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Title

Impact of age and *CYP2D6* genotype on exposure of zuclopenthixol in patients using long-acting injectable versus oral formulation - an observational study including 2,044 patients

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Abstract

Purpose

Zuclopenthixol is an antipsychotic available as oral and long-acting injectable (LAI) formulations. The aim of this study was to investigate the effect of age on zuclopenthixol exposure during oral and LAI administrations without and with adjustment for *CYP2D6* genotype.

Methods

Data on serum concentrations of zuclopenthixol and *CYP2D6* genotype (available for 28.2% of the population) from patients using oral or LAI zuclopenthixol were included retrospectively from a therapeutic drug monitoring service during the period 2005-2019. As a measure of exposure, dose-adjusted serum concentration (C/D ratio) was used. Based on age, patients were grouped to older (≥ 65 years) or younger (18-64 years). Linear mixed model analyses without and with adjustment for *CYP2D6* genotype were used.

Results

Serum concentrations of zuclopenthixol from 1,145 (14.1% older) and 899 patients (24.6% older) in the LAI and oral group were included, respectively. Compared to younger patients, older patients had higher C/D ratio of zuclopenthixol for LAI (+25-33%, $p < 0.001$) and oral formulation (+25-29%, $p \leq 0.003$) without and with adjustment for *CYP2D6* genotype. The doses were lower in older versus younger patients (Oral: -30%; LAI: -20%; $p < 0.001$). Compared to the younger LAI users without reduced CYP2D6 function, a higher C/D ratio was observed in the older LAI users with reduced CYP2D6 function (+104%, $p < 0.001$).

Conclusion

The present study showed that zuclopenthixol exposure increases in older patients, and that the older LAI users with reduced CYP2D6 function are exposed to high serum concentrations. Also, the present study showed that similar dose reductions are required for oral and LAI users.

Keywords

Zuclopenthixol, long-acting injectable, age, *CYP2D6* genotype

Introduction

Schizophrenia is a severe mental disorder affecting about 1% of the world's population [1]. Although persons with schizophrenia have reduced life expectancy [2, 3], an increasing number of persons are ageing with schizophrenia [4], and in a few years' time every fourth patient with schizophrenia will be 55 years or older [5].

Zuclopenthixol is a first-generation antipsychotic drug, which was licensed in 1982. Currently it is approved in 73 countries, including many European countries, Australia, New Zealand and Australia. The drug is available as oral formulation and two different solutions for injection (acetate and decanoate), with decanoate being the long-acting formulation (LAI).

Although the newer antipsychotics more often are utilized when starting antipsychotic treatment, zuclopenthixol is still a viable treatment option for the patients using first generation antipsychotics [6]. Although zuclopenthixol has been on the market for almost 40 years, the knowledge about which factors that should lead to dose adjustments is still limited. This is in particular true for the LAI formulation of zuclopenthixol and for the effects of ageing. In general, the use of LAI antipsychotics has been shown to reduce the risk of relapse and rehospitalisation compared with oral formulations [7, 8], and increased knowledge of factors contributing to tailored dosing for older individuals is important in a vulnerable and increasing patient group. To our knowledge, no previous studies have investigated the pharmacokinetics of zuclopenthixol in older LAI users. Since zuclopenthixol is metabolised primarily by cytochrome P450 (CYP) 2D6 and CYP3A4 [9], CYP2D6 polymorphisms could lead to high interindividual variability in drug exposure [10], and this factor should be corrected for when studying the effect of non-genetic variables on zuclopenthixol exposure. Also, a combination of more than one risk factor for increased drug exposure, like for instance high age and genetic polymorphisms, could theoretically lead to a substantial increase in plasma concentrations.

The aim of present study was to investigate the effect of age on serum concentrations of zuclopenthixol in users of oral compared to LAI formulations, after adjustment for gender and CYP2D6 genotype.

Methods

Subjects

The study was based on routine therapeutic drug monitoring (TDM) data collected at the Center for Psychopharmacology, Diakonhjemmet Hospital (Oslo, Norway) during 2005-2019. Sample information was extracted from the standard TDM requisition form, including concomitant medication, time interval between last dose and sampling, and daily dosage. Inclusion criteria were (i) serum samples of zuclopenthixol drawn 10-30 hours post last oral dose intake or 6-42 days post last LAI injection (representing 1-6 weeks dosing intervals), (ii) information regarding daily dosing, (iii) and zuclopenthixol concentrations above the lower limit of quantification (LLOQ) and below the upper limit of quantification (ULOQ). Exclusion criteria were (i) patient age <18 years, (ii) concomitantly use of both oral and LAI zuclopenthixol, (iii) use of enzyme inducers (carbamazepine, phenytoin and phenobarbital), (iv) or use of the enzyme inhibitors paroxetine, fluoxetine and bupropion, (v) intoxication or suspected compliance issues noted on the requisition form. In patients who had recorded TDM samples of both LAI and oral formulation at different time points during the study period, the oral formulation samples were excluded. The primary endpoint was the dose-adjusted serum concentrations (C/D ratio), in patient using long-acting injectable vs. oral formulation.

The study was approved by the Regional Committee for Medical and Health Research Ethics (2017/9121).

Serum concentration analyses of zuclopenthixol

During time course of the retrospective data collection, two slightly different, cross-validated routine LC-MS/MS methods were applied for zuclopenthixol analyses. For the routine LC-MS/MS methods, samples were prepared with protein precipitation using cold acetonitrile containing internal standard. Chromatographic separation was performed by an Acquity UPLC BEG shield RP18 column (1.7 μ m, 1.0 $\times$ 100 mm; Waters) using gradient elution at 40 °C with a mix of ammonium acetate buffer (pH=4.8) and acetonitrile (18–45 %) as mobile phase. The retention times were 2.7 and 2.5 min for zuclopenthixol, and promazine, respectively. Detection with multiple reaction monitoring was performed at the following transitions: m/z 401 $\rightarrow$ 271 for zuclopenthixol and m/z 285 $\rightarrow$ 212 for promazine. Reference standards were obtained from Sigma (St. Louis, MO, USA). LLOQ and ULOQ were 0.5 and 100 nmol/L, respectively.

Statistical analysis

The patients were divided into groups according to administration of zuclopenthixol as oral or LAI formulation as well as two age groups (18-64 years (young) vs. $\geq$ 65years (old)). If the patient was recorded in both age groups

during the time span of the study, only the analysis performed while the patient was ≥ 65 years were applied to avoid allocation of patients into multiple age groups. As a measure of exposure, absolute serum concentrations and dose-adjusted serum concentrations (C/D ratio) of zuclopenthixol were defined as outcome variables and analysed separately. For LAI users, the daily dose was calculated as the injected dose divided by the dosing interval. Linear mixed model analyses (using random intercept and the restricted maximum likelihood model) were used to allow for inclusion of multiple samples per patient. The following variables were tested within every group in the linear mixed model analyses: age group (18-64 years vs ≥ 65 years), gender and time interval between last dose intake and sampling. In a subsequent analysis, *CYP2D6* genotype subgroups were included in the analyses. The *CYP2D6* pharmacogenetic panel included the lack-of-function alleles (*Null*) *CYP2D6**3 (rs35742686), *CYP2D6**4 (rs3892097), *CYP2D6**6 (rs5030655); the reduced-function (*Red*) variants *CYP2D6**9 (rs5030656), *CYP2D6**10 (rs1065852) and *CYP2D6**41 (rs28371725), and copy number analysis to identify *CYP2D6**5 (whole gene deletion) allele and duplication of functional alleles (*CYP2D6*Wtx3). Absence of *Red* or *Null* variants was defined as a functional allele (*Wt*). We divided patients into the following *CYP2D6* genotype-defined categories according to the predicted increasing order of CYP2D6 metabolic capacity: poor metabolisers (PM; *CYP2D6*Null/Null), intermediate metabolisers (IM; *CYP2D6*Null/*Red* and *CYP2D6**Red*/*Red*); normal metabolisers (NM; *CYP2D6*Wt/Null, *CYP2D6*Wt/*Red*, and *CYP2D6*Wt/Wt), and ultrarapid metabolisers (UM: *CYP2D6*Wtx3). The patients without reduced function for CYP2D6 (i.e. UM and EM) and with reduced function (i.e. PM and IM) were merged in the analyses.

The proportions of females, patients ≥ 65 years or CYP2D6 genotype subgroups (UM, EM, IM and PM) were compared between the oral and LAI group using Chi-square tests. Linear mixed models were used to compare differences in age, TDM samples / patient or daily dose between the oral and LAI group, respectively.

All statistical analyses were performed in SPSS®, version 25.0 (IBM® SPSS® Statistics, Armonk, NY, USA). GraphPad version 4 was used for graphical presentations (GraphPad Software, San Diego, CA). Alpha was set as 0.05. The estimated means are given with 95 % confidence intervals.

Results

Table 1 shows the demographics of the study population. One thousand one hundred and forty-five patients (3428 serum samples) were included in the LAI group and 899 patients (2492 serum samples) were included in the oral group. The number of patients above 64 years was 161 in the LAI group and 221 in the oral group. During the timespan of the study period, 108 (LAI group) and 125 (tablet group) TDM samples were excluded from the multivariate analyses. These samples were from patients allocated to the old group (≥ 65 years) but were sampled before the patients' age of 65 years.

In figure 1, the relation between unadjusted and dose-adjusted serum concentrations (C/D ratio) and age is visualised. The regression lines indicate that the relation between C/D ratio and age is relatively similar for both LAI and oral formulation. This effect size is seen in table 2, showing a significant effect of age on C/D ratio for both LAI ($p < 0.001$, 25% increased C/D ratio in patients above 64 years) and oral formulation ($p < 0.001$, 25% increased C/D ratio in patients above 64 years). The C/D ratios were also slightly higher in females compared to males in both the oral ($p = 0.023$, 9% increased C/D ratio in females) and LAI groups ($p < 0.001$, 12% increased C/D ratio in females).

For the unadjusted serum concentrations, figure 1 and table 2 show that lower absolute concentrations are seen for the older patients in the oral group ($p = 0.013$, 15% decreased absolute serum concentration in patients above 64 years), but not in the LAI group ($p = 0.50$). The doses are significantly lower in patients above 64 years, both for LAI and oral users, but this is more pronounced in the oral group (30% lower doses compared to 20% in the LAI group).

For a subgroup of the patients (368 patients/1248 samples in the LAI group and 209 patients/578 samples in the oral group), information about CYP2D6 genotype was available. Table 3 shows the effect of age in the genotyped group. The effect of age on C/D ratio is approximately the same after adjustment for genotype groups for both LAI ($p < 0.001$, 33% increased C/D ratio in patients above 64 years) and oral formulation ($p = 0.003$, 29% increased C/D ratio in patients above 64 years).

In table 3, the effect of genotype group after adjustment for age is also seen, showing a significantly higher C/D ratio in the PM/IM group compared to the UM/EM patients both in the LAI group ($p < 0.001$, 28% increased C/D ratio in the PM/IM patients) and the oral group ($p < 0.001$, 48% increased C/D ratio in the PM/IM patients). Regarding CYP2D6 genotype, the PM/IM group received slightly lower doses both for the LAI and the oral group, but the difference was not statistically significant. The unadjusted concentrations were higher in the PM/IM group, but this was only significant among the LAI users, as seen in table 3.

Compared to younger UM/EM (1.99 nM/mg; 95%CI: 1.88, 2.10) or younger PM/IM (2.51 nM/mg; 2.22, 2.80) patients in the LAI group, respectively, older UM/EM (+30%; 2.58 nM/mg; 2.20, 2.97; $p=0.004$) or older PM/IM patients (+62%; 4.05 nM/mg; 3.56, 4.64; $p<0.001$) had increased C/D ratio of zuclopenthixol. Comparing the younger UM/EM versus older PM/IM showed a twofold increase in C/D-ratio (+104%, $p<0.001$) for the LAI group. For the younger UM/EMs versus the older UM/EMs in the tablet group, approximately the same effect was observed as for the LAI group (+40%, $p=0.002$; 1.47 (95%CI: 1.31, 1.63) vs 2.06 nM/mg (1.73, 2.40)). For the younger (2.36 nM/mg (2.02, 2.71)) versus the older PM/IMs (N/A) in the tablet group, calculations could not be performed since only 1 patient was identified as an older IM/PM patient.

Discussion

This study showed that increasing age affected the C/D ratio for zuclopenthixol relatively similar whether a LAI formulation or an oral formulation of the drug is used. The fact that 25-33% higher concentrations are seen in the oldest patients indicates that dose reductions should be performed in patients treated with this first-generation antipsychotic, regardless of drug formulation. The same effect of age was also found when correcting for *CYP2D6* genotype and the C/D ratios increased by the same order of magnitude in the PM/IM patients compared to the oldest patients. The older LAI patients with reduced *CYP2D6* function (PM/IM) deserve particular attention from clinicians, with twice the exposure compared to the younger LAI patients with no *CYP2D6* reduction (UM/EM).

Our results also show that dose adjustments in the oldest patients is performed, and in the oral group, the doses are reduced substantially, making absolute concentrations lower in the older than in the younger patients. This is in contrast to what is previously shown for risperidone [11] and some antidepressants [12], where higher or comparable absolute concentrations were seen in patients above 64 years. The present results are however in accordance with previous results for olanzapine; doses are reduced in older patients, leading to lower absolute concentrations [13]. Dose reductions for older patients are also previously documented for aripiprazole [14]. The present study showed more moderately reduced doses in the LAI group, making absolute concentrations more similar in the oldest and youngest patients. It could be concluded that clinicians in the present material are aware of the dose reduction requirements in the vulnerable group of old zuclopenthixol users, both when oral and LAI formulation is used. This is probably a result of general guidelines for psychopharmacological drugs in this population [15-17].

Although we found a significant, and also clinically relevant increase in drug exposure in older patients in the present study, the effect size was modest compared to what is previously found for older patients and antidepressants [12]. Increased concentrations in older patients when a similar dose is ingested are expected from theoretical considerations, as numerous changes in distribution and elimination of drugs occur at increasing age [16, 18]. One cannot however conclude from general expectations, and experimental data from each drug is necessary. The previous results are also somewhat conflicting, and one study concluded from a relatively small material that age had no impact on the pharmacokinetics of the antidepressant mianserin [19]. For antipsychotics, estimates comparable to what the present study found for zuclopenthixol are previously seen for oral use of clozapine, olanzapine, risperidone and quetiapine in 70 year old patients, while more pronounced effects were seen in 80 and 90 year old patients [20]. Another earlier study actually concluded that no effect of age was seen on C/D ratios for olanzapine or clozapine, but only patients above 50 years were studied specifically [21]. For

drugs like risperidone [11] and aripiprazole [14], the situation is shown to be complicated, with no age effect on the mother substance, but increased metabolite concentrations with age. The present study adds knowledge to previous results, placing zuclopenthixol among drugs where clinically relevant pharmacokinetic changes are seen in older patients, but of modest degree.

The aim of the present study was to compare the age effects in oral and LAI users. In particular, the two formulations differ in the way that presystemic effects are mostly eliminated during LAI use. In a previous study of the antipsychotic drug olanzapine, a significant age effect was found on the C/D ratio for oral but not for the LAI formulation [13]. This could indicate that an age effect on first pass metabolism, but not distribution and elimination, is present for olanzapine. The reason for this not being found for zuclopenthixol is not obvious, but may relate to the different LAI formulations with olanzapine being a crystalline salt and zuclopenthixol an oily decanoate and differences in gut expressions of the CYP1A2 and CYP2D6. The results are not corrected for bodyweight, which would impact the distribution. The age effects in zuclopenthixol users are to the best of our knowledge not previously compared between oral and LAI use, but a previous study investigated effects of CYP inhibitors and found similar results from oral and LAI users [9]. This indicates that the effect of age on zuclopenthixol exposure is similar after administration of the two formulations.

A strength of the present study was the relatively large material, not only of oral users, but also of LAI users of zuclopenthixol, a group of patients where pharmacokinetics is less previously studied. We also had the possibility to correct results for *CYP2D6* genotype in a subgroup of the population. One obvious weakness of the present study was the lack of clinical information about each patient, for instance, knowledge about renal and hepatic status as well as somatic comorbidities that would be of value. In addition, TDM measurements only represent systemic exposure in the circulation at steady state, and therefore do not capture potential differences in distribution.

In conclusion, the present study shows that the antipsychotic drug zuclopenthixol is subject to pharmacological changes in older patients, and that similar dose reductions are required for oral and LAI use. Clinicians should be particularly cautious when treating older patients with reduced CYP2D6 function (IM/PM).

Declarations**Funding**

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Conflict of interest

Prof. Molden has received speaker's honoraria from Lundbeck and Lilly. The other authors have no conflicts of interest to declare.

Ethics approval

The study was approved by the Regional Committee for Medical and Health Research Ethics (2017/9121) and Health Research Ethics and the Hospital Investigational Review Board.

Consent to participate

The use of historical patient data for the purpose of this study was approved by the Regional Committee for Medical and Health Research Ethics and the Hospital Investigational Review Board without consent from the patients, as the study was based on retrospective data only.

Consent for publication

All authors have approved the submission.

Availability of data and material

The data that supports the findings of this study are available upon reasonable request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

Code availability

Not applicable.

Author's contribution

All authors were involved in the ideation, conceptualizing and design of the study. RLS and MT collected and prepared the data material. RLS analysed the data. RLS, MT and GH interpreted the data. MT, RLS and GH drafted the manuscript. All other authors critically reviewed the manuscript and approved the submitted version.

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Tables

Table 1. Demographics of the study population.

	Long-acting injectable	Oral formulation	P value
No. of patients, n (TDM samples)	1145 (3428)	899 (2492)	
Male/female, n (TDM samples)	637 (1835) / 508 (1593)	450 (1268) / 449 (1224)	0.012
Age, years; mean (SD)	47.3 (15.4; 19, 97)	52.3 (17.5; 18, 97)	<0.001
Prop. of patients >64 years, n (TDM samples)	161 (581)	221 (617)	<0.001
Sampling time, mean (SD)	14.1 days (5.15)	15.0 hours (4.15)	
TDM samples / patient, median (SD)	2.99 (3.45)	2.77 (3.54)	0.159
Daily dose (mg)	11.6 (5.81)	13.1 (9.3)	<0.001
No. of patient with CYP2D6 genotype, n (%)	368 (32.1)	209 (23.1)	<0.001
UM, n (measurements)	14 (50)	2 (6)	
EM, n (measurements)	306 (1033)	179 (465)	
IM, n (measurements)	26 (77)	13 (45)	0.218
PM, n (measurements)	22 (138)	15 (78)	
TDM, therapeutic drug monitoring; Sampling time, time between last dose and blood sampling.			

Table 2. Effects of age and gender on unadjusted- and adjusted serum concentration as well as daily dose of zuclopenthixol in patients using long-acting injectable or oral formulation, adjusting for age, gender, and sampling time.

Variables	No. of patients (samples)	Unadjusted serum conc. (nM)	P	Daily dose (mg)	P	Dose-adjusted serum conc. (nM/mg)	P
Long-acting injectable:							
18-64y	984 (2845)	22.7 (22.0, 23.5)	0.50	12.1 (11.8, 12.4)	<0.001	2.02 (1.96, 2.08)	<0.001
>65y	161 (475)	23.4 (21.5, 25.3)		9.66 (8.89, 10.4)	1	2.52 (2.37, 2.68)	
Female	508 (1517)	23.7 (22.4, 24.9)	0.11	10.6 (10.1, 11.1)	0.099	2.40 (2.30, 2.49)	<0.001
Male	637 (1803)	22.5 (21.3, 23.8)		11.1 (10.6, 11.6)		2.15 (2.05, 2.25)	
Sampling time, days	1145 (3320)	-0.84 (-0.95, -0.74)	<0.001			0.036 (0.028, 0.045)	<0.001
Oral formulation:							
18-64y	678 (1875)	22.0 (20.7, 23.2)	0.013	14.8 (14.2, 15.5)	<0.001	1.63 (1.54, 1.71)	<0.001
>65y	221 (492)	18.6 (16.4, 20.9)		10.3 (9.12, 11.5)	1	2.03 (1.88, 2.18)	
Female	449 (1149)	19.9 (18.3, 21.5)	0.50	11.9 (11.1, 12.7)	0.022	1.91 (1.81, 2.01)	0.023
Male	450 (1218)	20.7 (18.9, 22.5)		13.2 (12.3, 14.2)		1.75 (1.63, 1.86)	
Sampling time, hours	899 (2367)	-0.50 (-0.66, -0.33)	<0.001			-0.0064 (-0.019, 0.0058)	0.30
The values are presented as estimate and 95% confidence intervals in parentheses. Estimated effects of sampling time were calculated for long-acting injectable (14.2 days) and oral formulation (15.4 hrs). P, P value							

Table 3. Effects of age and gender on unadjusted- and adjusted serum concentration as well as daily dose of zuclopenthixol in patients using long-acting injectable or oral formulation, adjusting for age, gender, CYP2D6 genotype subgroups and sampling time.

Variables	No. of patients (samples)	Unadjusted serum P conc. (nM)	Daily dose (mg) P	Dose-adjusted serum conc. (nM/mg) P
Long-acting injectable:				
18-64y	335 (1106)	26.0 (24.2, 27.8) 0.262	12.0 (11.3, 12.8) <0.00	2.33 (2.18, 2.49) <0.001
>65y	33 (142)	23.6 (19.6, 27.6)	8.68 (7.02, 10.3) 1	3.10 (2.77, 3.44)
Female	146 (546)	26.3 (23.8, 28.9) 0.017	10.4 (9.31, 11.4) 0.998	2.85 (2.64, 3.06) 0.010
Male	222 (702)	23.3 (20.7, 26.0)	10.4 (9.27, 11.4)	2.59 (2.37, 2.80)
CYP2D6 genotype:				
UM/EM	320 (1036)	22.8 (20.6, 25.0) 0.023	10.9 (9.97, 11.8) 0.163	2.39 (2.21, 2.57) <0.001
PM/IM	48 (212)	26.8 (23.4, 30.2)	9.85 (8.544, 11.3)	3.05 (2.77, 3.33)
Sampling time, days	368 (1248)	-0.84 (-1.02, -0.65) <0.001		0.048 (0.033, 0.064) <0.001
Oral formulation:				
18-64y	173 (492)	23.3 (19.8, 26.8) 0.473	13.8 (12.0, 15.6) <0.00	1.91 (1.71, 2.10) 0.003
>65y	36 (86)	20.9 (14.1, 27.7)	7.27 (3.80, 10.7) 1	2.46 (2.08, 2.84)
Female	108 (312)	20.4 (15.7, 25.1) 0.165	10.4 (7.93, 12.8) 0.787	2.18 (1.92, 2.44) 0.981
Male	101 (266)	23.8 (18.7, 29.0)	10.7 (8.07, 13.3)	2.18 (1.90, 2.46)
CYP2D6 genotype:				
UM/EM	181 (455)	19.7 (16.4, 23.0) 0.172	12.2 (10.5, 13.8) 0.074	1.76 (1.57, 1.94) <0.001
PM/IM	28 (123)	24.5 (17.4, 31.7)	8.90 (5.25, 12.5)	2.61 (2.22, 2.99)
Sampling time, hours	209 (578)	-0.51 (-0.84, -0.17) 0.003		-0.011 (-0.034, 0.013) 0.377
The values are presented as estimate and 95% confidence intervals in parentheses. Estimated effects of sampling time were calculated for long-acting injectable (*) and oral formulation (**): Zuclopenthixol (*14.2 days, ** 15.0 hrs). The estimated were calculated with linear mixed model adjusting for gender, age and sampling time as covariates.				

Figure legends

Figure 1. Impact of age and CYP2D6 genotype on serum concentrations of zuclopenthixol in long-acting injectable and oral formulation. Each dot (red= long-acting injectable (LAI); black= oral formulation) represents a single unadjusted (A) or dose-adjusted (B) serum concentration of zuclopenthixol. **A** and **B**. The trend lines are calculated by linear mixed model without any covariates [unadjusted serum concentration of LAI: $y = 26.1 - 0.044 \times \text{age}$; unadjusted serum concentration of oral: $y = 26.9 - 0.12 \times \text{age}$; dose-adjusted serum concentration of LAI: $y = 1.68 + 0.011 \times \text{age}$; dose-adjusted serum concentration of oral: $y = 1.22 + 0.010 \times \text{age}$].