ABCB1 and cytochrome P450 polymorphisms: clinical pharmacogenetics of clozapine

THESE

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Résumé

Polymorphismes *ABCB1* et *cytochrome P450*: Pharmacogénétique clinique de la clozapine

(*ABCB1* and *cytochrome P450* polymorphisms: clinical pharmacogenetics of clozapine)

Dans le but d'examiner les facteurs génétiques qui influencent la pharmacocinétique de la clozapine in vivo, 75 patients traités avec ce médicament antipsychotique ont été genotypés pour les polymorphismes CYP et *ABCB1*, et phénotypés pour l'activité de CYP1A2 et CYP3A. L'activité de CYP1A2 et les taux plasmatiques de clozapine en steady-state corrèlent d'une manière significative (r=0.61; p=1x10^-6), sans influence du génotype de CYP1A2*1F (p=0.38). Les métaboliseurs déficients CYP2C19 (génotype *2/*2 genotype) avaient des concentrations de clozapine 2,3 fois (p=0.036) plus élevées que les métaboliseurs rapides (non*2/*2). Chez les patients comédiqués avec la fluvoxamine, un fort inhibiteur de CYP1A2, les concentrations de clozapine et de norclozapine corrèlent significativement avec l'activité de CYP3A (r=0.44, p=0.075; r=0.63, p=0.007, respectivement). Les porteurs du génotype *ABCB1* 3435TT avaient des concentrations plasmatiques de clozapine 1,6 fois plus élevées que ceux qui ne présentaient pas ce génotype (p=0.046). En conclusion, cette étude montre pour la première fois, in vivo, le rôle significatif de CYP2C19 et celui du transporteur P-gp dans la pharmacocinétique de la clozapine. Le CYP1A2 est la forme principale de CYP impliquée dans le métabolisme de clozapine, tandis que le CYP2C19 joue un rôle modéré et que le CYP3A4 n'y contribue que chez les patients qui présentent une activité de CYP1A2 réduite. De plus, le polymorphisme de *ABCB1*, mais pas ceux de CYP2B6, CYP2C9, CYP2D6, CYP3A5 et CYP3A7, influence la pharmacocinétique de la clozapine.

*Mots-clé: clozapine; concentration plasmatique; CYP1A2; CYP2C19; CYP3A4; ABCB1*
ABCB1 and Cytochrome P450 Polymorphisms

Clinical Pharmacogenetics of Clozapine

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Abstract: To examine the genetic factors influencing clozapine kinetics in vivo, 75 patients treated with clozapine were genotyped for CYPs and ABCB1 polymorphisms and phenotyped for CYP1A2 and CYP3A activity. CYP1A2 activity and dose-corrected trough steady-state plasma concentrations of clozapine correlated significantly ($r = -0.61; P = 1 \times 10^{-5}$), with no influence of the CYP1A2*1F genotype ($P = 0.38$). CYP2C19 poor metabolizers ($2^*2^*$ genotype) had 2.3-fold higher ($P = 0.036$) clozapine concentrations than the extensive metabolizers ($2^*2^*$). In patients comedicated with fluvoxamine, a strong CYP1A2 inhibitor, clozapine and norclozapine concentrations correlate with CYP3A activity ($r = 0.44, P = 0.075$; $r = 0.63, P = 0.007$, respectively). Carriers of the ABCB1 3435TT genotype had a 1.6-fold higher clozapine plasma concentrations than noncarriers ($P = 0.046$). In conclusion, this study has shown for the first time a significant in vivo role of CYP2C19 and the P-gp transporter in the pharmacokinetics of clozapine. CYP1A2 is the main CYP isoform involved in clozapine metabolism, with CYP2C19 contributing moderately, and ABCB1 contributing only in patients with reduced CYP1A2 activity. In addition, ABCB1, but not CYP2B6, CYP2C9, CYP2D6, CYP3A4, or CYP3A7 polymorphisms influence clozapine pharmacokinetics.

Key Words: clozapine, plasma concentration, CYP1A2, CYP2C19, CYP3A4, ABCB1

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Because of the risk of hematologic adverse effects, clozapine is available as a second-line atypical antipsychotic drug despite its efficacy being considered superior to that of other antipsychotics. However, inadequate response to clozapine is estimated to be as high as 30%. Several studies confirmed the existence of a therapeutic window for clozapine and low plasma concentrations, despite adequate dosing, might explain some of the cases of nonresponse. High plasma concentrations are risk factors for side effects such as seizures.

Genetic and environmental factors contribute to the high interindividual variability in clozapine plasma concentrations. In vitro studies suggest that cytochrome P4501A2 (CYP1A2) is the most important CYP isoenzyme contributing to clozapine N-demethylation, leading to the formation of the main active metabolite norclozapine. Because smoking induces CYP1A2, this is in agreement with the lower plasma concentrations of clozapine measured in heavy smokers as compared with non-smokers. According to in vitro studies, CYP2C19 and CYP3A4 could also be of considerable importance in the metabolism of clozapine. In vivo, CYP3A4-inducing drugs such as carbamazepine reduce clozapine plasma concentrations, but it is unclear which CYP3A isoforms (ie, CYP3A4, CYP3A5, and/or CYP3A7) are implicated in clozapine metabolism. CYP2D6 probably plays a minor role in clozapine metabolism. Indeed, the pharmacokinetics of clozapine was not significantly different between 5 CYP2D6 poor metabolizers (PMs) and 5 extensive metabolizers (EMs) receiving a single oral dose of 10 mg clozapine. Similarly, and in contradiction to in vitro data, clozapine pharmacokinetics was not significantly different between CYP2C19 EMs and PMs receiving a single oral dose of 10 mg clozapine. Finally, an in vivo study suggested that clozapine is a substrate of the P-glycoprotein (P-gp) transporter, encoded by the ABCB1 gene, a finding that was subsequently contradicted. No in vivo study has yet evaluated whether genetic polymorphisms of the ABCB1 gene influence clozapine plasma concentrations.

The aim of this study was to examine the in vivo influence of genetic polymorphisms of CYP isoforms (CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, and CYP3A7) and ABCB1 on steady-state plasma concentrations of clozapine. As the activities of CYP1A2 and CYP3A are not partially reflected by genotyping tests, patients were also phenotyped with caffeine (CYP1A2) and midazolam (CYP3A). The impact of environmental factors such as smoking or comedication was investigated. Finally, as weight gain is one of the major side effects of clozapine and a risk factor for metabolic syndrome, and as norclozapine may be implicated in this effect, we examined this possible association.

MATERIALS AND METHODS

Patients

Seventy-five inpatients from 2 psychiatric clinics, aged 18 years or older, on stable clozapine treatment and unchanged medication for at least 2 weeks (4 weeks for fluoxetine) were included in the study. Exclusion criteria were any serious uncontrolled illness, any organic psychiatric illness, or substance dependence. To ensure compliance, patients took their medication under supervision of a nurse for 4 days before blood sampling. The study was approved by the local ethics committees of the 2 participating centers (Königsfelden and...
Blood Sampling
On the morning of day 1, before first drug intake, 75 µg oral midazolam was given to the patients for CYP3A phenotyping. A blood sample was taken 30 minutes later for determination of the 3′-O-midazolam/midazolam plasma ratio19 and trough clozapine and plasma concentrations. They then received their usual medication, together with 200 mg caffeine for CYP1A2 phenotyping.20 A second blood sample was performed 4 hours later for determination of the paraxanthine/caffeine plasma ratio.21

Clinical Assessments
Routine clinical chemistry and hematologic parameters were measured at baseline. All patients underwent a physical examination at screening; their medical history was recorded, and patients drank grapefruit juice. On days 1 and 7, vital signs, weight, spontaneously reported adverse events, and lifestyle factors (smoking, caffeine, and grapefruit intake) were collected retrospectively from the patient's medical files.

Statistical Analysis
Clozapine and nortriptyline blood concentrations were compared between different genotypes by nonparametric analysis (Kruskal-Wallis test for 2, Mann-Whitney U test for 2 groups). Correlations between plasma concentrations and CYP2A12 or CYP2B6 activity were assessed by Spearman test, and multivariate analyses were performed using linear regression (backward method). A P < 0.05 was considered to indicate statistical significance. All statistical tests were performed in the whole group of patients and in the 2 subgroups with and without fluvoxamine as influence by fluvoxamine could mask the potential influence of other factors. Statistical analyses were performed using SPSS version 15.0 (SPSS, Inc, Chicago, IL). For ABCB1 polymorphisms, Hardy-Weinberg equilibrium was tested, and linkage disequilibrium (Linkage D'Coefficient) was estimated with STATA (version 10; Stata Corporation, College Station, Tex). haplotypes were inferred using the expectation-maximization algorithm. As none of the inferred haplotypes had a posterior probability below 0.01, they can be considered as minimal. Genetic association studies were conducted using the haplo.score function in R (which uses generalized linear models and expectation maximization) with an additive effect and a Gaussian distribution for the trait.

RESULTS
Patient Characteristics
Three patients lost weight, 25 remained stable, and 13 increased weight gain. Three patients lost weight, 25 remained stable, and 13 increased weight. Ten patients reported a weight gain of 10%. During clozapine treatment, and 4 were diabetic before taking clozapine. In a multivariate analysis, the following factors were considered as relevant covariates: (maximal dose; number of patients: serotonin: clozapine 1.5 (SD 0.28, range 1.03–1.93) compared with nonsmokers (0.50 range, 0.10–1.55). Lower lower norclozapine (0.49 ng/mL x mg) was 0.31 ng/mL x mg in smokers and nonsmokers (P = 0.039), but not clozapine (1.35 ng/mL x mg) in smokers and nonsmokers (0.71, range 0.63–1.21 ng/mL x mg, in smokers and nonsmokers, respectively, (P = 0.011). The effect of smoking on clozapine or nortriptyline plasma concentrations was also investigated (n = 120, range, 0.28–1.25; P = 0.34, data not shown).

CYP and ABCB1 Genotyping
The observed genotype haplotypes of CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, and ABCB1 are presented in Table 1. Three patients were smokers (26 men and 17 women), and 74 were non-smokers (13 men and 17 women). The number of cigarettes smoked per day ranged from 10 to 60 (median, 20). Smoking induces CYP1A2 as shown by the 1.5–4-fold higher median paraxanthine-caffeine ratio (P = 0.013) in smokers (P = 0.008–0.49) compared with nonsmokers (0.50 range, 0.10–1.55). Lower lower norclozapine (0.49 ng/mL x mg) was 0.31 ng/mL x mg in smokers and nonsmokers (P = 0.039), but not clozapine (1.35 ng/mL x mg) in smokers and nonsmokers (0.71, range 0.63–1.21 ng/mL x mg, in smokers and nonsmokers, respectively, (P = 0.011). The effect of smoking on clozapine or nortriptyline plasma concentrations was also investigated (n = 120, range, 0.28–1.25; P = 0.34, data not shown).

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CYP1A2 and CYP3A Phenotyping and Clozapine Plasma Concentrations

A strong correlation was observed between CYP1A2 activity and plasma concentrations of clozapine, norclozapine, and clozapine-norclozapine in the whole population ($r = 0.10, p = 0.48$; $r = 0.17, p = 0.39$; $r = 0.40, p = 0.047)$, respectively). Multivariate analyses were obtained after adjusting for age and sex (data not shown). Haplotype analysis revealed a trend toward higher clozapine concentrations in patients with the genotype $*1/*17$, but this was not significant ($r = 0.05, p = 0.73$). Norclozapine was not performed because of the low observed genetic variability (Table 1). No significant correlation of the $CYP2D6$ polymorphism on clozapine plasma concentration was observed (data not shown). In addition, norclozapine and clozapine-norclozapine plasma concentrations did not differ significantly between different genotypes ($CYP2C9$ and $CYP3A5$) (data not shown).

Multivariate Analyses

Multivariate analyses between clozapine, norclozapine, and clozapine-norclozapine plasma concentrations and the CYP3A4 genotype were conducted using the highest levels of genotyping data available in our study ($n = 17$) and presented in Table 1. The main factors potentially influencing their kinetics were the following: CYP3A4 genotype ($P = 0.001$), smoking status ($P = 0.001$), and smoking dose ($P = 0.001$) were significant predictors of higher plasma concentrations. Smokers consumed significantly higher doses of clozapine ($P < 0.05$), and the CYP3A4 genotype was not detected. A second significant contribution was found between CYP2C19*7 or $CYP1A2*1$ genotype and $CYP3A4*17$ genotype as a significant covariate for higher clozapine (0.017) and norclozapine (0.03) plasma concentrations.

**DISCUSSION**

The measured trough plasma concentrations of clozapine, norclozapine, and clozapine-norclozapine corrected by daily dose presented a very high interindividual variability, with a 41-, 55-, and 33-fold variation, respectively. The determination of genetic and environmental factors contributing to this variation is therefore of clinical relevance, considering the existence of a narrow therapeutic window for clozapine (350-6000 ng/mL) with plasma levels more than 800 to 1000 ng/mL being associated with increased risk of side effects such as correlations.

In contrast to 2 previous studies, $^8$-10, $CYP3A4$ polymorphism, the effect of smoking and caffeine consumption, and comedication with psychotropic drugs were also of interest. In our study, CYP3A4*1F was associated with increased CYP2D6 activity in smokers, possibly because of increased inductive activity. In contrast to 2 previous studies, $^8$-10, but in agreement with 2 others, $^3$2 we could not confirm any influence of $CYP3A4*1F$ polymorphism on clozapine plasma concentrations in the whole group and in the group of smokers. A strong influence of this polymorphism on clozapine plasma concentrations seems therefore unlikely. On the other hand, the important inducing effect of smoking on CYP2D6 and $CYP2C19$ seems to be confirmed in our study. CYP2C19 activity and clozapine metabolism was confirmed in our study by the 1.5-fold higher CYP2C19 activity in smokers compared to non-smokers.
Based on in vivo studies, it has been suggested that clozapine is metabolized primarily by CYP1A2 and CYP2D6, with CYP1A2 having a more significant role in clozapine metabolism. CYP1A2 is responsible for the metabolism of clozapine and other drugs, such as fluvoxamine, which affects the metabolism of clozapine. The inhibition of CYP1A2 by fluvoxamine leads to higher plasma concentrations of clozapine, possibly by inhibition of the metabolism of clozapine. The question arises whether the blocking effect of fluvoxamine on CYP1A2 is due to its limited effect or a moderate inhibitor of CYP1A2.

Saturation of the inhibitory effect on CYP1A2 was found to be saturable at low doses. We investigated in an earlier case series and concluded that cotreatment with 150 mg fluvoxamine has the same blocking effect as 100 mg fluvoxamine in terms of the relationship between fluvoxamine, clozapine, and norclozapine plasma concentrations. Figure 1 shows the relationship between the plasma levels of clozapine and norclozapine. Saturation of the inhibitory effect on CYP1A2 activity is also observed in patients with high plasma concentrations of clozapine, possible by inhibition of CYP1A2. In the present study, at some time points, patients had low clozapine plasma concentrations, indicating that the effect of fluvoxamine on CYP1A2 is saturable at low doses.

In conclusion, this study examined thoroughly the in vivo inhibition of clozapine metabolism by fluvoxamine. The inhibitory effect of fluvoxamine on clozapine metabolism was not significant, as the plasma concentrations of clozapine were not significantly different between patients with and without fluvoxamine. However, a mild effect on the metabolism of clozapine by fluvoxamine was observed, as evidenced by the therapeutic drug monitoring and clinical response of treatment-refractory patients with schizophrenia. Further studies are needed to investigate the role of CYP1A2 in the metabolism of clozapine and other drugs.

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**AUTHOR DISCLOSURE INFORMATION**

The authors declare no conflict of interest.

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