Brief Communication

Influence of Cigarette Smoking Habit on Clozapine-to-Norclozapine Ratio in Male Patients

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ABSTRACT

Objective: This study aimed to evaluate the influence of cigarette smoking habit on the clozapine (CLZ)-to-norclozapine (norCLZ) ratio in male patients. **Methods:** The sample consisted of plasma concentration of CLZ and norCLZ data set. The mean values of CLZ, norCLZ, and CLZ-to-norCLZ, between male patients who smoke versus nonsmokers were compared. **Findings:** CLZ mean plasma level of 142 ± 80 ng/ml and 305 ± 159 ng/ml, norCLZ mean plasma level of 93 ± 72 ng/ml and 234 ± 62 ng/ml, and mean CLZ-to-norCLZ plasma level ratio of 2.1 ± 1.1 and 1.5 ± 0.5, were obtained respectively for male patients who smoke and nonsmokers. **Conclusion:** This study has shown a significant decrease in CLZ and norCLZ plasma levels, and an increase in the CLZ-to-norCLZ ratio, in smokers as compared to nonsmokers, due to an increase in the clearance of CLZ and norCLZ by smoking induction of CYP 1A2 and glucuronidation by uridyl glucuronyl transferase enzymes (UGT), mainly UGT 1A4, respectively, as the most probable cause.

KEYWORDS: *Cigarette, Clozapine-to-norclozapine ratio, schizophrenia*

INTRODUCTION

Clozapine (CLZ), mainly metabolized through cytochrome P450 1A2 (CYP1A2) to norclozapine (norCLZ), constitutes the treatment of choice for individuals with treatment-resistant schizophrenia, because of its superior efficacy in this regard.[1] The measurement of CLZ and norCLZ plasma concentrations is useful in assessing compliance, optimizing therapy, and minimizing toxicity; with sex, cigarette smoking habit, and body weight as the covariates that explain 48% of the observed variation in CLZ.[2] Although many studies have examined the influence of smoking behavior on CLZ and norCLZ, previous studies yielded conflicting results, partly because of limitations due to the influence of non-modifiable confounding factors (e.g., sex).[3] Because of this, the objective was to evaluate the influence of cigarette smoking habit (modifiable factor) on the CLZ-to-norCLZ ratio in male patients suffering from treatment-resistant schizophrenia and/or schizoaffective disorders.

METHODS

This retrospective and observational study was conducted from January 2020 to January 2021. The sample consisted of caucasian outpatients who met the diagnostic criteria for drug-resistant schizophrenia who were being treated with CLZ and belonged to a community mental health unit (Actur Sur Mental Health in Zaragoza, Spain) included in the therapeutic drug monitoring program to guarantee the safety and efficacy of the treatment. Since it was not an experimental work, no ethics committee review was required. However, it did need to meet the ethical standards of the Declaration of Helsinki 1975, as revised in 2013.

The analysis was performed on 74% of the initial 77 patients, due to patients failing to follow up, changing their psychiatrist or health area, and/or changing to another pharmacological analysis laboratory that did not include CLZ in its portfolio of services. Therefore, 20 patients were excluded from the original pool, resulting in a sample composed of 57 patients, 34 of whom were males, and constitute the final sample.

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Given that drugs metabolized through CYP1A2 are generally cleared more quickly in men, and that the sample was made up mainly of men, it was decided to eliminate the influence of gender and to carry out the study only on men.

We selected all patients from records held in the hospital's electronic prescriptions system, which incorporates help for metabolism-based interactions mediated by CYP450 and transport-based interactions mediated by P-glycoprotein.

The variables studied were the trough steady-state plasma concentration of CLZ, CLZ/dose, norCLZ, norCLZ/dose, and CLZ-to-norCLZ ratio. For the analysis of the influence of smoking, two groups were formed and compared: a smoking group and a nonsmoking group. Study patients were classified according to their smoking status as: "smokers," if they reported having smoked at least 100 cigarettes in their lifetime and continued to smoke; and "nonsmokers," if they had smoked <100 cigarettes, and had last cigarette at least 1 month ago, or had never smoked.

Blood samples for analysis were obtained in the morning before the administration of the corresponding dose (trough level), once a steady state had been reached. For the analysis of CLZ and norCLZ in the samples, the simple liquid chromatography/tandem mass spectrometer method in human serum was used. Blood samples (10 ml) were collected from patients in heparin-coated tubes in the early morning (between 06.30 and 08.00 h), before the next dose of CLZ. Samples were then centrifuged at 3000 g for 10 min, and plasma collected and stored at −20°C until assay, which was performed within 1 week. Chromatography was performed at ambient temperature, flow rate of 1.0 ml/min, and injection volume of 50 µl.

This assay is highly specific and sensitive for the simultaneous measurements of CLZ and norCLZ. The simplification of this assay makes it ideal for high throughput analyses of the patient samples in a routine clinical laboratory staffed with general medical technologists.[4]

Student's *t*-test was used as the statistical test, presenting the data as mean (± standard deviation). For this, the population distribution was assumed to be normal, with these basic assumptions: exactly two groups, the sample is normally distributed (data should resemble a bell-shaped curve when plotted graphically), and independent observations.

The observed statistical characteristics of the sample were, effect size (Cohen's *d*) = 0.5; probability level 0.05; sample size = 34; and beta level (two-tailed hypothesis) = 0.709. The statistical characteristics of the groups are as follows: CLZ level for nonsmokers = 305 ± 159 ng/ml; CLZ level for smokers = 142 ± 80 ng/ml; calculated sample size, nonsmokers, and smokers = 15; probability of type I error (α) = 0.05; probability of type II error (β) = 0.2; and power = 0.8.

Results

The total number of study patients was 34 male patients, 50% smokers (mean of 22 cigarettes/day), diagnosed with schizophrenia (87.5%) or schizoaffective disorder (12.5%), concomitant treatment with paliperidone (35%), aripiprazole (14%), and risperidone injection (14%). Furthermore, all prescribed drugs were revised to avoid drug–drug interactions mediated by CYP1A2.

The anthropometric data and CLZ doses administered were fairly comparable between the smoker and nonsmoker populations.

According to Table 1, CLZ was about 47% lower in smokers as compared to nonsmokers, and 30% of patients had levels below 200 ng/ml while 21% had levels above 400 ng/ml. The CLZ-to-norCLZ ratio was <0.5 in 14% of the patients, suggesting a lack of adherence, CYP1A2 enzyme induction, or presenting a "rapid metabolizer" phenotype as the most probable cause. The ratio was >3 in another 7% of patients, suggesting the saturation of their metabolism, enzymatic inhibition of CYP1A2, or

Table 1: Demographic and clinical characteristics of the study groups

	Total (*n*=34)	Smokers (*n*=17)	Nonsmokers (*n*=17)
Sex, males (%)	100	100	100
Age (years)	45 (10)	47 (11)	43 (8)
BMI (kg/m^2)	32 (5)	32 (5)	31 (5)
SBP (mmHg)	115 (10)	115 (9)	115 (8)
DBP (mmHg)	75 (9)	71 (9)	78 (7)
Total cholesterol (mg/dL)	187 (38)	177 (45)	197 (27)
LDL cholesterol (mg/dL)	133 (40)	123 (43)	145 (34)
CLZ (dose) (mg)	265 (84)	257 (109)	273 (52)
CLZ level (ng/mL)	231 (151)	142 (80)	305 (159)**
norCLZ level (ng/mL)	149 (97)	93 (72)	234 (62)**
CLZ level/dose (ng/mL*mg)	0.9 (0.6)	0.7 (0.6)	1.1 (0.5)*
norCLZ level/dose (ng/mL*mg)	3 (0.4)	0.2 (0.3)	0.3 (0.5)
CLZ-to-norCLZ ratio	1.9 (0.9)	2.1 (1.1)	1.5 (0.5)*

*Mean differences are significant at the 0.05 level (Student's *t*-test), **Mean differences are significant at the 0.01 level (Student's *t*-test). Data are presented as mean (± standard deviation), unless otherwise indicated. BMI=Body mass index, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, LDL=Low-density lipoprotein, CLZ=Clozapine

presenting a "slow metabolizer" phenotype as the most probable cause. Accordingly, 30% of patients in our sample would be below the therapeutic range, while 21% would be above 400 ng/ml, and thus nearly 50% of patients would require dose adjustment to avoid iatrogenic.[5]

Finally, the values of CLZ, CLZ/dose, norCLZ, norCLZ/dose, and CLZ-to-norCLZ ratio were analyzed [Table 1]; and intergroup differences (male smokers vs. male nonsmokers) reached statistical significance, except for norCLZ/dose. Surprisingly, the CLZ-to-norCLZ ratio was found to be higher in smokers (2.1 ± 1.1) as compared to nonsmokers (1.5 ± 0.5). This finding is not in line with the known induction of CLZ metabolism by smoking, which is expected to go along with a lower CLZ-to-norCLZ metabolic ratio.

Discussion

According to these results, CLZ and norCLZ data, a positive effect of tobacco on CLZ metabolism is observed; and adding to the obtained CLZ-to-norCLZ ratio data, this positive effect of smoking appears to be inverted and amplified. Therefore, overall, it could be due to a possible increase in the clearance of CLZ and norCLZ by smoking's induction of CYP1A2[6] and glucuronidation by uridyl glucuronyl transferase enzymes (UGT), mainly UGT 1A4, respectively, as the most probable cause.

The effect of smoking and smoking cessation on the pharmacokinetics of CLZ and its downstream metabolism is well-established in the public domain and reported in many articles over the last decades. The results of the present study are largely consistent with prior reports on this matter. Hence, the manuscript does provide new insights or aspects about the influence of smoking habit on induction of norCLZ glucuronidation, as novel information.

In short, lower values of CLZ and norCLZ have been found in male smokers, which leads to differences in the metabolic ratio, CLZ-to-norCLZ. Based on the results, we suggest that CLZ doses should be reduced by 30%–40% in nonsmoking men compared to smokers to obtain an equivalent concentration of CLZ and/or norCLZ. After adjusting the dose of CLZ, according to the previously stated criteria, and based on the Brief Psychiatric Rating Scale, at the end of the 12-month study period, 70.6% of patients were responders, without significant changes in negative symptoms in the total sample; Nor was the appearance of any serious adverse reaction observed that would lead to changes in the dose or suspension of treatment. There are several limitations regarding other confounding factors that were not analyzed, such as age, alcohol consumption, patient adherence, coffee consumption, and CLZ N-demethylation saturation, among others. Furthermore, major confounding factors (including adherence, concomitant treatments, and symptoms of severe mental illness) were not included in the analysis. It is possible that the difference in CLZ and norCLZ levels between smokers and nonsmokers could be due to differences in concomitant medications and adequate management of the symptoms of schizophrenia and schizoaffective disorder.

The results of the present study, a positive effect of tobacco on CLZ metabolism, are largely consistent with prior reports on this matter. However, the data on CLZ, norCLZ, and CLZ-norCLZ ratio, obtained in the present study, provide new knowledge or aspects about the influence of smoking on norCLZ glucuronidation, as novel information and, based on this, take into account drug interactions and other pathophysiological factors that may affect norCLZ glucuronidation.

Authors' Contribution

All authors have contributed equally.

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Conflicts of interest

There are no conflicts of interest.

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