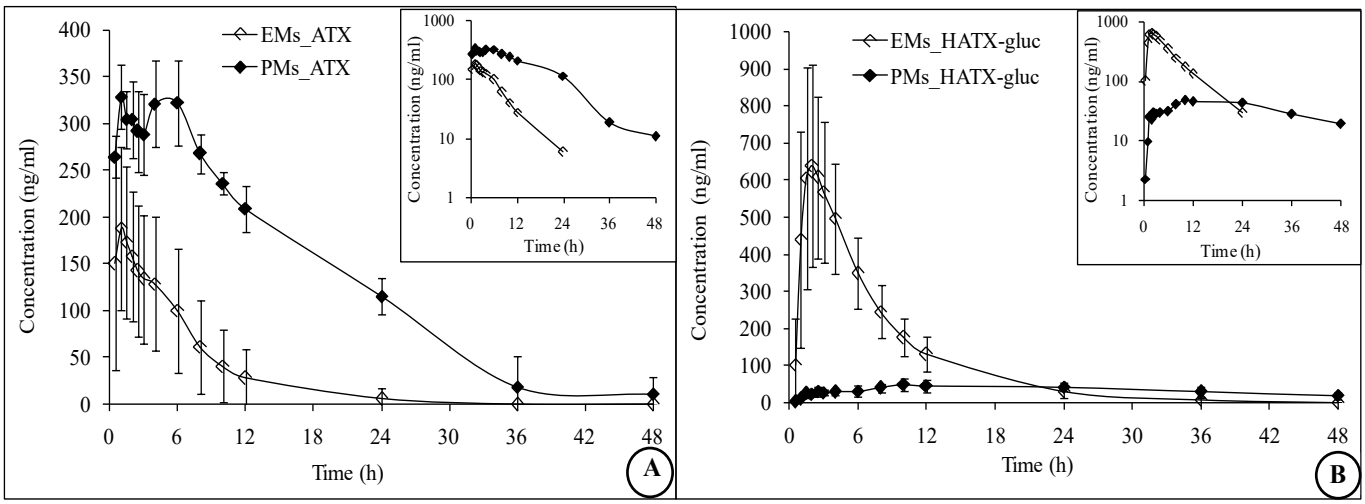

Figure 2. Mean ± SD plasma concentration–time curves of atomoxetine (ATX - (A) and of 4-hydroxyatomoxetine-O-glucuronide (HATX-gluc - (B)) corresponding to each phenotypic group, respectively extensive metabolizers (EMs) and poor metabolizers (PMs). Insert: semilogarithmic presentation

When the pharmacokinetic parameters of atomoxetine were calculated, a marked variability between the 2 phenotypic groups was observed. With regard to the pharmacokinetic data displayed in *Table III*, the mean C_{max} and AUC values were 1.5-fold and 4.5-fold higher, respectively the $t_{½}$ of the parent drug was about 4-fold longer, in subjects characterized as CYP2D6 PMs than in EMs. There were statistically significant differences for all pharmacokinetic parameters, except t_{max}, between the two phenotypic groups. Thus, all data suggest that exposure to atomoxetine is greater in PMs. The role of CYP2D6 in the metabolic fate of atomoxetine was previously investigated in a study conducted by Sauer *et al*. in healthy men. According to that research, after single dose intake (20 mg), the AUC of atomoxetine was 4-fold higher, while the $t_{½}$ was approximately 3.5-fold longer in PMs than in EMs [8], results which are similar with the ones reported in the present study. In addition, the previous investigation concluded that after repeated dosing, a 6-fold higher C_{max} at steady-state and 8-fold higher AUC values were determined for the PM phenotype [8].

The pharmacokinetic parameters of 4-hydroxyatomoxetine-*O*-glucuronide (*Table III*) corresponding to each metabolizer status came to confirm the interphenotype variability. As expected, a decrease in the rate of formation of 4-hydroxyatomoxetine was observed in the PM group. More precisely, for this phenotype, the values of C_{max} and AUC were 14-fold and 3.2-fold lower than the ones determined for EMs. Moreover, the following analysis concluded that the differences between the two groups were statistically different for all the pharmacokinetic parameters of the glucuronidated metabolite. With the exception of $C_{max,}$ these results can be viewed as similar to the ones found by Sauer *et al*. In that particular study, an approximately 4.7-fold decrease in C_{max} was calculated for the PM group after the administration of 20-mg repeated doses of atomoxetine [8]. The exposure to 4-hydroxyatomoxetine-*O*-glucuronide was about 3-fold lower in PMs due to the fact that the clearance of the parent drug was altered [8], a situation comparable with the one seen in the present investigation. Additionally, similarities also reside in the variation of the $t_{½}$ between the two groups. The mean $t_{½}$ of the active metabolite (glucuronidated form) was approximately 6 h in EMs and 21 h in PMs in the present study. Meanwhile, in the past, the same pharmacokinetic parameter was approximately 7 h in EMs and 19 h in PMs [8].

It is already acknowledged that there are pronounced differences not only in the prevalence of PMs, but also in the relative enzyme activity in different ethnic groups. Taking into account this aspect, the present research provided additional insight into the impact of CYP2D6 phenotype on atomoxetine bioavailability and metabolism. Furthermore, according to Teh *et al.*, the comparison of pharmacokinetic parameters between PMs and EMs may suggest the extent of interaction of a CYP2D6 substrate and strong inhibitors of the same isoenzyme and also indicate when clinical pharmacokinetic studies to investigate this aspect should be considered useful or not [21].

Although the present study did not intend to assess clinical aspects, information about the consequences of CYP2D6 polymorphism and on whether the efficacy and safety of atomoxetine are influenced by a certain phenotype, are available in the scientific literature. There is compelling evidence that while CYP2D6 PMs may have a better response to atomoxetine, they may also experience a higher frequency of adverse events as compared to CYP2D6 EMs [14]. Fijal *et al*. analyzed the differences between CYP2D6 PMs and non-PMs (IMs, EMs and UMs combined) in regard to safety and tolerability in a 12-week open-label study which included adult patients with ADHD. This research concluded that PMs had a higher prevalence of side effects such as decreased appetite, dry mouth, hyperhidrosis, insomnia, urinary retention and erectile dysfunction in men. In addition, significantly higher increases in cardiovascular parameters (blood pressure and heart rate) and a greater reduction in BMI were reported for the PM status [26]. Also, Michelson *et al*. evaluated if differences in CYP2D6 genotype/phenotype influence the clinical response to atomoxetine in children and adolescents with ADHD. In terms of efficacy, symptom reduction was greater in PMs versus EMs and fewer patients with a PM status suspended atomoxetine therapy due to lack of efficacy. When safety and tolerability aspects were considered, it was reported that greater increases in heart rate and diastolic blood pressure and smaller increases in weight were attributed to the PM group. Moreover, side effects like decreased appetite and tremor were reported more frequently for PMs [27].

Study limitations

The absence of any reference to the use of herbal medicines and supplements for the exclusion criteria should be acknowledged as a methodological deficiency and subsequently, as a study limit. This aspect needs to be acknowledged in this case as several herbs proved to influence the activity of CYP2D6, including goldenseal and dong quai [28,29]. Still, this interfering factor was later reviewed and no herbal remedy with potential to influence CYP2D6 activity was reportedly used by the study subjects.

Furthermore, lack of genotyping can be regarded as a study limitation, as this procedure could be useful in order to verify and validate the phenotypic data obtained in the present study. Another limit refers to the fact that no clinical monitoring was performed throughout the research. Nonetheless, essential information about the potential clinical consequences associated with atomoxetine intake by PMs was collected from the scientific literature and mentioned in the text.

Conclusion

Based on the MR_ AUC values and statistical tests, it was demonstrated that the study population comprised two phenotypic groups (EMs and PMs). Atomoxetine bioavail-

ability and metabolism were subjected to interphenotypic variation as PMs presented a 4.5-fold higher exposure to the parent drug and a 3.2-fold lower exposure to its metabolite in comparison to EMs.

Acknowledgements and funding

This work was supported by the National Research Council (CNCS) Romania – project PN-II-ID-PCE-2011-3-0731. All authors are full-time employees of the University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania.

Conflicts of interest

The authors declare no conflict of interest.

References

1. Corman SL, Fedutes BA, Culley CM. Atomoxetine: the first nonstimulant for the management of attention-deficit/hyperactivity disorder. Am J Health Syst Pharm. 2004;61(22):2391–99.
2. B Barton J. Atomoxetine: a new pharmacotherapeutic approach in the management of attention deficit/hyperactivity disorder. Arch Dis Child. 2005;90 Suppl 1:i26-9.
3. Sauer J-M, Ring BJ, Witcher JW. Clinical pharmacokinetics of atomoxetine. Clin Pharmacokinet. 2005;44(6):571–90.
4. Findling RL. Evolution of the treatment of attention-deficit/hyperactivity disorder in children: A review. Clin Ther. 2008;30(5):942–57.
5. Yu G, Li G-F, Markowitz JS. Atomoxetine: A review of its pharmacokinetics and pharmacogenomics relative to drug disposition. J Child Adolesc Psychopharmacol. 2016;26(x):1–13.
6. Ring BJ, Gillespie JS, Eckstein JA, Wrighton SA. Identification of the human cytochromes P450 responsible for atomoxetine metabolism. Drug Metab Dispos. 2002;30(3):319–23.
7. Simpson D, Plosker GL. Spotlight on atomoxetine in adults with attention-deficit hyperactivity disorder 1. 2004;18(6):397–401.
8. Sauer JM, Ponsler GD, Mattiuz EL, et al. Disposition and metabolic fate of atomoxetine hydrochloride: The role of CYP2D6 in human disposition and metabolism. Drug Metab Dispos. 2003;31(1):98–107.
9. Matsui A, Azuma J, Witcher JW, et al. Pharmacokinetics, safety and tolerability of atomoxetine and effect of CYP2D6*10/*10 genotype in healthy Japanese men. J Clin Pharmacol. 2012;52(3):388–403.
10. Cui YM, Teng CH, Pan AX, et al. Atomoxetine pharmacokinetics in healthy Chinese subjects and effect of the CYP2D6*10 allele. Br J Clin Pharmacol. Wiley-Blackwell; 2007;64(4):445–49.
11. Sistonen J, Sajantila A, Lao O, Corander J, Barbujani G, Fuselli S. CYP2D6 worldwide genetic variation shows high frequency of altered activity variants and no continental structure. Pharmacogenet Genomics. 2007;17(2):93–101.
12. Zhou S-F. Polymorphism of Human Cytochrome P450 2D6 and Its Clinical Significance. Clin Pharmacokinet. 2009;48(11):689–723.
13. Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. Pharmacogenomics J. 2005;5:6–13.
14. Brown JT, Bishop JR. Atomoxetine pharmacogenetics: associations with pharmacokinetics, treatment response and tolerability. Pharmacogenomics. 2015;16(13):1513–20.
15. LLerena A, Naranjo MEG, Rodrigues-Soares F, Penas-LLedo EM, Farinas H, Tarazona-Santos E. Interethnic variability of CYP2D6 alleles and of predicted and measured metabolic phenotypes across world populations. Expert Opin Drug Metab Toxicol. 2014;10(11):1569–83.
16. Zanger UM, Raimundo S, Eichelbaum M. Cytochrome P450 2D6: Overview and update on pharmacology, genetics, biochemistry. Naunyn Schmiedebergs Arch Pharmacol. 2004;369(1):23–37.
17. Gardiner SJ, Begg EJ. Pharmacogenetics, drug-metabolizing enzymes, and clinical practice. Pharmacol Rev. 2006;58(3):521–90.
18. Belle DJ, Ernest CS, Sauer J-M, Smith BP, Thomasson HR, Witcher JW. Effect of potent CYP2D6 inhibition by paroxetine on atomoxetine pharmacokinetics. J Clin Pharmacol. 2002;42(11):1219–27.
19. Shah RR, Smith RL. Addressing phenoconversion: The Achilles' heel of personalized medicine. Br J Clin Pharmacol. 2015;79(2):222–40.
20. Abraham BK, Adithan C. Genetic Polymorphism of Cyp2D6. Indian J Pharmacol. 2001;33(2):147–69.
21. Teh LK, Bertilsson L. Pharmacogenomics of CYP2D6: molecular genetics, interethnic differences and clinical importance. Drug Metab Pharmacokinet. 2012;27(1):55–67.
22. Frank D, Jaehde U, Fuhr U. Evaluation of probe drugs and pharmacokinetic metrics for CYP2D6 phenotyping. Eur J Clin Pharmacol. 2007;63(4):321–33.
23. Farid NA, Bergstrom RF, Ziege EA, Parli CJ, Lemberger L. Single-dose and steady-state pharmacokinetics of tomoxetine in normal subjects. J Clin Pharmacol. 1985;25(4):296–301.
24. Wang B, Yang L-P, Zhang X-Z, Huang S-Q, Bartlam M, Zhou S-F. New insights into the structural characteristics and functional relevance of the human cytochrome P450 2D6 enzyme. Drug Metab Rev. 2009;41(4):573–643.
25. Pan X, Jeong H. Estrogen-induced cholestasis leads to repressed CYP2D6 expression in CYP2D6-humanized mice. Mol Pharmacol. 2015;88(1):106–12.
26. F Fijal BA, Guo Y, Li SG, et al. CYP2D6 predicted metabolizer status and safety in adult patients with attention-deficit hyperactivity disorder participating in a large placebo-controlled atomoxetine maintenance of response clinical trial. J Clin Pharmacol. 2015;55(10):1167–74.
27. Michelson D, Read H, Ruff DD, Witcher J, Zhang S, McCracken J. CYP2D6 and clinical response to atomoxetine in children and adolescents with ADHD. J Am Acad Child Adolesc Psychiatry. 2007;46(2):242–51.
28. Wanwimolruk S, Prachayasittikul V, Phopin K, Prachayasittikul V. Cytochrome P450 enzyme mediated herbal drug interactions (Part 1). Excli J. 2014;13:347–91.
29. Wanwimolruk S, Phopin K, Prachayasittikul V. Cytochrome P450 enzyme mediated herbal drug interactions (Part 2). Excli J. 2014;13:869–96.