Nonresponse to Clozapine and Ultrarapid CYP1A2 Activity

Clinical Data and Analysis of CYP1A2 *Gene*

Chin B. Eap, PhD, Stefan Bender, MD,† Eveline Jaquenoud Sirot,‡ Gianni Cucchia, MD,* Michèle Jonzier-Perey,* Pierre Baumann, PhD,* Delphine Allorge, PhD,§ and Franck Broly, MD, PhD§*

Abstract: Clozapine (CLO), an atypical antipsychotic, depends mainly on cytochrome *P*450 1A2 (CYP1A2) for its metabolic clearance. Four patients treated with CLO, who were smokers, were nonresponders and had low plasma levels while receiving usual doses. Their plasma levels to dose ratios of CLO (median; range, 0.34; 0.22 to 0.40 ng × day/mL × mg) were significantly lower than ratios calculated from another study with 29 patients (0.75; 0.22 to 2.83 ng × day/ mL × mg; $P < 0.01$). These patients were confirmed as being CYP1A2 ultrarapid metabolizers by the caffeine phenotyping test (median systemic caffeine plasma clearance; range, 3.85; 3.33 to 4.17 mL/min/kg) when compared with previous studies (0.3 to 3.33 mL/min/kg). The sequencing of the entire CYP1A2 gene from genomic DNA of these patients suggests that the −164C > A mutation (*CYP1A2*1F*) in intron 1, which confers a high inducibility of CYP1A2 in smokers, is the most likely explanation for their ultrarapid CYP1A2 activity. A marked (2 patients) or a moderate (2 patients) improvement of the clinical state of the patients occurred after the increase of CLO blood levels above the therapeutic threshold by the increase of CLO doses to very high values (ie, up to 1400 mg/d) or by the introduction of fluvoxamine, a potent CYP1A2 inhibitor, at low dosage (50 to 100 mg/d). Due to the high frequency of smokers among patients with schizophrenia and to the high frequency of the −164C > A polymorphism, *CYP1A2* genotyping could have important clinical implications for the treatment of patients with CLO.

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*Unité de Biochimie et Psychopharmacologie Clinique, Département Universitaire de Psychiatrie Adulte, Hôpital de Cery, Prilly-Lausanne, Switzerland; †Klinik für Psychiatrie und Psychotherapie der Universität Essen, Essen, Germany; ‡Klinik Königsfelden, Psychiatrische Dienste des Kantons Aargau, Brugg, Switzerland; §Laboratoire de Biochimie et Biologie Moléculaire, Hôpital Calmette, Centre Hospitalier Régional et Universitaire de Lille, Lille, France.

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Address correspondence and reprint requests to Chin B. Eap, PhD, Unité de Biochimie et Psychopharmacologie Clinique, Departement Universitaire de Psychiatrie Adulte, Hôpital de Cery, CH-1008 Prilly-Lausanne, Switzerland. E-mail: Chin.Eap@inst.hospvd.ch.

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Clozapine (CLO) is an efficient atypical antipsychotic drug with a response rate of about 60% in patients with no or partial response to classic neuroleptics.[1] Several studies[1,2] have suggested optimum CLO plasma levels to be around 350 to 400 ng/mL, and the risks of adverse effects on the central nervous system (confusion, delirium, and generalized seizures) are increased by CLO concentrations above 1000 ng/mL.[2]

Cytochrome *P*450 1A2 (CYP1A2) is the major enzyme involved in the metabolism of CLO,[3–5] leading to the formation of *N*-desmethyl clozapine (NCLO).[5] There are pronounced interindividual differences in CYP1A2 activity among humans,[6,7] and it has been shown that CYP1A2 activity, as measured by a caffeine test, predicts CLO steady state concentrations in patients with schizophrenia.[4] Different factors such as gender, race, and environmental exposure to inducers or inhibitors are responsible for interindividual differences in CYP1A2 phenotype.[8] Induction of CYP1A2 expression by smoking and inhibition of activity by oral contraceptives, for example, partly explain the variation in in vivo enzyme activity.[8,9] We also recently identified a mutation in CYP1A2 gene responsible for a low CYP1A2 activity, determined by a caffeine phenotyping test, leading to CLO overdose in a patient receiving a standard dose of this drug.[10]

Other gene mutations have been identified, in particular, in the 5′-flanking region and in intron 1 of *CYP1A2*[11,12] (see also http://www.imm.ki.se/CYPalleles/cyp1a2.htm). Some of these polymorphisms may be associated with altered inducibility of gene expression in smokers.[11–13] Of particular interest is the −164C > A polymorphism (*CYP1A2*1F*) in intron 1, which confers a high inducibility of CYP1A2 in smokers.[12] High CYP1A2 activity may lead to low CLO plasma levels and to nonresponse as shown in 2 case reports.[14,15] These 2 patients, smokers, phenotyped as being ultrarapid CYP1A2 metabolizers, responded to their CLO treatment only when their CLO plasma levels were increased to therapeutic values by the addition of a low dose of fluvoxamine, a selective serotonin reuptake inhibitor antidepressant and a strong CYP1A2 inhibitor.[14,15] The presence of the *CYP1A2*1F* allele was confirmed in 1 patient.[15]

In the present report, clinical data and results of the phenotyping tests and genetic analysis from 4 patients treated with CLO, smokers and nonresponders to this drug, are presented.

METHODS

Subjects and Setting

One patient was included in Lausanne (patient 1), 1 in Königsfelden (patient 2), and 2 in Essen (patients 3 and 4). Clinical management of the patients (increase of CLO dose and/or introduction of fluvoxamine) was only based on CLO plasma levels and was performed independently of the phenotyping and genotyping tests. Patients or their legal representative gave their written informed consent to the phenotyping tests and to the genetic analysis. This study was carried out in accordance with the Declaration of Helsinki. Blood samplings for trough CLO plasma determinations were performed in steady state conditions ~12 hours after the intake of the evening dose and before the morning dose. Patients had normal hepatic and renal functions as assessed by standard clinical laboratory tests (alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, urea, and creatinine).

Analysis

Using genomic DNA samples from the 4 patients, we analyzed the nucleotide sequence of intron 1 of the CYP1A2 gene to identify the −164C > A polymorphism, as reported previously.[12] The presence of other mutations in the 5′-flanking region (from nucleotide −4078 to −870), the 7 exons, the 6 introns, and the exon-intron boundaries of the gene was also investigated by sequencing as described previously.[6,10] All nucleotide sequences were determined by using an automated DNA sequencer (Model 373A, Applied Biosystems, Foster City, CA) and the ABIPRISM Dye Terminator Cycle Sequencing Ready Reaction FS kit (Applied Biosystems) according to the manufacturer's instructions. Both strands of each DNA fragment were sequenced. Detection of defective alleles of *CYP2D6* (*CYP2D6*3*, *CYP2D6*4*, and *CYP2D6*6*) and of *CYP2D6* duplication was performed by allele-specific[16,17] and long[18] polymerase chain reaction, respectively.

For patients 1 and 2, CLO and NCLO were measured by gas chromatography with the use of a nitrogen phosphorus detector, with slight modifications of a previously published method.[19] Intraday and interday coefficients of variation for the determination of CLO and NCLO were between 5% and 10% and between 7% and 15%, respectively. The limit of quantification was 4 ng/mL for both substances. For patients 3 and 4, CLO levels were measured by high-performance liquid chromatography with ultraviolet detection in a commercial laboratory (unpublished data, available on request). Intraday and interday coefficients of variation for the determination of CLO were lower than 5% and 11%, respectively. The limit of quantification for CLO was 100 ng/mL. Fluvoxamine was measured by gas chromatography-mass spectrometry as described previously.[20] The caffeine phenotyping test was done by measuring the plasma paraxanthine/caffeine ratio 6 hours after the oral intake of 200-mg caffeine as described previously.[6,7] The test was performed in the morning, and no caffeine containing food or beverage was allowed during the day of the test until the blood sampling is completed. Caffeine and its major metabolite (paraxanthine) were measured by gas chromatography-mass spectrometry according to a method developed in our laboratory (unpublished data, available on request). Intraday and interday coefficients of variation for the determination of caffeine and paraxanthine were between 2% and 7% and between 6% and 15%, respectively. The limit of quantification was 0.8 ng/mL for both substances. The Mann-Whitney *U* test was used to compare the CLO plasma levels to dose ratios among the 4 patients of the present report and the ratios from a previously published study,[21] calculated with a mean daily dose of 400 mg (Release 4.5, Statsoft, Loll & Nielsen, Hamburg, Germany). A P value < 0.05 was considered as statistically significant.

RESULTS

Patients

The first patient, a 21-year-old male smoker (about 30 cigarettes/d), was hospitalized with the diagnosis of continuous undifferentiated schizophrenia (*International Classification of Diseases, 10th Revision*: F20.30). His first diagnosis of schizophrenia with psychotic symptoms and thematics of persecution and social withdrawal was established when he was 17 years old, leading to his first hospitalization. During the following years, several typical antipsychotics (pimozide, haloperidol, levomepromazine, and flupentixol) were administered resulting in a poor improvement of the psychotic symptoms. For 24 weeks, olanzapine, up to 40 mg/d, was also unsuccessfully administered. Olanzapine is an atypical antipsychotic, which is likely metabolized by CYP1A2,[22–24] although discrepant results have been published.[25] An olanzapine trough plasma level measurement performed at 20 mg/d was 22 ng/mL, which was considered as a rather low value for the dose.[26] During his last hospitalization, due to the poor response to previous antipsychotic treatments, CLO was introduced in dosages up to 600 mg/d, but the clinical state of the patient deteriorated, with distress and invading delirious perceptions. A CLO trough plasma level measurement performed at this dosage was 167 ng/mL for CLO and 151 ng/mL for NCLO. Figure 1 shows his CLO plasma level to dose ratio

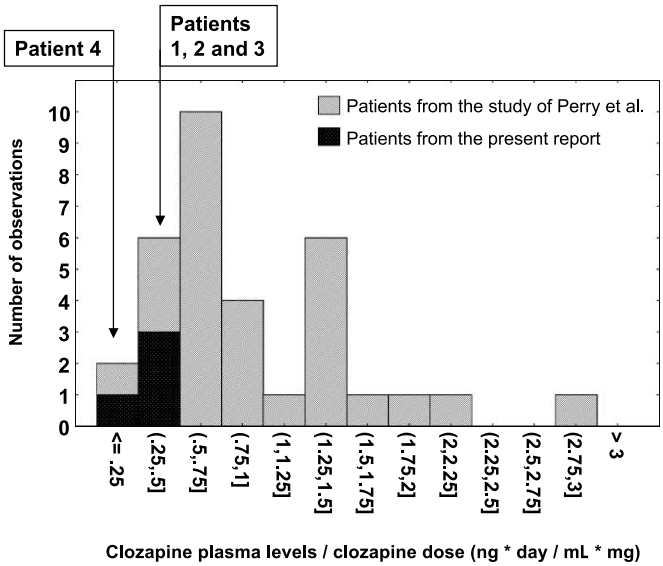

FIGURE 1. CLO plasma levels to dose ratios measured in 4 patients phenotyped with the caffeine test as being CYP1A2 ultrarapid metabolizers compared with previously published data. For each range, the (and] symbols before and after a number indicate that the number is included or not in the range, respectively.

as compared with previously published data.[21] A caffeine phenotyping test gave a paraxanthine/caffeine ratio of 2.0, corresponding to a value of 4.17 mL/min/kg for systemic caffeine plasma clearance[7] (medications at the time of the test: CLO, 600 mg/d; clorazepate, 10 mg/d; and atropine sulfate, 0.625 mg/d). Due to the low CLO plasma levels, fluvoxamine was introduced at a dosage of 50 mg/d, with doses of CLO simultaneously reduced to 400 mg/d. A further increase of fluvoxamine doses up to 100 mg/d (CLO plasma levels, 483 ng/mL; NCLO, 263 ng/mL) resulted in a clear improvement of the clinical state of the patient, with a decrease of the Clinical Global Impression (CGI) score from 7 to 5 after 4 weeks. Since then, the psychotic symptoms progressively and slowly regressed, which allowed the patient to be discharged from the outpatient setting, 7 months after the introduction of fluvoxamine. A 17-month follow-up period showed a stable clinical state of the patient with even a favorable evolution.

The second patient, a 44-year-old male smoker (>40 cigarettes/d), was hospitalized with the diagnosis of chronic paranoid schizophrenia (*International Classification of Diseases, 10th Revision*: F20.0). His first diagnosis of schizophrenia, with hallucinations and ego impairment disorder, was established when he was 18 years old (age of first hospitalization, 17 years). Since the age of 20, due to very poor performance in every domain of life, no autonomy, very aggressive, dangerous, and socially unacceptable behavior, the patient is chronically hospitalized. Several typical antipsychotics (fluphenazine, haloperidol, pimozide, flupentixol, chlorprotixene, levomepromazine, fluspirilene, and chlorpromazine) were administered which only helped to reduce relational and aggressive behavior toward other patients but were poorly tolerated, with massive extrapyramidal symptoms and difficulty in speech, and did not bring an amelioration of the psychotic symptoms. Electroconvulsive therapy was also unsuccessful. Among the antipsychotics received by the patient, CLO was the only medication that was relatively well tolerated, and therefore mainly prescribed, with some interruption due to a tachycardia episode and also once due to a grand mal seizure. Between the ages of 20 and 39, the daily doses of CLO ranged between 300 and 800 mg. When the patient was 39 years old, the dose of CLO was for the first time increased to 1000 mg/d (maximum recommended dose, 900 mg/d), leading to a first clear improvement of his clinical state, the patient being more communicative and open. A CLO plasma level determination performed at this dosage was 402 ng/mL for CLO and 143 ng/mL for NCLO. Due to this high dosage of CLO, an attempt was made once to reduce the dosage, resulting in an increase of psychotic symptoms, with return of rituals and of compulsive behavior. Comedications before the increase of CLO up to 1000 mg/d and after the increase were mostly stable with lithium, valproate (2400 mg/d), and laxatives. The paraxanthine/caffeine ratio was 1.90, corresponding to 3.96 mL/min/kg for systemic caffeine plasma clearance (medications at the time of the test: CLO, 1300 mg/d; lithium acetate, 1340 mg/s; valproate, 2400 mg/d; and lactitol, 20 g/d). Subsequently, CLO dosage was increased up to 1400 mg/d, with no abnormalities in the electroencephalogram. A determination of plasma levels gave the following values: CLO, 574 ng/mL; NCLO, 264 ng/mL (Fig. 1).

The third patient, a 31-year-old male smoker (about 40 cigarettes/d), was hospitalized with the diagnosis of paranoid schizophrenia, episodic course, with interepisodic residual symptoms (*International Classification of Diseases, 10th Revision*: F20.02). Since the onset of disease (21 years old), the patient was hospitalized in different hospitals and was unsuccessfully treated with several typical antipsychotics (haloperidol, benperidol, fluphenazine, and perazine) as well as with atypical antipsychotics (sertindole and risperidone). During the last hospitalization, due to an ongoing nonresponse to sertindole, CLO was introduced with doses up to 600 mg/d with no substantial improvement of the symptoms (CGI: 6). A CLO plasma level measurement performed at this dosage was CLO 240 ng/mL (Fig. 1). The paraxanthine/caffeine ratio was 1.61, corresponding to 3.33 mL/min/kg for systemic caffeine plasma clearance (medications at the time of the test: CLO, 600 mg/d; aluminium oxide, 690 mg/d; and magnesium hydroxide, 1200 mg/d). Fluvoxamine was introduced at 100 mg/d

(fluvoxamine plasma level, 51 ng/mL), leading to a strong increase of CLO plasma levels (1500 ng/mL). The paraxanthine/caffeine ratio measured 11 days after the introduction of fluvoxamine was 0.39, corresponding to 0.68 mL/min/kg for systemic caffeine plasma clearance (medications at the time of the test: CLO, 300 mg/d; fluvoxamine, 50 mg/d; metoclopramide, 30 mg/d; aluminium oxide, 690 mg/d; magnesium hydroxide, 1200 mg/d; and lactulose 20 g/d), indicating a reduction of CYP1A2 activity to about 20% of the original value. After an adaptation of CLO and fluvoxamine dosages, a remission of the symptoms was observed within the next months, with a substantial decrease of paranoid symptoms and hallucinations, a stabilization of affect, and an increase in quality of life (the patient stated that ''he had never felt so good since the beginning of the disease''). He was discharged 4.5 months after the introduction of fluvoxamine (CGI: 3).

The fourth patient, a 38-year-old female smoker (about 40 cigarettes/d), was hospitalized with the diagnosis of schizophrenia, paranoid type, episodic with interepisodic residual symptoms, and with prominent negative symptoms (*International Classification of Diseases, 10th Revision*: F20.02). The first diagnosis of schizophrenia was made when she was 13 years old. The patient was first hospitalized when she was 36 years old. The last hospitalization was due to exacerbation of severe residual negative symptoms (emotional and social withdrawal, blunted affect, and lack of spontaneity), severe cognitive dysfunction, obsessive thoughts, agitation, and ego disturbance. Since her first hospitalization, the patient had been treated with CLO. On admission, she received 450 mg/d of CLO. Two plasma level determinations performed during the hospitalization gave a value below the limit of quantification (see ''METHODS''); the limit of quantification was thus used to calculate the plasma levels to dose ratio for this patient (Fig. 1). The paraxanthine/caffeine ratio was 1.80, corresponding to 3.74 mL/min/kg for systemic caffeine plasma clearance (medications at the time of the test: CLO, 450 mg/d; clonazepam, 0.5 mg/d). Fluvoxamine was introduced at 25 mg/d (CGI before fluvoxamine: 6) and subsequently increased to 50 and 100 mg/d, which led to a strong increase of CLO plasma levels (1100 ng/mL). The paraxanthine/caffeine ratio 24 days after the introduction of fluvoxamine was 0.06, indicating an almost complete inhibition of CYP1A2 activity (medications at the time of the test: CLO, 450 mg/d; fluvoxamine 100 mg/d). A complete inhibition of CYP1A2 activity by fluvoxamine (plasma level of fluvoxamine, 63 ng/mL), leading to a saturation of CLO metabolism and to nonlinear kinetics, could partially explain why, at the dosage of 450 mg/d of CLO and 100 mg/d of fluvoxamine, successive plasma level determinations of CLO gave very variable values, ranging from 420 to 1000 ng/mL. Despite the variable CLO levels, possibly because they were always above the postulated therapeutic threshold of CLO concentration, the clinical state of the patient improved, and the patient was discharged 4.5 months after the introduction of fluvoxamine (CGI: 4).

Genetic Analyses

The 4 patients were genotyped as CYP2D6 extensive metabolizers (3 patients, *CYP2D6*1*/*CYP2D6*1* and 1 patient, *CYP2D6*1*/*CYP2D6*4*). No patient was found to be an ultrarapid metabolizer for the CYP2D6 isozyme. Sequencing of intron 1 of the CYP1A2 gene showed the 4 patients to be homozygous for the −164C > A polymorphism. No other nucleotide differences in exons, introns, or the 5′-flanking region (up to about −4.0 kb) were found when compared with a reference sequence of *CYP1A2* (Genbank NT_010374.5). Specifically, polymorphisms, which have been previously suggested to alter CYP1A2 inducibility in smokers,[11,13] were not detected in any of the patients.

DISCUSSION

The present case report describes 4 schizophrenic patients treated with CLO, who were nonresponders to this drug, with low CLO plasma levels, despite usual dosages. Their low plasma levels were confirmed by several blood samplings with intake supervised by nurses to check compliance (for 3 patients, CLO tablets were also dissolved in water before intake). The plasma levels to dose ratios of CLO in these 4 patients (median; range, 0.34; 0.22 to 0.40 ng × day/mL × mg) were significantly lower than ratios calculated from a previous study with 29 schizophrenic patients[21] (0.75; 0.22 to 2.83 ng × day/mL × mg; $P < 0.01$; see Fig. 1). These ratios are also among the lowest measured in patients receiving this drug when comparing the present data with those of another study.[27]

A low plasma level of CLO, despite usually administered dosages, suggests an ultrarapid metabolism of this drug. As CLO metabolism is mainly mediated by CYP1A2,[3] a caffeine phenotyping test was performed, which confirmed a very high CYP1A2 activity in these 4 patients. Thus, the systemic caffeine plasma clearance found in these 4 patients (median; range, 3.85; 3.33 to 4.17 mL/min/kg) indicates a very high CYP1A2 activity when compared with previously published data (0.3 to 3.33 mL/min/kg).[7] In these 4 nonresponders to CLO, an increase in CLO blood levels beyond the postulated therapeutic threshold, either by an increase of CLO daily dosage beyond the maximum recommended dose (1 patient) or by the administration of fluvoxamine (3 patients), led to a marked (patients 1 and 3) or a moderate (patients 2 and 4) improvement of their clinical state.

To evaluate the possible genetic basis of such a high CYP1A2 activity, the sequence of the CYP1A2 gene was

studied. Specifically, the presence of the *CYP1A2*1F* allele was investigated. The *CYP1A2*1F* allelic variant harbors a unique point mutation in intron 1 (−164C > A) and has been related to higher inducibility of CYP1A2 expression in smokers.[12,13,28] The A/A genotype identified in the 4 patients would be the most likely explanation for their high CYP1A2 activity, even if the molecular mechanism underlying this marked inducibility remains unclear. Moreover, no other nucleotide differences that could explain the observed high CYP1A2 activity in the 4 patients were found in any exons, introns, or 5′-flanking region of the gene.

Metabolism of CLO by other cytochrome *P*450 enzymes than CYP1A2[3] is most probably of less clinical relevance. Some studies suggest an involvement of CYP3A4[5,29,30]; however, itraconazole, nefazodone, or erythromycin, which are potent CYP3A4 inhibitors, do not inhibit the metabolism of CLO.[31–33] CYP2D6 is most probably not involved[34,35] despite evidence of an in vitro metabolism of CLO by CYP2D6.[36] This is in agreement with the present report where none of the patients were found to be CYP2D6 ultrarapid metabolizers. With regard to CYP2C19, despite the results of an in vitro study suggesting a possible involvement,[37] in vivo data do not confirm such a result.[34]

In summary, we described 4 patients treated with CLO, smokers, nonresponders to this drug, and with low plasma levels despite being treated with usual doses. These patients were confirmed as being CYP1A2 ultrarapid metabolizers by a caffeine phenotyping test. Sequencing of the entire CYP1A2 gene suggests that the *CYP1A2*1F*/*CYP1A2*1F* genotype is the most likely explanation for their very high CYP1A2 activity. A marked or a moderate improvement of their clinical state occurred after the increase of CLO plasma levels above the therapeutic threshold by the increase of CLO doses to very high values or by the introduction of fluvoxamine. However, these results should be confirmed by prospective studies using a larger number of patients. Due to the high frequency of smokers among patients with schizophrenia (>80%)[38] and to the high frequency of the −164C > A polymorphism (about 45% of homozygotes A/A vs. 10% of homozygotes C/C),[12] *CYP1A2* genotyping could have important clinical implications for the treatment of patients with CLO. Finally, the present report, as well as previous case reports,[14,15,39] strongly suggests a nonlinear kinetics of CLO when given with fluvoxamine, which might lead to marked variations of CLO plasma levels after only small modifications of the dosage. Due to the increased risks of adverse effects at high CLO plasma levels,[2] fluvoxamine or very high doses of CLO should be given only to nonresponders when low levels of CLO have been measured, when noncompliance or poor compliance has been excluded, and/or when very high CYP1A2 activity has been measured.

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