

**PHARMACOGENETICS OF CYP2D6: CLINICAL  
IMPLICATIONS IN PSYCHIATRIC PATIENTS  
TREATED WITH ANTIPSYCHOTIC DRUGS**

By

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## INTRODUCTION

### *1. Cytochrome P450 enzymes and drug metabolism*

Drug therapies are frequently associated with great inter- and intraindividual differences in therapeutic response. This variability may result in diminished clinical response or, conversely, increased incidence of adverse drug reactions (side-effects). The role of *pharmacokinetic factors* in the variability of treatment outcome has been a field of thorough investigation for the last decades. Differences in plasma concentrations between individuals after the same dose of a drug may be attributed to several factors, however, the majority of interindividual differences are related to differences in drug metabolism. Drugs which are mainly excreted unchanged do not exhibit such a pronounced variability in their disposition kinetics; therefore, drug metabolism is thought to be the source of the variability. With a few exceptions (such as lithium), drugs used in the treatment of psychiatric disorders are highly lipophilic and thus subject to an extensive metabolic biotransformation in the body. Interindividual differences in drug metabolism are mainly determined by genetical factors. In 1959 a new discipline, *pharmacogenetics* emerged, which deals with the study of genetically determined variations in drug metabolism and response. The genetical variability of drug metabolizing enzymes can exist as polymorphism or as a rare trait. The allelic variant that is responsible for the genetic determinant of the isoenzyme can be determined by mutation-specific PCR.

Besides genetical factors, drug disposition and thus therapeutic efficacy and/or side-effects can be modified by various other, non-genetical factors, such as physiological (age, gender, pregnancy, exercise, etc.), pathological (fever, diseases, infections, etc.) or environmental ones (diet, tobacco smoking, alcohol intake, xenobiotics).

Drug metabolizing enzymes are present abundantly in the human body. The first step of the drug metabolism is usually referred to as a Phase I reaction and this process is catalyzed mainly by the cytochrome P450 enzyme family, which is the most important group of enzymes in drug disposition. Every cytochrome P450 enzyme is encoded by a separate gene. A large number of these enzymes have been described (currently more than 50 human enzymes), and divided into 14 families. The division is based on sequence homology. Genes that have 40% or greater homology are classified in the same family and are named with the root CYP (derived from cytochrome P450) followed by an Arabic number, which refers to the family. Genes within an enzyme family with greater than 55% homology are classified in the same subfamily signed by upper case Latin letters (A, B, C, D, etc.). Separate genes within the same subfamily are designated by Arabic numbers (1, 2, 3, 4, etc.). Drug metabolizing enzymes are known in the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> CYP enzyme family.

The catalytic activity of the cytochrome P450 enzymes can be determined *in vivo* by the urinary Metabolic Ratio (MR) by a test of “phenotyping”. In this test the drug metabolizing capacity is measured by giving to the subjects a single dose of a test-drug specifically metabolized by the CYP enzyme in question. The metabolic ratio reflects the actual activity of the specific enzyme.

Different isoenzymes involved in the metabolism of antipsychotic drugs have been described in humans. At present CYP2D6, CYP1A2, CYP2C19 and CYP3A4 have been shown to be the most important enzymes in psychopharmacology because of their implication

in psychotropic drug biotransformation, although the role of other CYP isoenzymes can not be ruled out (like CYP2C9, or CYP2E1).

The activity of CYP2D6 enzyme, studied in the present work is bimodally distributed in Caucasian populations. People with decreased or absent activity of the enzyme have high MRs, while the rest have normal. On the basis of population studies, a cut-off point to distinguish between subject with high or low MR can be defined. Individuals with low enzyme activity are referred as slow or poor metabolizers (poor metabolizer=PM), while the others are rapid or extensive metabolizers (extensive metabolizer=EM) of the enzyme. The different phenotypes are related to the genotypes (polymorphic allelic variants) of the *CYP2D6* enzymes. Around seven percent of the Caucasians are classified as PMs. Recently, another group of individuals called ultrarapid metabolizers (UM) have been described. These individuals carry an extra *CYP2D6* gene, i.e. three or more active *CYP2D6* genes are present and expressed, and this leads to a higher metabolic activity.

## 2. Antipsychotic drugs and CYP enzymes

Antipsychotic drugs are highly lipophilic substances, and therefore they are substrates of the polymorphic cytochrome P450 enzymes. The involvement of the specific cytochrome enzymes is different for each drug. The extent of involvement and the specificity of a CYP enzyme in the metabolism are determined by the chemical properties of the substance. Unfortunately, *in vitro* studies can not predict the exact metabolic fate of a substance in the human beings due to the various environmental and endogenous factors that may influence the process. Part of the information comes from healthy volunteers' studies, where the differences in plasma levels between extensive and poor metabolizers are analyzed by comparing single dose kinetics. The final clinical relevant information about the implication of CYP enzyme polymorphism in the metabolism of antipsychotic drugs should be obtained from studies in patients during steady-state conditions.

## 3. Inhibition of cytochrome P450 enzyme activity by antipsychotic drugs

Several classical antipsychotic drugs are not only metabolized by CYP enzymes but they also inhibit the enzyme activity. Chlorpromazine, levomepromazine, perphenazine and thioridazine are the strongest inhibitors of CYP2D6, but *in vitro* virtually all the antipsychotic drugs have the potential to inhibit CYP2D6 enzyme activity. Antipsychotic drugs exhibit a striking selectivity for CYP2D6 inhibition compared with other isoforms, which may reflect a structural commonality of the therapeutic target of these drugs.

The inhibition can be due to enzyme saturation, as a consequence of the use of high doses of an antipsychotic drug metabolized by the CYP enzyme. A second potential explanation for the inhibition could be the result of a mutual competitive inhibition caused by the coadministration of drugs metabolized by the same CYP enzymes. There are also some precedents for the inhibition of CYP2D6 by drugs which are not metabolized by that enzyme.

## 3. Cytochrome P450 enzymes polymorphism in the metabolism of antipsychotic drugs

The present Thesis is focused on the involvement of CYP2D6 enzyme in the metabolism of three antipsychotic agents, two typical drugs: thioridazine and haloperidol, and an atypical one: risperidone. One reason for selecting these particular drugs was that they are used extensively in everyday clinical practice, but our selection was also guided by previous

studies that had suggested important implications of pharmacogenetic factors in their metabolism.

### 3.1. Thioridazine

The thioridazine is first metabolized by side chain sulfoxidation to thioridazine 2-sulfoxide (mesoridazine) and then from mesoridazine to thioridazine 2-sulfone (sulforidazine). Another metabolic pathway is the thioridazine-5-sulfoxidation of thioridazine, resulting in thioridazine-ring-sulfoxide. Mesoridazine and sulforidazine are both active pharmacologically, while thioridazine-ring-sulfoxide has no antipsychotic effect but this metabolite was related to cardiovascular side-effects.

Almost 70% patients under thioridazine treatment were phenotypically PMs with respect to CYP2D6, i.e. phenotypic conversion may have occurred due to the inhibition. In PMs the thioridazine peak serum concentration was 2.4 times higher and the elimination half-life twice longer than in EMs. The mesoridazine/thioridazine ratio correlates to the debrisoquine MR, while the sulforidazine/mesoridazine does not. Thus, it is probable that the thioridazine-mesoridazine metabolic step is catalyzed by CYP2D6.

### 3.2. Haloperidol

In the human body the main pathway of the metabolism of haloperidol is oxidative N-dealkylation resulting in two inactive metabolites: p-fluorobenzoyl-propionic acid (FBPA) and 4-(4-chlorophenyl)-4-hydroxypiperidine. Another important metabolic pathway is the reduction of the ketone group to another metabolite, reduced haloperidol. In humans the interconversion between haloperidol and reduced haloperidol also exists.

Among healthy volunteers after a single oral dose of haloperidol, the plasma half-life of the drug was significantly longer and the plasma clearance was lower in PMs than in EMs. The frequency of PMs of CYP2D6 found in patients receiving haloperidol therapy was significantly higher than the 5-10% of PMs found among Caucasian healthy volunteers. Several authors have reported that smokers had significantly lower plasma levels of haloperidol than non-smokers and that the clearance of haloperidol was higher in smokers.

### 3.3 Risperidone

In humans the two main metabolic pathways for risperidone are: (1) alicyclic hydroxylation of the tetrahydropyrido-pyrimidinone ring at the 7- and 9-positions and (2) oxidative N-dealkylation of both risperidone and 9-hydroxy-risperidone to acidic metabolites. Hydroxylation of risperidone at the 9-position is by far the most important metabolic pathway, accounting for up to 31% of the dose excreted in the urine. 9-hydroxy-risperidone is also the most abundant fraction in the plasma of humans.

In an *in vitro* study performed with human liver microsomes the involvement of CYP2D6 in the metabolism of risperidone to 9-hydroxy-risperidone was reported. The mean total body clearance of risperidone in healthy volunteers is reduced about sevenfold in PMs. Due to the fact that 9-hydroxy-risperidone is an active metabolite, pharmacokinetic data have been calculated using the sum of risperidone and 9-hydroxy-risperidone content (active moiety). The absolute oral bioavailability of the active moiety is 100%, irrespective of the metabolic status.

#### 4. Clinical implications of CYP2D6 polymorphism in psychiatric patients

The clinical importance of polymorphic drug metabolizing CYP enzymes is increasingly recognized. In the everyday clinical situation psychiatric drugs usually exert their effect when steady-state conditions are reached. The importance of the cytochrome enzymes polymorphic metabolism is related to the activity (clinical or side-effects) of the parent drug and /or its metabolite(s).

**Poor metabolizers might be more prone to side-effects** because the plasma levels of the parent drug could be higher than expected and the plasma concentration of the metabolites lower. The incapability of elimination may be due to a genetically impaired enzyme activity (PMs) or to non-genetical factors such as:

- a. environmental, e.g. ethanol consumption or dietary factors.
- b. a dose dependent inhibition of the drug on the cytochrome enzymes.
- c. an inhibition due to drug interactions (inhibitors or competitive substrates).

The frequency of CYP2D6 PM subjects was found to be three times higher among schizophrenic patients with longitudinal tardive dyskinesia (TD), as compared with the group of patients with fluctuating or no TD. This indicates that genetically impaired CYP2D6 metabolism may be a contributory factor in the development of persistent TD.

**The lack of therapeutic effect may also be the result of genetic polymorphism**, as in the case of codeine. Codeine is metabolized to morphine by CYP2D6; therefore, in PM patients the analgesic effect is lower than in EMs. Furthermore, since morphine is a drug that is frequently abused, the metabolic status is also a factor in determining the patient's liability for addiction.

**Increased elimination of drugs, resulting in lower than expected plasma concentration** of the parent drug, can be expected in *genetically ultrarapid metabolizers* or it may be caused by other, non-genetical factors, such as:

- a. environmental factors inducing the cytochrome enzymes, e.g. tobacco and CYP1A2.
- b. autoinduction when a drug accelerates its own metabolism by induction of the cytochrome enzyme involved in its disposition (e.g. carbamazepine).
- c. use of concomitant inducers (e.g. carbamazepine), which diminish the plasma concentration of the active drug.

In clinical practice there have been reports on cases of ultrarapid metabolizers in whom therapeutic response could be elicited only with megadosis of the drug.

The findings of pharmacogenetics may provide a tool to predict unexpected side-effects or interactions and to differentiate between pharmacodynamic or pharmacokinetic effects of antipsychotic drugs. In the present Thesis the possible application of pharmacogenetics to clinical problems were also studied. The cardiac effect of antipsychotic drugs is clinically important in terms of potentially dangerous side-effects. Due to the potentially fatal arrhythmias and sudden deaths reported during antipsychotic treatments these side-effects are clinically of utmost importance. Pharmacogenetic factors (like CYP enzyme activity) might be involved in the occurrence of cardiac side-effects, therefore their relevance were also explored.

#### 4.1. Side-effects: QTc Interval lengthening during thioridazine treatment

The risk of cardiac side-effects by non-antiarrhythmic drugs has become a matter of public concern. There are several published reports of torsade de pointes type arrhythmias and sudden deaths associated with antipsychotic treatment. It has been claimed that the risk is substantially higher in patients treated with thioridazine. The cardiotoxicity of thioridazine has been proved in both animal and *in vitro* studies. There are several published case reports of torsade de pointes type arrhythmias and sudden deaths associated with prolongation of the heart-rate corrected QT interval (QTc) after thioridazine overdose. However, so far only three studies have studied in humans the association of thioridazine or its metabolite, mesoridazine dosage, plasma concentration and ECG changes.

The relationship between thioridazine dose and QTc has been found in a large population study. This dose-effect relationship has also been shown in a clinical trial of nine healthy volunteers, however no correlation between the plasma concentration of thioridazine and metabolites and QTc interval lengthening was found. Plasma concentration increase due to overdose and/or due to drug interactions at the cytochrome P450 enzymes, which produce elevations in thioridazine plasma levels may increase QTc interval and the risk of ventricular arrhythmias.

The activity of CYP2D6 enzyme will determine the plasma levels of thioridazine and its metabolites and thus may also have an influence on the risk of cardiac side-effects. Unexpectedly high plasma concentration of thioridazine may be the consequence of an impaired activity of CYP2D6 due to genetic factors or enzyme inhibition.

#### 4.2 Clozapine withdrawal symptoms

Withdrawal symptoms for typical antipsychotics are generally mild and self-limited. However, following withdrawal of clozapine serious symptoms with rapid onset have been repeatedly reported. Based on their main characteristics, the withdrawal effects can be categorized as follows: (1) somatic symptoms - sweating, nausea, vomiting, diarrhoea, diaphoresis; (2) extrapyramidal symptoms - dystonia, dyskinesia, worsening of tardive dyskinesia, akathisia; (3) psychiatric symptoms - insomnia, restlessness, agitation, anxiety, delusions, disorganized thinking and hallucinations. Concerning severity and onset, the clozapine withdrawal symptoms seem to be different from the withdrawal effects of classical neuroleptics. Psychotic symptoms usually occur within two weeks after the cessation of clozapine therapy; therefore, they can be considered as rebound psychosis and not a relapse. Relapse occurs only in 5% of the patients within this short time interval after discontinuation of a classical antipsychotic medication. However, the origin of these symptoms is still not known and the involvement of pharmacokinetic factors can not be ruled out.

## AIMS OF THE STUDIES

The general aim of our studies was to determine the implications of the CYP2D6 enzyme polymorphism and environmental factors (dose-dependent inhibition, concomitant medications, tobacco smoking) for the interindividual variability in the plasma concentration of antipsychotic drugs, and the clinical implications of these findings for the occurrence of side-effects (thioridazine, haloperidol, risperidone and clozapine).

### *Specific aims of the studies*

- 1) To develop and adapt simple, reliable HPLC methods for measurement of two typical antipsychotic drugs: thioridazine and haloperidol, and two atypicals - risperidone and clozapine - in plasma.
- 2) To determine and compare the inhibitory effect of thioridazine, haloperidol and risperidone on the CYP2D6 enzyme activity and to determine the extent of dose dependency of the CYP2D6 enzyme inhibition by these drugs.
- 3) To determine the relationship between the activity of the CYP2D6 enzyme and the steady-state plasma levels of the above mentioned antipsychotic drugs.
- 4) To study the possible use of parent drug/metabolite ratios for the assessment of the actual CYP2D6 enzyme capacity.
- 5) To study the effects of tobacco smoking on the plasma concentrations of the investigated drugs.
- 6) To study the pharmacokinetic interactions between risperidone and concomitant treatment with drugs metabolized by the CYP2D6 enzyme.
- 7) To determine the relationship between plasma concentrations of the thioridazine, haloperidol and risperidone, debrisoquine MR, drug metabolite ratios and extrapyramidal side effects.
- 8) To determine the influence of thioridazine plasma levels and CYP2D6 enzyme activity on the QTc interval in patients.
- 9) To study the usefulness of measuring drug plasma levels in case of clinically important side-effect (clozapine withdrawal syndromes).

## **MATERIALS AND METHODS**

### *1. General design of the studies*

Three antipsychotic drugs used extensively world-wide, viz. thioridazine, haloperidol, and risperidone, were studied. In the present studies the following parameters were determined: a) plasma concentrations of drugs and metabolites, b) the CYP2D6 phenotypes by debrisoquine c) in selected cases the CYP2D6 genotypes d) clinical evaluation of side-effects and in selected cases evaluation of the QTc interval on ECG. The clinical relevance of the use of plasma concentration monitoring will also be evaluated in a forth drug: the atypical antipsychotic clozapine. The studies listed below has been designed to achieve the aims of the present thesis:

**Study I.** The implications of CYP2D6 enzyme activity for the metabolism of thioridazine – in 65 psychiatric patients receiving thioridazine monotherapy.

**Study II.** The effect of thioridazine dosage on CYP2D6 enzyme activity in psychiatric patients – a subset of the population of Study I, consisted of 16 psychiatric patients.

**Study III.** The use of therapeutic drug monitoring of thioridazine as a marker of CYP2D6 enzyme capacity – in 27 psychiatric patients receiving thioridazine monotherapy.

**Study IV.** The effect of the CYP2D6 enzyme activity and smoking on the plasma concentrations of haloperidol – in 27 psychiatric patients receiving haloperidol monotherapy.

**Study V.** The relationship between the plasma concentrations of risperidone and 9-hydroxy-risperidone and CYP2D6 enzyme activity in psychiatric patients – in 43 psychiatric patients receiving risperidone therapy.

**Study VI.** The relationship between the QTc interval lengthening and the dose and plasma concentration of thioridazine and CYP2D6 activity – the psychiatric patients enrolled in Study I.

**Study VII.** Clozapine withdrawal symptoms in a schizophrenic patient after switching to sertindole – a case report.

### *1.1. CYP2D6 phenotyping*

The metabolic phenotype was determined by the administration of the test drug, debrisoquine. The subjects took a single oral dose of 10 mg debrisoquine sulphate and the urine was collected over the next eight hours. Debrisoquine and its metabolite, 4-hydroxy-debrisoquine, were analyzed with the method developed originally by Lennard. Samples were derivatized by acetyl-acetone and the derived pyrimidines were extracted and analyzed by gas chromatography with hydrogen flame ionization. The debrisoquine oxidation phenotype was determined by calculation of the urinary MR, which is the ratio between percentages of doses eliminated as debrisoquine and 4-hydroxy-debrisoquine. Individuals with a debrisoquine MR over 12.6 were considered as PMs.

### *1.2. CYP2D6 genotyping*

The genotyping of CYP2D6 was carried out in collaboration with the Department of Molecular Biology and Genetics of the University of Extremadura. The CYP2D6 genotype was determined using genomic DNA purified from peripheral blood leukocytes and the QIAamp<sup>®</sup> DNA Mini Kit. The AmpliTaq Gold<sup>™</sup> System was used to amplify the *CYP2D6*\*3, *CYP2D6*\*4 and *CYP2D6*\*6 alleles by tetra-primer PCR, whereas Expand<sup>™</sup> Long Template PCR System was used to amplify the *CYP2D6*\*5 allele by multiplex PCR. The PCR products were separated by electrophoresis in agarose gels and were visualized by staining with ethidium bromide.

### *1.3. Determination of plasma levels of antipsychotic drugs and metabolites*

Ten ml-s of venous blood samples were drawn from patients before they took the morning dose of the studied drug. The samples were immediately centrifuged to separate plasma and they were stored at –20° C in refrigerator until plasma concentration measurements of the drugs.

Plasma concentrations of **thioridazine** and its metabolites, mesoridazine and sulforidazine were measured in duplicates by high performance liquid chromatography with UV detection according to the method developed in our laboratory. Briefly, to 500µl of plasma 25 µl of internal standard (5-(pirolodiniilpropyliden)-10-11-dihydro-5H-dibenzo (a,d) cycloheptane, IS) was added. The extracted residues were then resuspended in mobile phase and 20 µl were injected on a straight-phase Spherisorb Silica column. Peaks were detected by ultraviolet absorbance at 267 nm.

Plasma concentrations of **haloperidol** were measured in duplicates by high performance liquid chromatography (HPLC) with UV detection according to a previously published method with some minor modification.

Plasma concentrations of **risperidone** and 9-OH-risperidone were determined by HPLC based on a method from Huddinge Hospital, Sweden with some modifications: To 1

ml of plasma 40 µl of internal standard (IS) was added (metoxy-risperidone at concentration of 5µg/ml), 1 ml of 0.5 M sodium hydroxide buffer and 4 ml of isoamyl-alcohol in diisopropyl-ether. The tube was briefly mixed and after centrifugation the aqueous layer was collected and 175 µl of acetic acid (25 mM) was added. The mixture was centrifuged and the organic layer discarded, then 500 µl of heptane was added. The heptane layer was aspirated and evaporated in a gentle stream of nitrogen. A 30 µl aliquot was injected into the chromatograph. The analytical column was packed with Hypersil ODS coated with C18 groups. The flow-rate was 0.8 ml/min and the detection wavelength was at 278 nm.

An HPLC method for the determination of **clozapine** and its main metabolite N-desmethylclozapine has been developed. Sample preparation was carried out by liquid-liquid extraction. 2 ml of human plasma was pipetted into a 10 ml polypropylene tube and 100 µl of 0.005 mg/ml protriptyline (IS) and 200 µl of 2 M sodium hydroxide were added. The plasma was mixed and then 5 ml of the hexane:isoamyl-alcohol (98.5:1.5 v/v) was added to the tubes. The tubes were centrifuged and the organic phase was drawn off and 120 µl of 0.1 M hydrochloric acid was added. The tubes were vortexed and centrifuged again, then the organic layer was drawn off and evaporated at 40° C under N<sub>2</sub> atmosphere. The residue was redissolved in 110 µl of 0.1 M hydrochloric acid and an aliquot of 100 µl of this solution was injected onto the HPLC system for analysis.

#### *1.4. Clinical evaluation of the patients*

Patients were evaluated clinically by a senior psychiatrist, and this included the following:

- a. Clinical exploration (both general and psychiatric) with detailed data on patient's weight, height, arterial pressure, general examination and psychiatric exploration.
- b. A laboratory battery was performed within two days before or after the study involving biochemical and haematological examination.
- c. On the day of the blood sampling side effects were determined with the clinically validated Utvalg for Kliniske Undersogelser (UKU) Side Effect Scale.
- d. Routine ECG was performed with an automated equipment in each patient on the day of the blood-sampling.

#### *1.5. Pharmacogenetics of thioridazine (Study I-III)*

##### *1.5.1. Implications of CYP2D6 enzyme activity for the metabolism of thioridazine (Study I)*

The pharmacogenetics of thioridazine was studied in 65 Caucasian, chronic psychiatric patients. They were hospitalized at Mérida Psychiatric Hospital (Extremadura, Spain). The patients were on continuous oral antipsychotic monotherapy with thioridazine. The administered dose range was 20 to 500 mg/day and the average dose was 140±93 mg/day (mean±S.D.). Of the study population 47 (72%) patients were tobacco smokers, defined as smoking regularly more than 5 cigarettes per day. For each patient the debrisoquine metabolic ratio (MR) and the plasma levels of thioridazine, mesoridazine, and sulforidazine were determined as described before.

##### *1.5.2. Effects of thioridazine dosage on CYP2D6 enzyme activity in psychiatric patients (Study II)*

A subset of the population of the thioridazine study (Study I) was involved consisting of sixteen patients. In these patients the dose of thioridazine was decreased or the administration of the drug was stopped due to clinical considerations. The initial dose range was 20 to 300 mg/day, the average dose was  $126 \pm 68.5$  mg/day. 12 patients had two dose reductions and 4 patients had one. Finally, 10 patients became completely drug free. The CYP2D6 genotypes and phenotypes and the plasma concentrations of thioridazine, mesoridazine, and sulforidazine were determined after the dose changes as described before.

### *1.5.3. The use of therapeutic drug monitoring of thioridazine as a marker of CYP2D6 enzyme capacity (Study III)*

In order to determine the relationship between CYP2D6 enzyme activity and the plasma concentrations and ratios of different metabolites of thioridazine, twenty-seven Spanish, Caucasian, chronic psychiatric patients hospitalized at the Psychiatric Hospital of Mérida (Extremadura, Spain) were studied. The patients were on a continuous oral thioridazine monotherapy of at least 14 days. The daily dose of thioridazine was 50 mg (n=13) or 100 mg (n=14). For each patient the CYP2D6 genotype, the debrisoquine metabolic ratio (MR) and the plasma levels of thioridazine, mesoridazine, and sulforidazine were determined as described before.

### *1.6. The effect of CYP2D6 enzyme activity and smoking on the plasma concentrations of haloperidol (Study IV)*

Twenty-seven Spanish, Caucasian psychiatric patients hospitalized at Mérida Psychiatric Hospital (Extremadura, Spain) were studied. The patients were on continuous oral neuroleptic monotherapy with haloperidol. The dose range was 1.5 to 30 mg/day and the average dose was  $7 \pm 5$  mg/day. Of the 27 enrolled patients 20 were tobacco smokers. The plasma levels of haloperidol and debrisoquine MR were determined as described previously.

### *1.7. The relationship between the plasma concentrations of risperidone and 9-hydroxy-risperidone and CYP2D6 enzyme activity in psychiatric patients (Study V)*

Forty-three Caucasian (12 Hungarian and 31 Spanish), schizophrenic patients were studied. They were hospitalized at the Psychiatric Hospital of Mérida (Extremadura, Spain) and University of Debrecen, Department of Psychiatry (Debrecen, Hungary). The patients had received at least 7 days of continuous oral risperidone therapy with an average daily dose of  $4.6 \pm 2.4$  mg (range: 1.5-9). Of 40 patients, 27 (67.5%) were receiving antipsychotic monotherapy with risperidone. 9 patients (22.5%) were receiving an associated antipsychotic and 3 (7.5%) patients antidepressants. The plasma levels of risperidone, its metabolite and CYP2D6 phenotypes were determined as described previously.

### *1.8. The relation between the QTc interval lengthening and the dose and plasma concentration of thioridazine and CYP2D6 activity (Study VI)*

In order to determine the influence of the thioridazine dose and plasma concentration and the CYP2D6 enzyme activity on the cardiac side effects of thioridazine, cardiological examinations were performed in the patient population of Study I with a standard ECG apparatus used in clinical practice, which calculated the QTc intervals automatically. The upper normal limit of the QTc interval was set at 420 msec. The cut-off value for limit of risk of arrhythmia was set at 456 ms.

### *1.9. Clozapine withdrawal symptoms in a schizophrenic patient after change to sertindole – a case report (Study VII)*

In a case of a severe clozapine withdrawal syndrome plasma concentrations of clozapine and its metabolite were studied in order to determine the involvement of pharmacokinetic factors in the syndrome. A 30-year-old male patient with paranoid schizophrenia was on clozapine therapy for more than five years. Discontinuation of clozapine and an attempt to change his medication to sertindole has led to serious psychotic and somatic symptoms. After readministration of clozapine the psychotic symptoms rapidly disappeared. The change of his medication from clozapine to sertindole was unsuccessful. The clinical status of the patient was evaluated by the Brief Psychiatry Rating Scale (BPRS) on days 8, 16, 23 and 30. Plasma level measurement of clozapine and N-desmethylclozapine were carried out on day 16, 30 and 52.

## RESULTS

### *1. Determination of plasma concentrations of antipsychotic drugs and their metabolites*

Plasma concentration of **thioridazine** and its metabolites were quantifiable in 100 % of the samples. The recovery was 95.1% for sulforidazine, 73% for thioridazine and 59% for mesoridazine. The limit of detection was 100 nmol/l for sulforidazine, 75 nmol/l for thioridazine and 180 nmol/l for mesoridazine. The precision was calculated by the coefficients of variation (%CV). The %CV of the calibration points was under 10% for all substances.

The method for the plasma concentration measurement of **haloperidol** was the adaptation of a previously published method. The intra-day and inter-day precisions expressed by the coefficient of variations (%CVs) were below 15%. The limit of detection was 0.17 ng/ml for haloperidol, and the recovery was 85.3%. Plasma concentration of haloperidol was quantifiable in 100 % of the samples.

**Risperidone** was present in measurable amounts in 67% of the samples, whereas 9-OH-risperidone was present in 100% of the total number of samples. The intra-day and inter-day precision and accuracy were less than 2% for risperidone and 9-hydroxy-risperidone. The lower levels of detection for risperidone and 9-OH-risperidone were 1.5 and 2.5 nmol/L, respectively. The accuracy (corrected recovery) ranged from 95 to 100% in the experimental series with an overall average of 99.9%.

Plasma concentrations of **clozapine** and its metabolite were quantifiable in 100% of the samples. The absolute recoveries of clozapine and N-desmethylclozapine were 92% and 37%, respectively. The within-day precision (%CV) was under 3.2% for clozapine and under 2.6% for N-desmethylclozapine at all calibration points. The limit of detection was 3 ng/ml for clozapine and 5 ng/ml for N-desmethylclozapine.

### *2. Influence of antipsychotic drug treatment on debrisoquine phenotypes*

Previously we found an inhibitory effect on CYP2D6 in patients treated with thioridazine. This population is compared with patients treated with haloperidol and risperidone. Of the 65 patients on thioridazine monotherapy, 53 had log debrisoquine MR>1.1

and thus were phenotypically PMs of CYP2D6. In the haloperidol patient group consisting of 27 patients, 4 were phenotypically PMs of CYP2D6. The frequency of the PM phenotype found in patients treated with thioridazine (81.5%) or haloperidol (15%) was significantly ( $p<0.001$ ) higher than in the control population of healthy volunteers (5.4%) Among the patients on risperidone monotherapy ( $n=27$ ), no one was phenotypically PM (0%), thus, no phenotypic conversion occurred in this patient sample.

### *3. The dose of antipsychotic drugs and the debrisoquine hydroxylation phenotype*

Although there was great interindividual variability, the debrisoquine MR correlated significantly with the dose of haloperidol ( $r=0.47$ ,  $p<0.001$ ). In patients receiving risperidone monotherapy ( $n=27$ ) the administered dose did not correlate with debrisoquine MR. Previously, we found in patients treated with thioridazine a correlation of debrisoquine MR with the dose of thioridazine ( $p<0.001$ ).

#### *3.1. The effect of thioridazine dose changes on the debrisoquine Metabolic Ratio (MR)*

The debrisoquine MR decreased according to dose changes. Fourteen subjects (87.5%) had debrisoquine MR $>12.6$  (PM phenotype) at the initial dose level. After the complete withdrawal of thioridazine in ten patients, only two remained phenotypically PMs. It is worth noticing that these patients were also genotypically PMs ( $*4/*4$ ) for the CYP2D6 gene. Among *wt/wt* patients the proportion of EMs was related to the dose: during complete withdrawal 100% of the patients were EMs, and at doses of 50 and 100 mg, the proportion of EMs decreased to 33% and 29%, respectively. Moreover, at a daily dose of 150 mg (or higher), none of the patients remained EMs. In heterozygous patients (*wt/\*4*), however, even at a dose of 50 mg/day, 100% were classified as PMs.

### *4. Variability of plasma concentrations of antipsychotic drugs*

Previously we found great interindividual differences in the plasma levels of thioridazine in patients during steady-state conditions. The steady-state, dose-corrected plasma concentrations (C/D) of thioridazine showed approximately 23-fold interindividual variation, mesoridazine (15.7-fold variation) and sulforidazine (24.1-fold variation). There was a great interindividual variability in haloperidol plasma levels. The steady-state, dose-corrected plasma concentrations (C/D) of haloperidol showed approximately 8-fold interindividual variation (from 0.12 to 0.93  $\mu\text{g}/\text{ml}/\text{mg}$ ). The dose corrected mean plasma concentration (C/D) was  $2.97\pm 4.04$  ng/ml/mg (range: 0.22-17.38) for risperidone and  $12.04\pm 11.31$  ng/ml/mg (range: 0.78-49.98) for 9-OH-risperidone. The average C/D of the active moiety (R+9-OH-R) was  $15.01\pm 13.15$  (range: 1.28-60.31).

#### *4.1. Relationship between the dose and the and plasma concentration of the antipsychotic drugs*

Thioridazine plasma levels correlated with the administered dose of thioridazine ( $r=0.6$ ,  $p<0.001$ ) and sulforidazine, but not with mesoridazine. The plasma levels of haloperidol and the daily dose correlated significantly ( $r=0.8$ ,  $p<0.001$ ). The plasma concentration of risperidone showed a weak correlation with the administered dose ( $r=0.37$ ,  $p=0.02$ ), while the plasma concentration of 9-OH-risperidone and the risperidone and 9-OH-risperidone (total active moiety) indicated a stronger relationship with the administered dose with a correlation of  $r=0.48$  ( $p<0.01$ ) and  $r=0.47$  ( $p<0.01$ ), respectively.

#### 4.2. CYP2D6 enzyme activity and plasma levels of antipsychotic drugs

Previously we found that the C/Ds of thioridazine correlated with the debrisoquine MR, while mesoridazine or sulforidazine did not. The correlation between the debrisoquine MR and the plasma levels of haloperidol was weaker, but statistically significant ( $r=0.38$ ,  $p<0.05$ ). The debrisoquine MR showed a correlation with the C/D of risperidone among patients receiving risperidone monotherapy ( $r=0.63$ ,  $p<0.001$ ). No correlation was found with C/D-s of 9-OH-risperidone, or the total active moiety.

#### 5. Correlation between the debrisoquine metabolic ratio and the drug/metabolite ratio

The plasma ratios of thioridazine/mesoridazine correlated significantly with the debrisoquine MR ( $r=0.6$ ,  $p<0.001$ ), while the mesoridazine/sulforidazine ratios did not. Also the risperidone/9-OH-risperidone ratio showed a great interindividual variability among patients (0.03-6.95) and correlated strongly with the debrisoquine MR.

#### 6. The effects of smoking on the elimination of antipsychotic drugs

The dose-corrected plasma levels of thioridazine were significantly lower among smokers than in non-smokers. As regards the debrisoquine MR, no significant difference was found between smokers and non-smokers. The C/D of haloperidol was not different in the group of smokers and non-smokers ( $0.44\pm 0.21$  versus  $0.45\pm 0.30$  ng/ml/mg). However the log debrisoquine MR was significantly ( $p<0.05$ ) higher in non-smokers ( $1.0\pm 0.9$ ), than in smokers ( $0.2\pm 0.6$ ). No significant difference was observed on the plasma concentration of risperidone, or 9-OH-risperidone between smokers and non smokers.

#### 7. The effects of concomitant drug treatment on the elimination of antipsychotic drugs

Drugs which are known as strong inhibitors of the CYP2D6 activity had a substantial effect on the plasma concentration of risperidone and its metabolite, and also on the risperidone/9-OH-risperidone ratio. Among the patients receiving concurrently strong inhibitors of CYP2D6, the debrisoquine MR ( $p<0.01$ ) and the risperidone/9-OH-risperidone ratio were increased. The risperidone plasma concentration/dose ratio was almost six times higher in comedicated patients than in the group treated with monotherapy ( $p<0.01$ ).

#### 8.1. Extrapyramidal side effects

Of 65 patients in the thioridazine group, a total of 8 patients (15%) had extrapyramidal side-effects. All of them were PMs of CYP2D6. No correlation with plasma levels of thioridazine or its metabolites, the debrisoquine MR was found; however, patients with extrapyramidal side-effects tended to have higher plasma concentration of thioridazine ( $0.67$   $\mu\text{mol/liter}$ , [95%CI 0.3-1.06] versus  $0.41$   $\mu\text{mol/liter}$ , [95% CI 0.33-0.50],  $p=0.08$ ). Among 27 patients receiving haloperidol treatment 10 (37%) had extrapyramidal side-effects. These patients were all EMs. No correlation with the plasma levels or the debrisoquine MR was found. One patient (2.5%) had extrapyramidal side effect in the risperidone patient group. This patient had the highest log debrisoquine MR and risperidone/9-OH-risperidone ratio.

### 8.2. QTc changes during thioridazine monotherapy

QTc interval over 420 msec, the physiologically normal level, was found in 35 patients out of 65 (54%). There was a correlation between the daily dose of thioridazine ( $p < 0.05$ ) and the QTc interval. The mean dose of thioridazine among patients with QTc > 420 msec ( $n = 35$ , 54%) was significantly higher ( $167 \pm 106$  [95%CI: 130-203] versus  $109 \pm 61$  [95%CI 86-132],  $p < 0.05$ ) than among patients with QTc less than 420 msec ( $n = 30$ , 46%). The plasma concentration of thioridazine also correlated to the QTc interval ( $p < 0.05$ ), while the mesoridazine and sulforidazine plasma concentrations did not ( $r = 0.21$ ,  $r = 0.19$ , respectively). The debrisoquine MR and the ratio of thioridazine to the metabolite mesoridazine (markers of CYP2D6 enzyme activity) also correlated to the QTc interval ( $p = 0.01$  and  $p < 0.05$ , respectively). Among 65 patients, 53 (81.5%) had log debrisoquine MR > 1.1 and were PMs. All patients over 150 mg daily dose of thioridazine were PMs. QTc interval between 420 and 456 msec was found in 30 patients, and 90% of them were PMs.

### 8.3. Clozapine plasma levels in a patient with withdrawal symptoms

Several plasma samples were taken from the patient at different time points after reaching steady-state in order to monitor the plasma levels of clozapine and to correlate them with the clinical symptoms. The Plasma concentrations were clozapine and N-desmethylclozapine were 76 ng/ml and 56 ng/ml, (at 100 mg/day clozapine dose), while they were 100 ng/ml and 78 ng/ml (at 175 mg/day clozapine dose), respectively.

## DISCUSSION

The present results indicate the importance of cytochrome enzymes in the metabolism of antipsychotic drugs. The involvement of CYP2D6 is proven in the metabolism of the drugs investigated: thioridazine, haloperidol, and risperidone. The drugs are not only metabolized by the enzyme but they also exert an inhibition on the activity of CYP2D6, and in this respect thioridazine was more potent than haloperidol, while risperidone had a negligible effect at therapeutic doses. The CYP2D6 activity affects the plasma levels of the thioridazine, haloperidol and risperidone and, therefore, may influence the therapeutic and side-effects, as it was observed with thioridazine for the risk of ventricular arrhythmias. The metabolism of these drugs is also influenced by smoking (in case of thioridazine and haloperidol) and concomitant medications (as proven with risperidone), which both modify plasma concentrations. It is also concluded that the plasma level ratio of thioridazine and risperidone and their metabolites may provide a tool for evaluating CYP2D6 enzyme activity in psychiatric patients.

### 1. HPLC methods for the determination of antipsychotic drugs in plasma

New HPLC methods have been developed for the plasma concentration measurement of **thioridazine**, **risperidone** and **clozapine** and their metabolites. The most significant advantage of the present methods is that chromatographic conditions are simple to adapt for the analysis of the most commonly used psychotropic drugs. These methods are therefore suitable for use in a clinical practice for therapeutic drug monitoring of antipsychotic drugs.

## 2. Inhibition of CYP2D6 enzyme activity by antipsychotic drugs in patients

The present studies have demonstrated that thioridazine, haloperidol and risperidone have different potential for inhibiting the CYP2D6 enzyme activity. In the present study we could confirm the results of previous studies, which demonstrated, that **thioridazine** was a strong inhibitor of the CYP2D6 enzyme, and apparently was able to transform EMs to phenotypically PMs. Almost 70% of the patients receiving thioridazine treatment are phenotypically PMs of debrisoquine (CYP2D6), i.e. phenotypic conversion occurs owing to the inhibition, in spite of their CYP2D6 EM genotype.

The inhibitory effect on the enzyme activity seemed to be the result of thioridazine treatment, since by decreasing the thioridazine dose, the inhibition also diminished, except in two patients who remained PMs even after thioridazine treatment was ceased. These two PM subjects were genotypically PMs, having two copies of the *CYP2D6\*4* allele. As it was expected, the dose capable of modifying the phenotype was related to the inactivating mutated alleles. Among *wt/wt* patients treated with 150 mg/day or more 100% were PMs, although for *wt/\*4* patients this dose was 50 mg/day. The inhibition may carry clinical consequences, such as drug interactions and/or side-effects (i.e. cardiotoxicity) due to higher than expected plasma levels of thioridazine and/or its metabolites.

The results also show that enzyme inhibition disappears one week after thioridazine withdrawal. To explain this observation, it should be borne in mind that the inhibition of the CYP2D6 activity takes place through a reversible mechanism (competitive inhibition). Considering the present data, as well as previous results, it can be stated that after this period no therapeutical consequences of the former CYP2D6 enzyme inhibition are to be expected in clinical practice.

In the present work **haloperidol** also proved to have a dose-dependent inhibitory effect on the CYP2D6 enzyme activity under steady-state conditions, which was reflected by the higher ratio of PMs among patients (15%) than that observed in a previously phenotyped sample of healthy volunteers among Spaniards (PMs: 6.6%). The present study confirms the inhibition of CYP2D6 by haloperidol under steady-state conditions in patients.

No inhibition of the enzyme activity was observed with **risperidone** among patients, which supports the in vitro data from the literature. It seems that at therapeutical dose levels the inhibition of the CYP2D6 enzyme activity is not relevant.

## 3. The dosage of antipsychotic drugs and the CYP2D6 activity

The present results show that thioridazine and haloperidol inhibited CYP2D6 enzyme activity dose-dependently in psychiatric patients, while there was no apparent inhibition during risperidone treatment. **Thioridazine** was a more potent inhibitor in patients than the other drugs, and there were no phenotypically EM patients at thioridazine doses above 150 mg/day. The importance of this fact is that this dose is well under the recommended daily maximum dose, thus, side-effects due to higher than expected plasma levels may. At therapeutical doses the dose-dependent inhibition is less pronounced with **haloperidol** and negligible with **risperidone**.

#### *4. Correlation between the plasma levels of drugs and CYP2D6 enzyme activity*

The interindividual differences in plasma levels of **thioridazine** may be attributed to the activity of the polymorphic CYP2D6 enzyme, as previously suggested based on data from healthy volunteers. This study confirms in patients that the plasma level of thioridazine is determined by the CYP2D6 genotype and by the debrisoquine MR, which supports the assumption that this enzyme is involved, at least partly, in thioridazine disposition.

The results proved a modest, but statistically significant effect of CYP2D6 enzyme activity on the steady-state plasma levels of **haloperidol**. This result suggests that CYP2D6 is involved in the metabolism. However, the CYP2D6 activity by itself does not explain all the interindividual variability among patients, therefore, the involvement of other enzymes must be considered.

The metabolism of **risperidone** to 9-OH-risperidone seems to be related to the activity of the CYP2D6 enzyme. The present data agree with the previously reported association between the CYP2D6 PM genotype and increase of the risperidone/9-OH-risperidone ratio, since the ratio of risperidone/9-OH-risperidone correlated strongly with the debrisoquine MR. It has been stated that the CYP2D6 enzyme polymorphism is not important in the clinical effect of risperidone, since there is no difference in the active moiety between EMs and PMs. However, according to a recent study CYP2D6 poor metabolizers, who were enzyme deficient, did not appear to tolerate risperidone well.

#### *5. Correlation between the debrisoquine metabolic ratio and the drug/metabolite ratio*

One of the findings of the present work was that the plasma concentration ratio of **thioridazine/mesoridazine** correlated significantly with the debrisoquine MR. Thus, the CYP2D6 enzyme capacity, i.e. the debrisoquine hydroxylation phenotype can be estimated from the levels of thioridazine and mesoridazine in plasma. The thioridazine/mesoridazine ratio can be used in clinical practice to predict drug interactions caused by CYP2D6 inhibition. We suggest that the thioridazine/mesoridazine ratio should be monitored when additional drugs are administered to a patient on thioridazine therapy.

The strong correlation between the debrisoquine MR and the **risperidone/9-OH-risperidone** ratio may allow us to assess the CYP2D6 enzyme capacity by monitoring the therapeutic drug levels of risperidone and its metabolites. Since 9-OH-risperidone seems to have a very important role in the clinical efficacy of risperidone, the evaluation of the risperidone/9-OH-risperidone ratio might be important in a given patient if a variation in the clinical status is observed or a new medication is added to the therapeutic regimen. Thus, in clinical practice the evaluation of the risperidone/9-OH-risperidone ratio is a potential therapeutic tool for improving the trade-off between clinical efficacy and side-effects during the use of risperidone.

#### *6. The effect of smoking on the elimination of antipsychotic drugs*

The dose-corrected plasma levels of **thioridazine** and its metabolites were lower in smokers than in non-smokers. The pronounced effect of smoking on the plasma levels of thioridazine suggests that a smoking inducible enzyme is involved in the disposition of this drug. We have previously described that the concomitant use of fluvoxamine increased thioridazine plasma levels markedly. Since fluvoxamine is a potent inhibitor of the CYP1A2 activity and CYP1A2 can be induced by smoking, we suggest that CYP1A2, in addition to

CYP2D6, is involved in the metabolism of thioridazine. Since tobacco smoking is a determinant factor of the plasma levels of thioridazine, therefore, in clinical practice the smoking habits of the patients on thioridazine therapy should also be considered.

The findings of this study could not confirm previous results on the significant effect of smoking on plasma concentrations of **haloperidol**. However, the debrisoquine MR was significantly higher in non-smokers than in smokers. Further research is needed to elucidate the role of the different CYP enzymes in the metabolism of haloperidol in patients.

No apparent influence of smoking on the plasma levels of **risperidone** or 9-OH-risperidone was observed, which confirms previous results.

### *7. Drug interactions and the CYP2D6 enzyme activity*

The importance of pharmacokinetic drug interactions that involve cytochrome enzymes has been increasingly emphasized, as CYP2D6 plays an important role in the metabolism of several important psychotropic agents and other drugs (i.e. beta-blockers, antidepressants, etc.). According to the results of the present study, a concomitant drug treatment increased the plasma concentrations of **risperidone** and the risperidone/9-OH-risperidone ratios. Thus, clinicians should be aware that metabolic interactions can occur and, consequently, potentially severe side-effects or an unexpected decrease in clinical efficacy (due to the plasma concentration changes of the drug and/or its metabolites) should be reckoned with when new medications (CYP2D6 inhibitors) are introduced during risperidone treatment.

### *8.1 Extrapyramidal side-effects*

No correlation between the plasma levels of drugs and extrapyramidal side-effects were found; however, patients in the thioridazine group tended to have higher plasma levels than other patients.

The CYP2D6 PM genotype and phenotype were previously found to be related to a higher risk of extrapyramidal symptoms. In the present studies no relationship was observed between the debrisoquine MR and the extrapyramidal side-effects. However, the reason for this negative finding may be that the patient populations studied here consisted of chronic psychiatric in-patients in stable conditions; therefore, the extrapyramidal symptoms could also be controlled by dose adjustments (non-fix dose studies).

Notwithstanding, the only patient who had extrapyramidal side-effects and was on an antiparkinsonian medication in the risperidone patient group had the highest debrisoquine MR and risperidone/9-OH-risperidone ratio. The obtained results show that patients with an impaired CYP2D6 enzyme activity due to genetic factors or enzyme inhibition have six times higher plasma concentrations of risperidone than EM patients. This high concentration may influence the occurrence of side-effects. Thus, further investigation is needed to explore the differences in the clinical efficacy and side-effect profile of risperidone and 9-OH-risperidone in humans.

### 8.2. QT changes in patients treated with thioridazine

The present study shows that the CYP2D6 activity, the thioridazine dose and plasma concentration can all influence the QTc interval. Generally, a QTc interval of 420 msec is considered to be the normal physiological limit; therefore, a QTc prolongation greater than 456 msec indicates a high risk of arrhythmias and sudden deaths. According to the present data, 54% of the patients were at risk of cardiac side-effects. Nevertheless, they were treated at the clinically recommended doses (average 167 mg/day). The importance of this fact is that this dose is well below the maximum recommended daily dose. Thus, treatment with the drug at clinically used doses gives rise to an increased risk of arrhythmia and sudden death.

The QTc interval correlated with the thioridazine dose, which confirms the results of previous studies investigating the dose-dependent QTc prolongation caused by thioridazine. The results indicate that the plasma concentration of thioridazine is related to the lengthening of QTc interval among psychiatric patients receiving antipsychotic treatment with thioridazine. No correlation was found between the plasma levels of mesoridazine and sulforidazine and the QTc intervals, which may suggest that the cardiotoxic effect is related to some other metabolites, presumably to thioridazine-5-sulfoxide, as proposed earlier. The elevated plasma concentration of the 5-sulfoxide metabolite might come from the high plasma levels of thioridazine.

According to the present results, both the debrisoquine MR and the ratio of thioridazine/mesoridazine correlate with the QTc intervals, a fact that supports the involvement of CYP2D6 in the cardiotoxicity of thioridazine. Thus, according to the present data, PMs are prone to the risk of thioridazine cardiotoxicity. As CYP2D6 enzyme is involved in the metabolism of thioridazine an increase in the drug plasma concentrations may occur when the enzyme activity is impaired genetically in PMs. Notwithstanding, the CYP2D6 enzyme inhibition may also result from a dose-dependent inhibition by thioridazine or from concomitant medications which are inhibitors or substrates of CYP2D6. Considering that thioridazine is frequently used among elderly patients, and that this population is usually co-medicated with several drugs, the risk of drug interactions and sudden death is higher.

Since thioridazine/mesoridazine ratio correlates with the CYP2D6 enzyme activity the CYP2D6 enzyme capacity can be estimated from plasma concentrations of thioridazine and mesoridazine, and thus the thioridazine/mesoridazine ratio can be used in clinical practice to predict the impaired activity of CYP2D6, and can be useful tool in the clinical management of this potentially fatal side-effect.

### 8.3. Clozapine withdrawal syndrome

The present results confirmed that after the discontinuation of clozapine no measurable amount of the drug or its main metabolite was present in the plasma of the patient. After the reintroduction of 100 mg clozapine, the plasma level reached 76 ng/ml, and that further increased to 100 ng/ml on day 56 when the patient was receiving a clozapine dose of 175 mg/day. Several authors have suggested that a minimum threshold plasma level of 300-350 ng/ml clozapine has to be achieved to obtain an optimal therapeutic response. Interestingly, the plasma level of our patient was far below this threshold; still, the somatic and psychotic symptoms of clozapine withdrawal completely disappeared.

Relationship between the plasma levels of clozapine and the changes in the clinical status of the patient confirmed that the patient's severe psychotic and somatic symptoms

resulted from the discontinuation of clozapine treatment. This finding also suggests that the rapid disappearance of the clozapine withdrawal symptoms after the reintroduction of low-dose clozapine is not related to the clozapine plasma levels suggested for optimum antipsychotic effect.

## CONCLUSIONS

CYP2D6 is an important enzyme in the metabolism of numerous antipsychotic drugs. Its genetically inherited activity is modified by several environmental factors (dose-dependent inhibition by the administered drug, concomitant drug treatment, smoking). Interindividual variability in the enzyme activity has clinical implications in the everyday clinical practice.

### *1. Specific conclusions*

- 1) The HPLC methods developed in the present work (for thioridazine, risperidone and clozapine) are useful and reliable tools for determining the plasma concentration of different antipsychotic drugs and their metabolites.
- 2) At clinically used dosage thioridazine and haloperidol seem to inhibit the CYP2D6 enzyme activity in a dose-dependent manner. Thioridazine and haloperidol are potent and moderate inhibitors respectively, however risperidone seems not to exert a clinically relevant inhibition on CYP2D6 enzyme activity at clinically used dosage. In clinical practice it is important to consider the differences in the dose-dependent inhibitory effect of antipsychotic drugs on the CYP2D6 enzyme.
- 3) The CYP2D6 enzyme activity influences the plasma concentrations of thioridazine, haloperidol and risperidone and, therefore it is an important factor in its metabolism. The extent of this influence is different for each specific drug.
- 4) In patients the risperidone and thioridazine drug/metabolite ratio can be a useful tool for monitoring the actual enzyme capacity of CYP2D6.
- 5) Smoking decreases markedly the thioridazine levels in plasma, a similar but less effect was observed in the case of haloperidol, and the risperidone level was not influenced by smoking.
- 6) Concomitant drug treatment influences the disposition of risperidone. Significant changes in drug disposition can be observed in patients receiving risperidone and other concomitant drugs, which are strong inhibitors of CYP2D6.
- 7) The extrapyramidal side-effects might be related to the plasma levels of risperidone.
- 8) The QTc lengthening effect of thioridazine is dependent on the dose, the plasma concentration of the drug and the CYP2D6 activity.
- 9) Pharmacokinetic measurements may help clinicians to explore the possible involvement of pharmacokinetic factors in clinically important unexpected adverse events. This has been probed in a clinical case report in a patient treated with clozapine.

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