DOCTORAL THESIS

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

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ABSTRACT

It is frequently observed in clinical practice that there is a certain variability in clinical efficacy and in the occurrence of side-effects in patients treated with the same doses of a drug. This interindividual variability in the response to antipsychotic drugs can be partly attributed to differences in plasma concentration of the drug and/or its metabolites. One of the major factors influencing drug elimination and, thus, plasma concentration is the activity of the cytochrome P450 (CYP) enzymes. Previously several authors (including us) have shown that the genetic polymorphism of the different CYP enzymes is important in the determination of the plasma concentration of antipsychotic drugs. Furthermore environmental factors (such as concomitant treatment, smoking, diet etc.) also may modify the activity of these enzymes. One of these cytochrome P450 enzymes, CYP2D6 plays a major role in the elimination of several antipsychotic drugs (e.g. haloperidol, thioridazine, risperidone). Several studies have shown in healthy volunteers and in patients that the individuals with impaired enzyme activity (poor metabolizers, 5–10% of Caucasian population), with normal activity (extensive metabolizers), and the ultrarapid metabolizers with excessive activity of CYP2D6 have different plasma concentrations of these drugs and its metabolites after giving the same doses.

In the studies presented in this thesis the hypothesis evaluated is that poor metabolizers - due to genetic or environmental factors (dose-dependent inhibition, drug interaction with concomitant medication) - have higher plasma concentrations of the parent drug than extensive metabolizers, and also examined whether environmental factors, such as smoking or drug inhibition, can effect the disposition of drugs. The clinical implication of this fact is related to the relevance of drug or metabolite concentrations in therapeutic activity and/or side-effects. The relevance of CYP enzyme phenotyping and genotyping to the prediction of side-effects is also evaluated. Finally, it is also hypothesized that it may be possible to use therapeutical drug monitoring for predicting the activity of drug metabolising enzymes, by using different drug–metabolite ratios.

Three antipsychotic drugs used extensively world-wide, thioridazine, haloperidol, and risperidone were studied. These drugs were selected partly on the basis of their frequent use and also because previous studies had suggested important implications of pharmacogenetic factors in their metabolism. In the present studies the following parameters were determined:
a) plasma concentrations of drugs and metabolites, b) the CYP2D6 hydroxylation phenotypes by using debrisoquine c) in selected cases the CYP2D6 genotypes, and 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 obtained results indicate that CYP2D6 is an important factor in the metabolism of the antipsychotic drug haloperidol, thioridazine and risperidone. The drugs are not only metabolised by the enzyme but they also exert an inhibition on the activity of CYP2D6 in treated patients, and in this respect thioridazine (87.5% with impaired activity compared to the 5-10% in the normal population) was more potent than haloperidol (15% of PM at clinically used doses), while risperidone had a negligible effect at therapeutical doses. Thioridazine (p<0.001) and haloperidol (p<0.001) seems to exert a dose-dependent inhibition on CYP2D6 enzyme activity. The CYP2D6 activity affects the plasma concentrations of thioridazine (p<0.001), haloperidol (p<0.05) and risperidone (p<0.001), therefore, may influence side-effects, as it was observed with thioridazine for the risk of ventricular arrhythmias (over 150 mg daily dose of thioridazine patients have more risk). The metabolism of these drugs is also influenced by smoking (in case of thioridazine and haloperidol) and concomitant medications (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 (linear correlation coefficient; r=0.6 for thioridazine, and r=0.74 for risperidone, p<0.001) in psychiatric patients. In the view of the present data, assessment of the CYP2D6 status might be a useful aid for clinical psychiatrists to predict interindividual variability in the plasma concentration of antipsychotic drugs and to tailor therapeutic regimens to the individual patient.

Nevertheless, at present in clinical practice if unexpected side-effects occur or therapeutic failure is observed with antipsychotic drugs, the possible involvement of pharmacogenetic factors should also be considered and the adequate examinations be carried out.
“Es imposible ser feliz si uno actúa en contra de sus convicciones.

El que sepa como se llega a ser un hombre feliz, intentara hacerlo.

Por ello, quien sabe lo que esta bien, también hará el bien,
pues ninguna persona querrá ser infeliz.”

Sócrates

“Lehetetlen úgy boldognak lenni, hogy valaki saját meggyőződése ellen tegyen.

Aki tudja, hogyan tud boldog lenni, megpróbál az lenni,
ezért, aki tudja mi a jó, jót is fog tenni,
hiszen senki sem akar boldogtalan lenni”

Szókratesz
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ABBREVIATIONS

AUC: area under the curve
BPRS: Brief Psychiatric Rating Scale
C/D: dose corrected plasma concentration
CYP: cytochrome P450 enzyme
DSM: Diagnostic and Statistical Manual for Mental Disorders
EM: extensive metabolizer
fMRI: functional magnetic resonance imaging
HAL: haloperidol
HPLC: high-performance liquid chromatography
ICD: International Classification of Diseases
IS: internal standard
MR: metabolic ratio
NADPH: nicotinamid adenine dinucleotidephosphate
PCR: polymerase chain reaction
PET: positron emission tomography
PM: poor metabolizer
QTc: corrected QT interval
RFLP: restriction fragment length polymorphism
RHAL: reduced haloperidol
TDM: therapeutical drug monitoring
TD: tardive dyskinesia
UKU Scale: Utvalg for Kliniske Undersogelser Side Effect Scale
UM: ultrarapid metabolizers
9-OH-risperidone: 9-hydroxy-risperidone
%CV: Coefficient of variation
I. INTRODUCTION
1. INTERINDIVIDUAL DIFFERENCES IN DRUG METABOLISM

1.1. General considerations

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 goal of the pharmacological therapy is to achieve an effective drug concentration at the site of the action. Following administration of the drug, the substance and the human organism enter into an interaction. The drug evokes, modifies or inhibits the aimed physiological processes (and usually also some other, undesirable ones) at receptor level (effects of drug on the organism). The study of these processes belongs to the discipline of pharmacodynamics. On the other hand, pharmacokinetics deals with the effects of the human body on the administered compound (Rowland and Tozer, 1995). To achieve and to maintain the optimal drug concentration at the receptor level for a sufficient period of time, it is essential to know the dynamic balance between the drug input (dose regimen) and output (drug disposition).

At present, our knowledge of the role of pharmacodynamic factors in the interindividual variability of drug response is still fairly limited. This is largely due to methodological and ethical problems, since it is difficult to study pharmacodynamic processes in vivo at receptor level. However, sophisticated brain imaging methods developed recently, such as functional magnetic resonance imaging (fMRI), or positron emission tomography (PET), and their use in dynamic or activation studies has opened a completely new field in the investigation of drug effects at receptor level in humans.

The role of pharmacokinetic factors in drug treatment has been a field of thorough investigation for the last decades. Differences in plasma levels 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 (Vesell, 1977). Drugs which are mainly excreted unchanged do not exhibit such a pronounced variability in their disposition kinetics; therefore, metabolism, which is determined by environmental or genetical factors, 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. This transformation yields more polar metabolites, which are more easily excreted in the urine or bile.

Interindividual differences in drug metabolism are mainly determined by genetical factors (Meyer, 1990; Kalow, 1990). As early as 1959 a new discipline, **pharmacogenetics** emerged, which deals with the study of genetically determined variations in drug metabolism and response. The term pharmacogenetics was coined by Vogel et al. (1959) and the first book about this field entitled: „Pharmacogenetics: heredity and the response to drugs” was edited by Werner Kalow (1962). Since that time our knowledge of the importance of genetical factors in drug metabolism has grown remarkably.

Recently the term of **pharmacogenomics** has been introduced, and it is defined as the utilization of genetic information to predict the outcome of drug treatment (therapeutic and side-effects) (Pickar and Rubinow, 2001). The pharmacogenomic strategy aims to identify genes that influence clinical response to drug treatment (Catalano, 1999). Although this strategy traditionally has focused on genes that influence drug disposition, the broader appeal of pharmacogenomics is the possibility of predicting drug response (efficacy) or limiting side-effect profiles. The ability to predict drug response would allow individualized pharmacotherapy that could maximize the chance of optimal drug choice for each patient, and, consequently, could offer savings in both time and cost of care, and substantially improve the patients’ long-term prognosis.

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) (Vesell, 1984; Pelkonen and Sotaniemi, 1987; LLerena et al., 1996).

**1.2. Biotransformation of drugs in the human body – the role of cytochrome P450 enzymes**

Drug metabolizing enzymes are present abundantly in the human body. Although drug metabolism occurs most prominently in the liver, it also takes place in many other tissues including lungs, skin, intestines, and kidney. Lipophilic substances, and most drugs belong to
this class, are usually metabolized in the liver. The first step of this process - which can be oxidation, reduction, dealkylation, desulfation or deamination – is usually referred to as a Phase I reaction. These reactions are catalyzed mainly by the cytochrome P450 enzyme family, which is the most important group of enzymes in drug disposition. The members of the cytochrome P450 enzyme family have the following common features: a) they contain a heme group b) they are situated in the endoplasmatic reticulum of the liver c) they utilize NADPH and oxygen.

The Phase II reactions are catalyzed by transferases, which transfer an active moiety, such as glucuronic acid, sulphate and gluthation, to the compound, usually after they have been metabolized in Phase I reactions. These reactions serve to transform the hydrophobic compound into a form that is more water-soluble and can be easily eliminated through the urine or bile.

Every 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 (Nelson et al., 1996). 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 1st, 2nd and 3rd CYP enzyme family.

The catalytic activity of a cytochrome enzyme 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. Then, the concentrations of the drug and its metabolite are determined in the collected urine, and the metabolic ratio (MR) is calculated:

\[
MR = \frac{\text{percentage of drug recovered as parent drug}}{\text{percentage of drug recovered as metabolite}}
\]
The metabolic ratio reflects the actual activity of the specific enzyme. Jackson et al. (1986) demonstrated by computer simulation that this index is adequate to evaluate the drug metabolizing capacity of a CYP enzyme.

The genetical variability of drug metabolizing enzymes can exist as polymorphism or as a rare trait. By definition, polymorphism is a Mendelian or monogenic trait that exists in the population in at least two phenotypes (and presumably at least two genotypes), neither of which occurs with a frequency less than 1%. If the frequency is lower than this, it is called a rare trait (Bertilsson, 1995). The allelic variant that is responsible for the genetic determinant of the isoenzyme can be determined by mutation-specific PCR and/or mapping restriction endonucleases (Gonzalez and Idle, 1994). The polymorphic or mutant alleles which are different from the most common allele (i.e. wild type = wt) are designated by an asterix followed by an Arabic designation number. The wt allele is always the *1 allele, for example CYP2D6*1 is the wild-type enzyme.

The activity of CYP2D6 enzyme, studied in this Thesis, 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. This point can be calculated from bimodal distribution and it is called the antimode (Evans et al., 1980; Evans et al., 1983). This value makes it possible to define separate phenotypes. 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 of the CYP2D6 enzymes (Gonzalez and Idle, 1994; Meyer and Zanger, 1997).

2. GENETIC POLYMORPHISM OF THE MAIN CYTOCHROME P450 DRUG METABOLIZING ENZYMES

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).

2.1. CYP2D6

The debrisoquine hydroxylase polymorphism (first name for this enzyme) is by far the most thoroughly studied genetic polymorphism of the drug-metabolizing enzymes. The polymorphism of this enzyme was discovered independently by two different groups in the late 1970s. Mahgoub et al. (1977) studied the metabolism of debrisoquine, a post-ganglionic adrenergic blocking antihypertensive drug, while Eichelbaum et al. (1979) investigated that of sparteine, an alkaloid, antiarrhythmic and oxytocic drug. Both groups found that polymorphism existed in the metabolism of these drugs, which was in the 4-hydroxylation enzymatic step for debrisoquine and in the N1-oxidation for sparteine metabolism. The autosomal recessive inheritance of PM phenotype was established by family studies (Evans et al., 1980). Later it was proved that these drug metabolic polymorphisms were both based on interindividual variability of the debrisoquine hydroxylase enzyme, CYP2D6 (Eichelbaum et al., 1982).

The distribution of debrisoquine urinary metabolic ratio (debrisoquine/4-OH debrisoquine) in Caucasian populations is bimodal. The antimode has been calculated to be 1.1 (log_{10} debrisoquine MR) (Evans et al., 1980; Evans et al., 1983). Around seven percent of the Caucasians have higher than 1.1 log_{10} debrisoquine MR values, i.e. the amount of the excreted metabolite is extremely low or negligible, they are classified as PMs. Different drugs, such as debrisoquine, sparteine, dextromethorphan, metoprolol, have been used to determine the activity of CYP2D6 in healthy volunteers studies (Alván et al., 1990; LLerena et al., 1996).

The frequency of debrisoquine hydroxylation PM phenotype has been studied in different populations. In European, American and Australian Caucasian populations the frequency of PM phenotype is between 5-10%. In Caucasian populations the frequency of PMs of debrisoquine type and also the distribution of the enzyme activity seem to be homogenous. On the basis of debrisoquine urinary MR determinations among 8,764 healthy volunteers, Alván et al. (1990) calculated that the overall frequency of PM phenotype in the European Caucasian populations is 7.4%. In the Spanish population, among 925 healthy volunteers the rate of PMs was 4.9% (LLerena et al., 1993c). Data from Hungarian population is consistent
with the European figures. In an adult (Gachályi et al., 1986) and in an infant population (Szórády and Sánta, 1987) the frequency of PM phenotype was 10% and 8%, respectively. Interethnic differences in the activity of CYP2D6 enzyme in various populations are shown in Table 1.

<table>
<thead>
<tr>
<th>Country</th>
<th>Population</th>
<th>Number of individuals</th>
<th>Test drug</th>
<th>PMs (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUROPE</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Denmark</td>
<td>Caucasian</td>
<td>301</td>
<td>sparteine</td>
<td>7.3</td>
<td>Brøsen et al. 1985</td>
</tr>
<tr>
<td>Finland</td>
<td>Caucasian</td>
<td>211</td>
<td>debrisoquine</td>
<td>5.2</td>
<td>Arvela et al. 1988</td>
</tr>
<tr>
<td>Hungary</td>
<td>Caucasian</td>
<td>100</td>
<td>debrisoquine</td>
<td>10</td>
<td>Gachályi et al. 1986</td>
</tr>
<tr>
<td>Spain</td>
<td>Caucasian</td>
<td>924</td>
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<td>4.9</td>
<td>LLerena et al. 1993c</td>
</tr>
<tr>
<td>Sweden</td>
<td>Caucasian</td>
<td>1011</td>
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<td>6.9</td>
<td>Bertilsson et al. 1992</td>
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<td>USA</td>
<td>Afro-American</td>
<td>106</td>
<td>dextrometorphan</td>
<td>1.9</td>
<td>Relling et al. 1991</td>
</tr>
<tr>
<td>Panama</td>
<td>Indian</td>
<td>51</td>
<td>sparteine</td>
<td>0</td>
<td>Arias et al. 1988</td>
</tr>
<tr>
<td>AUSTRALIA</td>
<td>Caucasian</td>
<td>100</td>
<td>debrisoquine</td>
<td>6</td>
<td>Peart et al. 1986</td>
</tr>
</tbody>
</table>

Table 1. Frequency of poor metabolizers (PM) of CYP2D6 in different population studies among healthy volunteers

2.1.1. CYP2D6 genotype

The bimodal distribution of the debrisoquine hydroxylation phenotypes (CYP2D6 activity) suggests a possible monogenic inheritance of the trait. Theoretically, the distribution of phenotypes in a monogenic recessive trait should be trimodal (as homozygote recessive, heterozygote and homozygote dominant) with three peaks. The reason for the bimodality in this case is that the heterozygotes and recessive homozygotes can not be functionally separated by phenotyping methods, i.e. the distribution curves are overlapping.

Eichelbaum et al. (1987) in a linkage study mapped the gene for debrisoquine hydroxylase on the long arm of the 22 chromosome in linkage with the blood factor P1. Independently,
another group (Gonzalez et al., 1988a) using CYP2D6 cDNA probe and somatic cell hybrids of mouse–human and hamster–human cells also mapped the CYP2D6 locus to the long arm of the 22 chromosome. In the liver of PM individuals some mRNA variants of CYP2D6 with different length were found (Gonzalez et al., 1988b). After cloning and sequencing of the CYP2D6 gene (Kimura et al., 1989) it became possible to search for mutations in PM individuals, and to develop allele specific polymerase chain reaction (PCR) tests (Heim and Meyer, 1990).

CYP2D6 gene is located in a structure of three tandemly arranged genes (CYP2D7 and CYP2D8P), each containing 9 exons. There is no evidence that either CYP2D7 or CYP2D8P is expressed in human liver, furthermore CYP2D8P can be considered a pseudogene with many gene-disrupting mutations (Gonzalez and Meyer, 1991).

In Caucasian populations the most frequent mutation are: CYP2D6*4 (21% of the Caucasians), which leads to a substitution, and, consequently, to defect splicing (Kagimoto et al., 1990), CYP2D6*5, which leads to complete gene deletion (Gaedigk et al., 1991), and CYP2D6*3, which contains a frame-shift mutation due to a deletion (Kagimoto et al., 1990).

The differences of CYP2D6 activity observed in the Asian population could be attributed also to genetical factors. In a Chinese population Johansson et al. (1994) described the existence of two mutations, the CYP2D6*9 and CYP2D6*8, which have high allele frequency (51% and 37%, respectively) and lead to the instability of the enzyme. This, in turn, causes a diminished catalytic activity and thus provides a firm explanation for the higher MRs observed in the Chinese population.

Bertilsson et al. (1993) investigated the genetical basis of ultrarapid metabolism in healthy individuals. In ultrarapid metabolizers (UM) there is an extra CYP2D6 gene, i.e. two ore more active CYP2D6 genes are present and expressed, and this leads to a higher metabolic activity. The duplicated allele is not a rare mutation, the overall frequency of the duplicated/amplified CYP2D6-allele is about 1% in the Swedish (Dahl et al., 1995) and 3%-10% in the Spanish population (Agúndez et al., 1995; Bernal et al., 1999). Also, alleles carrying two or three extra CYP2D6*1 or *2 genes yielding in a total of three or four active CYP2D6 genes have been described. Dahl et al. (1995) reported that three persons, who had the lowest MR in a Swedish population (consisting of more than 1.000 subjects), were found
to possess 12 extra active genes. The frequency of different CYP2D6 alleles is shown in Table 2.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency (%)</th>
<th>Nucleotide change</th>
<th>Enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1</td>
<td>32-36</td>
<td>wild type</td>
<td>normal</td>
</tr>
<tr>
<td>*1xn</td>
<td>0.5</td>
<td>multiplication</td>
<td>increased</td>
</tr>
<tr>
<td>*2</td>
<td>25-32</td>
<td>substitution</td>
<td>decreased/normal</td>
</tr>
<tr>
<td>*2xn</td>
<td>1.4</td>
<td>multiplication</td>
<td>increased</td>
</tr>
<tr>
<td>*3</td>
<td>2.0</td>
<td>deletion + frameshift</td>
<td>absent</td>
</tr>
<tr>
<td>*4</td>
<td>16-20</td>
<td>substitution splicing</td>
<td>absent</td>
</tr>
<tr>
<td>*4xn</td>
<td>≤0.1</td>
<td>multiplication</td>
<td>absent</td>
</tr>
<tr>
<td>*5</td>
<td>2-7</td>
<td>entire gene deleted</td>
<td>absent</td>
</tr>
<tr>
<td>*6</td>
<td>0.9-1.1</td>
<td>deletion + frameshift</td>
<td>absent</td>
</tr>
<tr>
<td>*7</td>
<td>≤0.1</td>
<td>substitution</td>
<td>absent</td>
</tr>
<tr>
<td>*8</td>
<td>≤0.1</td>
<td>substitution</td>
<td>absent</td>
</tr>
<tr>
<td>*9</td>
<td>1.8-2.7</td>
<td>deletion</td>
<td>decreased</td>
</tr>
<tr>
<td>*10</td>
<td>1.5</td>
<td>substitution</td>
<td>decreased</td>
</tr>
<tr>
<td>*11</td>
<td>≤0.1</td>
<td>substitution splicing</td>
<td>absent</td>
</tr>
<tr>
<td>*12</td>
<td>≤0.1</td>
<td>substitution</td>
<td>absent</td>
</tr>
<tr>
<td>*13</td>
<td>≤0.1</td>
<td>deletion + insertion</td>
<td>absent</td>
</tr>
<tr>
<td>*14</td>
<td>≤0.1</td>
<td>substitution</td>
<td>absent</td>
</tr>
<tr>
<td>*15</td>
<td>≤0.1</td>
<td>insertion</td>
<td>absent</td>
</tr>
<tr>
<td>*16</td>
<td>≤0.1</td>
<td>deletion + insertion</td>
<td>absent</td>
</tr>
<tr>
<td>*17</td>
<td>≤0.1</td>
<td>substitution</td>
<td>decreased</td>
</tr>
</tbody>
</table>

Table 2. The frequency of CYP2D6 alleles in Caucasian populations (after Sachse et al., 1997 and Marez et al., 1997, with modifications)

The presently available methods, using PCR and RFLP together, allow us to achieve a 98% specificity in rendering the PM phenotype to the genotype (Chen et al., 1996; Marez et al., 1997). It is reasonable to believe that in the near future with the incorporation of rare mutations it will be possible to find out the phenotype of any subject.

2.1.2. Clinical implications of CYP2D6 polymorphism

The CYP2D6 enzyme polymorphism has clinically relevant consequences. At average drug doses poor metabolizers are prone to side-effects due to unexpectedly high plasma levels, while lack of therapeutic effect may occur in ultrarapid metabolizers as a consequence of very low plasma concentrations.
The present Thesis is focused on the involvement of CYP2D6 enzyme in the metabolism of important antipsychotic drugs frequently used in the everyday practice, but this enzyme is also implicated in the metabolism of other important drugs (Table 3).

<table>
<thead>
<tr>
<th>Drugs</th>
<th>CYP2D6</th>
<th>Drugs</th>
<th>CYP2D6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antipsychotics</strong></td>
<td>chlorpromazine</td>
<td><strong>Cardiovascular</strong></td>
<td>bisoprolol</td>
</tr>
<tr>
<td></td>
<td>haloperidol</td>
<td></td>
<td>flecainide</td>
</tr>
<tr>
<td></td>
<td>thioridazine</td>
<td></td>
<td>metoprolol</td>
</tr>
<tr>
<td></td>
<td>risperidone</td>
<td></td>
<td>mexiletine</td>
</tr>
<tr>
<td></td>
<td>zuclopenthixol</td>
<td></td>
<td>propafenon</td>
</tr>
<tr>
<td></td>
<td>haloperidol</td>
<td></td>
<td>metoprolol</td>
</tr>
<tr>
<td></td>
<td>thioridazine</td>
<td></td>
<td>mexiletine</td>
</tr>
<tr>
<td></td>
<td>risperidone</td>
<td></td>
<td>propafenon</td>
</tr>
<tr>
<td></td>
<td>zuclopenthixol</td>
<td></td>
<td>metoprolol</td>
</tr>
<tr>
<td></td>
<td>haloperidol</td>
<td></td>
<td>mexiletine</td>
</tr>
<tr>
<td></td>
<td>thioridazine</td>
<td></td>
<td>propafenon</td>
</tr>
<tr>
<td></td>
<td>risperidone</td>
<td></td>
<td>metoprolol</td>
</tr>
<tr>
<td></td>
<td>zuclopenthixol</td>
<td></td>
<td>mexiletine</td>
</tr>
<tr>
<td></td>
<td>haloperidol</td>
<td></td>
<td>propafenon</td>
</tr>
<tr>
<td></td>
<td>thioridazine</td>
<td></td>
<td>metoprolol</td>
</tr>
<tr>
<td></td>
<td>risperidone</td>
<td></td>
<td>mexiletine</td>
</tr>
<tr>
<td></td>
<td>zuclopenthixol</td>
<td></td>
<td>propafenon</td>
</tr>
<tr>
<td><strong>Antidepressants</strong></td>
<td>amitriptyline</td>
<td><strong>Others</strong></td>
<td>codeine</td>
</tr>
<tr>
<td></td>
<td>clomipramine</td>
<td></td>
<td>dextrometorphan</td>
</tr>
<tr>
<td></td>
<td>imipramine</td>
<td></td>
<td>metamphetamine</td>
</tr>
<tr>
<td></td>
<td>mianserin</td>
<td></td>
<td>methadone</td>
</tr>
<tr>
<td></td>
<td>fluoxetine</td>
<td></td>
<td>tramadol</td>
</tr>
<tr>
<td></td>
<td>paroxetine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>venlafaxine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. CYP2D6 enzyme substrates (Michalets, 1998 with modifications)

This fact is important since potentially dangerous pharmacological interaction may occur, when two or more of CYP2D6 substrates are administrated together.

2.2. CYP1A2

CYP1A2 is one of the major CYP enzymes, accounting for 15% of the total P450 content in the human liver (Shimada et al., 1994). There is a wide interindividual variability of CYP1A2 activity among human subjects (Kalow and Tang, 1991a and 1991b). The variation may be due to the enzyme induction and inhibition by other drugs or by environmental exposures. Tobacco smoking, coffee and alcohol consumption have a substantial contribution to the variability. To measure the metabolic activity of CYP1A2, caffeine has been suggested to be used as phenotyping probe in vivo (Fuhr et al., 1996). Although the metabolism of caffeine is complex, the major metabolic steps to metabolites, such as 1,7-dimethylxanthine, 1-methylxanythine, are catalyzed by CYP1A2 and the measurement of urinary metabolites after the ingestion of caffeine can be used to calculate metabolic ratios. However, in psychiatric patients phenotyping may be cumbersome because the incidence of heavy smoking and coffee consumption is particularly high (Kellermann et al., 2000), and these interfere with the procedure.
There is little information on interethnic differences in the enzyme activity (Butler et al., 1992; Bartoli et al., 1996). No genetic polymorphism resulting in an altered protein sequence for CYP1A2 had been described until recently when a rare mutation (CYP1A2*2) was reported in a Chinese sample (Huang et al., 1999). The enzyme is well known for its role in the metabolic activation of environmental and food-borne carcinogens, including arylamines and heterocyclic amines (Eaton et al., 1995).

CYP1A2 is involved in the metabolism of a large number of drugs, including psychotropic drugs, like imipramine, mianserin, nortriptyline, clozapine, olanzapine and tacrine (Koyama et al., 1996; Koyama et al., 1997; Olesen and Linnet, 1997; Bertilsson et al., 1994; Ring et al., 1996; Benoit et al., 1997).

### 2.3. CYP2C19

CYP2C19 is the source of S-mephenytoin oxidation polymorphism that is bimodally distributed in the population (De Morais et al., 1994a). In EM individuals the S-enantiomer of mephenytoin is rapidly hydroxylated to its metabolite, while the R-enantiomer is slowly N-demethylated. In PM individuals, the rate of S-mephenytoin hydroxylation is lower due to the altered enzyme activity, and, therefore, the amount of S-mephenytoin is higher, while the rate of N-demethylation is the same in the two phenotypes. The mephenytoin S/R enantiomer ratio determined from the urine collected during the 8-hour period following the ingestion of the racemate is used for phenotyping. In EM persons the S/R ratio is always below 0.9, while in PMs it is around 1.0. Omeprazole is reported to have certain advantages over mephenytoin as a probe drug for CYP2C19, because of the lower incidence of side-effects (Chang et al., 1995). CYP2C19 is not inducible by xenobiotics.

The prevalence of PMs of S-mephenytoin was 1.34% in 373 unrelated, healthy Spanish Caucasian individuals (Reviriego et al., 1993). Combined data of the 22 homogeneous studies show that the frequency of poor metabolizers in healthy, unrelated Caucasians determined by phenotyping is 2.8%, but in Orientals (Japanese, Chinese, Koreans) the incidence is about 14–20% (Nakamura et al., 1985; Xie et al., 1999; Xie, 2000). Among 103 black Zimbabweans 4% were poor metabolizers (Masimirembwa et al., 1995).
The impaired activity of the CYP2C19 enzyme is inherited as an autosomal recessive trait and it is independent of CYP2D6 enzyme activity (Inaba et al., 1986a; Ward et al. 1987), as it was demonstrated in a Spanish family phenotyped with mephenytoin (LLerena et al., 1993a). The two most common defects are two null alleles: the first is a mutation in exon 5 (CYP2C19*2), and the second is a single base transition in exon 4 (CYP2C19*3). These two defects account for >99% of the defective alleles in the Oriental populations but only for ~87% of the defective alleles in Caucasians (De Morais et al., 1994a; De Morais et al., 1994b; Broersen et al., 1995). Three different point mutations have also been reported that result in single amino acid substitutions: CYP2C19*4 which accounts for an additional 3% of the defective alleles in Caucasian, and the rare mutations (~1.5% of Caucasian PM alleles) CYP2C19*5 and CYP2C19*6 (Ferguson et al., 1998; Ibeanu et al., 1998a; Ibeanu et al., 1998b). Recently two additional alleles of the poor metabolizer phenotype for S-mephenytoin 4-hydroxylation in Caucasians (CYP2C19*7, *8) have been described (Ibeanu et al., 1999).

The CYP2C19 polymorphism is of clinical importance because CYP2C19 catalyses the metabolism of several pharmacologically important tricyclic drugs, including amitriptyline, clomipramine, imipramine and also diazepam, citalopram and moclobemide (Bertilsson et al., 1995). On the other hand, no inhibitory effect of antidepressants or neuroleptics on the activity of CYP2C19 enzyme has been demonstrated in psychiatric patients (LLerena et al., 1993b).

2.4. CYP3A4

The CYP3A gene subfamily is the most abundant cytochrome enzyme in the liver accounting for up to 30% of the total cytochrome P450 content of the adult human liver (Shimada et al., 1994). The two most important enzymes are CYP3A3 and CYP3A4, which have a 98% amino acid similarity and oxidize the same drugs. It is currently an unsettled question whether they are encoded by deferent genes, or represent the allelic variants of the same genetic locus. There is a marked interindividual heterogeneity in the expression of CYP3A genes. Phenotyping of this enzyme is difficult because the CYP3A enzyme is abundantly present in the small intestine, and thus any oral test designed for the evaluation of liver metabolism will be influenced by the activity of the CYP3A enzyme present in the intestines. The most widely accepted and tested CYP3A probes are erythromycin and midazolam. However, none of the current phenotyping procedures are ideal (Streetman et al., 2000).
The initial studies of in vivo nifedipine oxidation (marker drug of CYP3A4 activity) showed an apparent polymorphism in a set of 53 healthy individuals (Kleinbloesem et al., 1984), but subsequent pharmacokinetic studies with a larger group did not confirm this result (Schellens et al., 1988). An intriguing observation of dramatic ethnic differences in nifedipine oxidation was made in a study comparing South Asians and Caucasians (Ahsan et al., 1991) and also both in vitro and in vivo differences between Caucasians and Japanese were found with regard to CYP3A4 activity (Shimada et al., 1994). To date, there are no reports in humans of a definitive evidence for a CYP3A4 genetic polymorphism related to the catalytic activity (Horsmans et al., 1992). Recently, an in vitro study has revealed polymorphic alleles (CYP3A4*2, *3) with altered catalytic activity (Sata et al., 2000).

As with CYP1A2, the CYP3A4 enzyme is highly inducible by a number of drugs, and diet; therefore, it difficult to find out the exact role that genetic factors may have in its activity. CYP3A4 plays a significant role in the metabolism of approximately half of the drugs in use today (Guengerich, 1999). Several psychotropic drugs, including carbamazepine, midazolam, triazolam, diazepam, clomipramine, imipramine, are also metabolized by the CYP3A4 enzyme (Michalets, 1998).

3. ANTIPSYCHOTIC DRUGS

3.1. General considerations

In 1891 Paul Ehrlich observed the antimalarial effects of methylene blue, a phenothiazine derivative, but during the next six decades its „neuroleptic” effect of this chemical class had not been discovered. The first antipsychotic drug, chlorpromazine, was synthesized by Charpinter at the pharmaceutical company Rhône-Poulenc in 1950. One year later Laborit and Huguenard administered the aliphatic phenothiazine, chlorpromazine, to patients to try to use its potential anaesthetic effect in surgery. Shortly thereafter, Hamon and Delay extended the use of the drug by giving it to psychiatric patients and serendipitously discovered its antipsychotic activity (Shen, 1999). Between 1954 and 1975 about 40 antipsychotic drugs were introduced. The dramatic impact that these drugs had on the treatment of hitherto
intractable psychotic disorders has revolutionized the psychiatric therapy. Since the introduction and widespread use of antipsychotics, hospitalization periods and relapse rates have declined spectacularly.

However, clinicians are still faced with many difficulties in antipsychotic drug treatment regarding incomplete efficacy, especially in negative (or deficit) syndromes, and unpleasant side-effects, such as undue sedation, autonomic symptoms, weight gain, and movement disorders. The motor side-effects of the antipsychotic drugs are frequent, and they include drug-induced parkinsonism, akathisia, dystonias, and tardive dyskinesia in chronically treated patients.

A new therapeutic class of antipsychotic drugs emerged with the development of clozapine. This drug was the first of the so-called atypical antipsychotic drugs, named so because they induced fewer movement disorders (Livingstone, 1994). Clozapine has a unique side-effect profile and it is more efficient against the negative syndromes than the classical neuroleptics. During the last ten years, several new atypical antipsychotic drugs have been developed, and the first of them was risperidone. The main indication of antipsychotic drug treatment is schizophrenia, although several other psychotic disorders have been also indicated (e.g. paranoia, psychotic depression, folie a deux, etc.) (DSM-IV, 1994; ICD-10, 1992). The pharmacodynamic basis of the action of antipsychotic drugs is still poorly understood. To our present knowledge, the main site of action is at receptor level. The classical antipsychotic drugs shared a common effect: the inhibition of the central nervous system dopamine receptors, and this inhibition was considered to be essential to decrease the “positive” symptoms (formal and content thought disorder, hallucinations, agitation). However, the classical antipsychotic drugs do not diminish the “negative” symptoms (blunted affect, affective flattening, poverty of speech, social isolation). The new atypical antipsychotic drugs (clozapine, risperidone, olanzapine) also decrease the negative symptoms, and their effect has been possibly related to their action on serotonin receptors.

In the antipsychotic drug treatment we still face with severe problems:

a) the aetiology of psychotic disorders is largely unknown (diagnostic heterogeneity)
b) delayed onset of action (usually 2-3 weeks)
c) no definitive outcome measures (surrogate markers)
d) side-effects may mimic the symptoms of diseases (e.g. akathisia – anxiety, catatonia – extrapyramidal side-effects).

A better understanding of the pharmacodynamic and pharmacokinetic aspects of antipsychotic drug treatment may help in overcoming some of these problems. It is particularly important to be aware that intrinsic pharmacodynamic variability can also be very pronounced (Levy et al., 1994) and that major advances in antipsychotic drug therapy may come from this *terra incognita* in the near future.

### 3.2. Pharmacokinetics of antipsychotic drugs

The antipsychotic drugs are highly lipophilic and pass through lipid membranes. The orally ingested drugs are absorbed well and eliminated to a great extent pre-systematically by first-pass mechanism in the liver. The bioavailability of these drugs is between 10-70%. On the other hand, the intramuscular formulations are well absorbed and their bioavailability is substantially higher.

Owing to their liposolubility, antipsychotic drugs are highly distributed in the human body and a high proportion of them is bound to plasma proteins (75–99%). Their main route of elimination is hepatic metabolism, and only a small proportion of them is excreted in the urine in unchanged form. Several metabolic pathways can be involved in their disposition, although there are some common pathways in the metabolism of the different antipsychotics. During biotransformation several metabolites are formed, and in some cases the metabolites are also active pharmacologically (Dahl, 1982).

There is a great interindividual variability in the steady-state plasma levels of antipsychotic drugs after the same dose regimen (Dahl, 1986). This variability can partly be attributed to genetical factors, but other factors (i.e. age, tobacco smoking) may also influence different pharmacokinetic parameters (Balant-Georgia et al., 1993). Antipsychotic drugs are frequently used among elderly people. Elderly people may have higher plasma levels of drugs, as it has been shown after a single dose of thioridazine compared to young patients (Cohen and Sommer, 1988). CYP2D6 enzyme activity (one of the major enzymes involved in the
metabolism of thioridazine) is not influenced by age (Szórády and Sánta, 1987; LLerena et al., 1996).

The inducer effect of tobacco smoking on hepatic cytochrome enzymes has been increasingly recognized (Goff et al., 1992). Psychiatric patients in general and schizophrenic patients in particular tend to be heavy smokers. The incidence of smokers (50–92%) is much higher than in the general population (25–33%) (Goff et al., 1992; Kellermann et al., 2000). The high incidence of smoking has been related to several consequences, e.g. decrease in the plasma levels of drugs, the decrease of extrapyramidal symptoms, and the partial improvement of neurocognitive deficit may reinforce its utilization (Shoaf and Linnoila, 1991; Goff et al., 1992). Tobacco, or more specifically, its polycyclic carbohydrate ingredients are potent inducers of hepatic microsomal enzymes (e.g. CYP1A2); therefore, the plasma concentration of the antipsychotic drugs metabolized by this enzyme may decrease. This effect may partly explain clinical observations that smokers may require higher doses of psychotropic drugs than non-smokers (Vinarova et al., 1984; Decina et al., 1990; Goff et al., 1992). A double dose of chlorpromazine was needed in smokers to achieve a therapeutic effect similar to that in non-smokers. However, the incidence of somnolence was inferior in smokers (Vinarova et al., 1984). The steady state plasma levels of antipsychotic drugs may be lower in smokers than in non-smokers using the same dose regimen (Ereshefsky et al., 1985; Jann et al., 1986, Centorrino et al., 1994.).

Smoking seems to have a pharmacodynamic effect as well on the central cholinergic and dopaminergic receptors. It was reported that smokers have a lower risk of acquiring idiopathic Parkinson disease than non-smokers (Baron, 1996). Similarly, the parkinsonian extrapyramidal side-effects of antipsychotics are less frequent in smokers (Goff et al., 1992). Nevertheless, the risk of antipsychotic drug induced tardive dyskinesia is increased in smokers (Yassa et al, 1987; Binder et al., 1987; Goff et al., 1992). However, the risk of akathisia seems not to be affected by smoking status (Goff et al., 1992).
3.3. Relevance of cytochrome isoenzyme polymorphism to antipsychotic drug treatment

3.3.1. Therapeutical drug monitoring in psychiatry

Although the concentration of psychotropic drugs in the brain can not be directly measured, pharmacokinetic drug monitoring can provide information on the concentration of these drugs in the plasma.

Monitoring plasma concentration (Therapeutic Drug Monitoring, TDM) can be helpful in clinical practice for several reasons (Perry, 2000; Dahl and Sjöqvist, 2000):

a) adjusting the patient to a defined plasma concentration the knowledge of response threshold or therapeutic window increase the probability of response;
b) large interindividual variability between drug dose and plasma concentration of the drug makes it improbable that all patient can be treated with the same dose regimen;
c) excessively high drug concentrations in plasma may be toxic for the patient;
d) drug interactions may lead to a sudden loss of efficacy or to an abrupt onset of drug toxicity;
e) psychiatric patients often comply poorly with the drug regimen and plasma concentration monitoring also assures compliance;
f) helps to differentiate between side-effects and symptoms.

The classical TDM concept tries to correlate and monitor the correlation between plasma concentration and pharmacological effect (Point B and C, see Fig. 1). The correlation between dose and plasma concentrations (Point A and Point B) (and consequently between Point A and Point C) is the relationship where the interindividual variability will appear due to the factors influencing CYP enzyme activity (genetical and environmental), and also to the variability of other pharmacokinetic factors (absorption, distribution, etc.)
3.3.2. Cytochrome P450 enzymes and antipsychotic drug metabolism

Twin and family studies some 30 years ago showed that steady-state plasma concentrations of tricyclic antidepressants, such as nortriptyline and desmethyl-imipramine are under genetic control (Alexanderson et al., 1969; Åsberg et al., 1971).

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 came form 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 enzymes polymorphism in the metabolism of antipsychotic drugs should be obtained.
from studies in patients during steady-state conditions. However, at present few studies have been published. An updated review of the literature of the implication of CYP enzymes on antipsychotic drugs metabolism is given in Table 3.

<table>
<thead>
<tr>
<th>Drug</th>
<th>In vitro</th>
<th>Healthy volunteers</th>
<th>In patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP1A2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chlorpromazine</td>
<td>+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>haloperidol</td>
<td>+</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>clozapine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>olanzapine</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
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<tr>
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<td>NA</td>
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<tr>
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<td>risperidone</td>
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Table 4. Major human drug-metabolizing enzymes and their antipsychotic drug substrates (+ = positive results, - = negative results, NA = no information available) (Michalets, 1998, with modification).
3.3.3. Inhibition of cytochrome P450 enzyme activity by antipsychotic drugs

Several classical antipsychotic drugs inhibit the CYP2D6 enzyme activity in vitro (Von Bahr et al., 1985). In patients Syvälathy et al. (1986) reported for the first time a substantial inhibitory effect of thioridazine and levomepromazine treatment on the CYP2D6 enzyme activity. The debrisoquine MR was significantly higher in the patient group than in the controls, which reflects that the activity of the enzyme is decreased by the competitive inhibition of the concomitant drug therapy.

Our group confirmed in several studies the inhibition of the CYP2D6 enzyme in psychiatric patients receiving antipsychotic drug therapy (LLerena et al., 1987; Benítez et al., 1989, Spina et al., 1991; LLerena et al., 1993b). Chlorpromazine, levomepromazine, perphenazine and thioridazine are the strongest inhibitors, but in vitro virtually all the antipsychotic drugs have the potential to inhibit CYP2D6 (Shin et al., 1999). 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 (LLerena and Kiivet, 1994; Ito et al., 1998). There are also some precedents for the inhibition of CYP2D6 by drugs which are not metabolized by that enzyme. Quinidine and halofantrin compete for the substrate binding of CYP2D6 but are not metabolized by the enzyme (Otton et al., 1988, Halliday et al., 1995). Another example is the antipsychotic drug pimozide, which is metabolized by CYP3A4 and CYP1A2, but it does inhibit CYP2D6 activity (Desta et al., 1998). These inhibition are reversible and competitive inhibitions, the extent of inhibition is dependent on the affinity of the substrate to the enzyme. The CYP enzyme inhibition may lead to increased plasma concentration of coadministered drugs and thus to drug interaction.

3.3.4. Cytochrome P450 enzymes polymorphism in psychiatric patients
Of the typical antipsychotic drugs, haloperidol, trifluoperazine and fluphenazine have demonstrated relatively consistent and significant correlation between plasma concentration and therapeutic response (Ereshefsky, 1999). With the exception of clozapine, little data is available as yet about the atypical antipsychotics. Several studies have reported a minimum threshold response rate for clozapine at 310-420 µg/L, and that above this plasma concentration there is an increased probability that the patient will respond to the medication (Perry et al., 1991).

The activity of CYP enzymes influences the plasma concentration of antipsychotic drugs and their metabolites. Thus, the metabolic phenotype of the patients might be important in the disposition of different drugs and in the therapeutical response. In PM subjects normal doses of drugs might result in unexpectedly high plasma concentrations and thus dose-dependent side-effects, or even toxic symptoms may occur. On the other hand in ultrarapid metabolizers (UM) the disposition of drugs might be accelerated compared to normal subjects; therefore, following the administration of an average daily dose, the plasma concentrations of the drug might be lower and a therapeutic failure may occur.

### 3.3.5. Cytochrome P450 enzymes and antipsychotic drug interactions

Drug interactions are related to pharmacodynamic or pharmacokinetic factors. However, it is increasingly recognized that the competitive inhibition of the cytochrome enzymes may lead to drug interactions of clinical importance. These interactions may increase the plasma concentration of drugs that have low therapeutic indices, and cause serious adverse effects (Goff and Baldessarini, 1993; Michalets, 1998). Although in vitro studies may predict possible interaction, the observed plasma concentration changes and clinical symptoms do not necessarily coincide with the predicted effects. The discrepancy between the results obtained in in vitro and in vivo conditions may be attributed to several factors. Drugs may accumulate in the liver of the patient, e.g. the concentration of haloperidol was reported to be 900 times higher in the liver than in the plasma (Forsman et al., 1981). Similarly, the concentration of thioridazine and its metabolites in liver tissue was 3 to 20 times higher than that in the blood obtained post-mortem (Dinovo et al., 1978). This accumulation may lead to an underestimation of the in vivo interaction potential of these drugs. On the other hand, extrapolation of in vitro data to in vivo circumstances is also hampered by the presence of
metabolites, which may contribute to the enzyme inhibition, and be involved in pharmacokinetic interactions.

The importance of pharmacokinetic drug interactions involving cytochrome enzymes has been increasingly emphasized (LLerena and Kiivet, 1994). As CYP2D6 involved in the metabolism of several important psychotropic and other drugs frequently used in clinical practice including psychiatric patients (e.g. beta-blockers, antidepressants, antidiabetics, antiarrhythmics etc.), theoretically substantial, and potentially dangerous, drug interactions may occur during the coadministration of drugs which are substrates or inhibitors of the enzyme. Drug interaction may also lead to therapeutic failure if the plasma concentration of an active metabolite is decreased by induction, or if the formation of a potent active metabolite is blocked by inhibition.

The most important drug interactions described so far generally come from single clinical case reports or observations without direct pharmacogenetic measurement of enzyme activities and/or the plasma levels of the drugs involved. Therefore, these reports should be interpreted with caution, because the interactions may be influenced by other factors, and not only by drug metabolizing cytochrome enzymes.

### 3.3.6. Cytochrome P450 enzyme genotyping versus phenotyping in patients

Using modern amplification techniques, genotyping allows characterization of many significant CYP genes from a single venous blood sample (Meyer and Zanger, 1997). However, genotyping is only clinically relevant to the degree with which it predicts phenotypes (McLeod, 1994; Tucker, 2000). Consequently, the optimal method for describing real-time enzyme activity is phenotyping, in which the metabolism of a carefully selected probe compound is used to estimate the activity of one or more enzyme(s) involved in the metabolism (Streetman et al., 2000). Phenotyping provides the most relevant information for clinical practice, because it reflects the combined effects of genetic, environmental and endogenous factors on CYP activity.

The research described here was focused on 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 (LLerena et al., 1992a; LLerena et al., 1992b; Von Bahr et al., 1991; Huang et al., 1993). In the following chapter our present knowledge of the pharmacogenetic factors implicated in the metabolism of thioridazine, haloperidol and risperidone are reviewed.
4. CYTOCHROME P450 ENZYMES AND THE METABOLISM OF THIORIDAZINE, HALOPERIDOL, AND RISPERIDONE

4.1. Thioridazine

The thioridazine is first metabolized by side chain sulfoxidation to thioridazine 2-sulfoxide (mesoridazine) and then from mesoridazine to thioridazine 2-sulfone (sulforidazine) (Lin et al., 1993).

Another metabolic pathway is the thioridazine-5-sulfoxidation of thioridazine, resulting in thioridazine-ring-sulfoxide (Fig. 2). Mesoridazine and sulforidazine are both active pharmacologically, and mesoridazine is even registered in some countries as a different drug entity. The thioridazine-ring-sulfoxide has no antipsychotic effect but this metabolite was related to cardiovascular side-effects (Hale and Poklis, 1986).

The thioridazine seems to be a potent inhibitor of CYP2D6. In an in vitro study (Von Bahr et al., 1985) was described that thioridazine inhibited the desipramine 2-hydroxylation reaction, which cosegregates with the debrisoquine hydroxylase activity. Additional data supporting this hypothesis came from the result that almost 70% patients under thioridazine treatment were phenotypically PMs with respect to CYP2D6, i.e. phenotypic conversion may have occurred due to the inhibition (LLerena et al., 1987; LLerena et al., 1993b).

Figure 2. The metabolism of thioridazine
Von Bahr et al. (1991) studied thioridazine metabolism in a panel of healthy volunteers consisting of 13 EMs and 6 PMs of CYP2D6. 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 correlated significantly to the debrisoquine MR, while the sulforidazine/mesoridazine did not. Thus, it is probable that the thioridazine-mesoridazine metabolic step is catalyzed by CYP2D6. This hypothesis was also supported by a study among patients (Von Bahr et al., 1991).

The involvement of CYP2C19 in the metabolism of thioridazine to an unknown metabolite has been reported (Eap et al., 1996). Also the possible implication of the CYP1A2 enzyme was suggested based on the findings that fluvoxamine (a CYP1A2 inhibitor) had a substantial influence on the plasma levels of thioridazine and its metabolites (Carrillo et al., 1999).

Dahl et al. (1986) found no correlation between the plasma concentration of thioridazine and its therapeutic response. However in a clinical report (Meyer et al., 1990) the PM phenotype was associated with the occurrence of side-effects. To the best of our knowledge, no data is available on the relationship between the CYP2D6 genotype and thioridazine metabolites. Also the effect of smoking on thioridazine has not been explored.

4.2. Haloperidol

The metabolism of haloperidol has been extensively studied (Fig. 3), however the involvement of different drug-metabolizing enzymes is still not well understood, and the in vitro and in vivo results are partly controversial.
Figure 3. The metabolism of haloperidol


Enzymes: KR: cytosolic carbonyl reductase

In the human body the main pathway of the metabolism of haloperidol is oxidative N-dealkylation. During the oxidative N-dealkylation the side chain joined to the tertier nitrogen breaks up resulting in two inactive metabolites: p-fluorobenzoyl-propionic acid (FBPA) and 4-(4-chlorophenyl)-4-hydroxypiperidine (Forsman and Larsson, 1978; Gorrod and Fang, 1993). This is thought to be the most important metabolic step, since FBPA and its degradation products (p-fluorophenylacetic acid and p-fluorophenaceturic acid) are the major excreted metabolites in the urine (Soudijn et al., 1967).

Another important metabolic pathway is the reduction of the ketone group to another metabolite, reduced haloperidol (RHAL) (Inaba and Kovacs, 1989). This metabolite has some pharmacological activity (Korpi and Wyatt, 1984). After the administration of a single dose, about 23% of the biotransformation of haloperidol occurs through this reduction (Jann et al., 1990). In humans the interconversion between HAL and RHAL also exists (Midha et al., 1987). Both HAL and RHAL undergo glucuronidation (Oida et al., 1989). A minor pathway of metabolism accounting for only 0.4–2.3% of the administered dose is the formation of a pyridinium (HPP⁺) metabolite and a reduced pyridinium metabolite (RHPP) (Fang and
Gorrod, 1991; Eyles et al. 1994). In spite of the small ratio, this metabolic pathway may be of special interest since the pyridinium metabolites have neurotoxic effects both in vitro and in vivo (Rollem et al., 1994; Fang et al., 1995). This conversion of haloperidol to HPP+ seems to be similar to the activation of the dopaminergic neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to N-methyl-4-phenyl pyridinium ion (MPP+). MPP+ is responsible for the damage of striatal dopaminergic neurons induced by MPTP in humans and animals. HPP+ was also shown to be toxic to dopaminergic cells in vivo in a time and concentration dependent matter (Fang et al., 1995), thus it might contribute to the extrapyramidal side-effects of haloperidol.

Haloperidol inhibits the activity of CYP2D6 in liver microsomes, which suggests that it may be a substrate of the enzyme (Inaba et al., 1985). In an in vitro study Tyndale et al. (1991) reported that the metabolic conversion of RHAL to HAL was inhibited by quinidine indicating that CYP2D6 might be involved in the metabolism of haloperidol. However, in vivo Young et al. (1993) found that quinidine did not affect the interconversion of RHAL to HAL.

In a healthy volunteers study 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 (Llerena et al., 1992a). The authors later reported that in the same study population the plasma levels of reduced haloperidol were also higher (Llerena et al., 1992b). This latter study showed that the disposition of haloperidol was associated with the hydroxylation of debrisoquine.

Additional results support the previous hypothesis, 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 (Gram et al., 1989; Spina et al., 1991; Llerena et al., 1993b).

However, in patients no difference was found between the steady-state plasma level of haloperidol in EMs and PMs of CYP2D6 enzyme activity (Gram et al., 1989). On the contrary, coadministration of fluoxetine (a known inhibitor of CYP2D6) and haloperidol resulted in increased plasma levels of HAL, a finding that further corroborates the involvement of CYP2D6 in the metabolism of the drug (Goff et al., 1991). However, the
clinical implication of the involvement of CYP2D6 in haloperidol metabolism remains unclear.

In an in vitro investigation using expressed CYP enzymes in a human cell line it was found that CYP2D6 is involved in several metabolic steps of haloperidol metabolism, e.g. in the conversion of HTP to HPP$^+$ and haloperidol to CPHP (Fang et al., 1997). Nevertheless, Pan et al. (1997) using several in vitro methods (human liver microsomes, chemical and immunoinhibition of specific isoforms, c-DNA) could not confirm the involvement of CYP2D6 in the dealkylation of haloperidol, and only a minor involvement in the biotransformation of haloperidol and reduced haloperidol (Pan and Belpaire, 1999).

Pan et al. (1997) also observed that in vitro CYP3A4 was the major enzyme involved in the dealkylation of haloperidol. In another in vitro study it was reported that CYP3A4 was the major enzyme of the biotransformation of haloperidol and reduced haloperidol (Pan and Belpaire, 1999). In a further in vitro study the back oxidation of RHAL to HAL was reported in human cytochrome isoenzymes expressed in a human cell line (Fang et al., 1997). This study also suggested that CYP3A4 might be involved in the metabolism of HAL to HTP, and in its further conversion to HPP$^+$, as well as in the N-dealkylation of RHAL.

Carbamazepine, which is a strong inducer of the CYP3A4 enzyme, significantly decreased the plasma levels of haloperidol in patients (Hesslinger et al., 1999). On the other hand, itraconazole, a potent inhibitor of CYP3A4, when it was coadministered with haloperidol to patients, increased significantly the plasma concentrations of haloperidol and reduced haloperidol (Yasui et al., 1999). This inhibition also correlated with a significantly higher incidence of neurological side-effects.

Several authors have reported that smokers had significantly lower plasma levels of haloperidol than non-smokers (Jann et al., 1986; Shimoda et al., 1999) and that the clearance of haloperidol was higher in smokers (Miller et al., 1990). Since CYP2D6 is only slightly inducible by smoking, if at all, these findings suggest the involvement of other tobacco inducible enzymes (e.g. CYP1A2). The coadministration of fluvoxamine, a potent CYP1A2 inhibitor, was found to increase haloperidol plasma levels (Daniel et al., 1994; Vandel et al., 1995), which also supports that CYP1A2 may be involved in the metabolism of haloperidol. However, a recent study concluded that the CYP1A2 activity does not play an important role.
in controlling the steady-state plasma concentration of haloperidol or reduced haloperidol (Mihara et al., 2000).

4.3. 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 (Fig. 4).

Figure 4. The main metabolic pathways of risperidone disposition
1: alicyclic hydroxylation, 2: oxidative N-dealkylation

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 (Heykants et al., 1994). With the use of standard pharmacological testing in animal models and by prolactin secretion in healthy volunteers, 9-hydroxy-risperidone was shown to have a pharmacological activity similar to that of risperidone, while other metabolites did not show any pharmacological activity (Huang et al., 1993). However, there are no published clinical data in humans on the therapeutical effects or side-effects of 9-hydroxy-risperidone.
In an *in vitro* study performed with human liver microsomes the involvement of CYP2D6 but not of CYP1A1, CYP1A2, CYP2C9, CYP2C19 or CYP3A4 in the metabolism of risperidone to 9-hydroxy-risperidone was reported (Prior et al., 1999). In another *in vitro* study CYP2D6, 3A4 and 3A5 were all able to metabolize risperidone to 9-hydroxy-risperidone (Fang et al., 1999).

The pharmacokinetics of risperidone and its main metabolite, 9-hydroxy-risperidone, was studied in healthy volunteers phenotyped with dextromethorphan (Huang et al., 1993). The mean total body clearance of risperidone was 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 (Huang et al., 1993).

In one study thirty-seven schizophrenic patients on monotherapy with risperidone were genotyped for the CYP2D6 gene and the steady-state plasma levels of risperidone and 9-hydroxy-risperidone were analyzed. The steady-state plasma concentrations of risperidone were significantly higher in PM patients than in patients with other genotypes. However, no significant differences were found in the sum of the plasma concentrations of risperidone and 9-hydroxy-risperidone between the genotype groups. The authors concluded that the lack of relationship between the genotype and the sum of risperidone and 9-hydroxy-risperidone (the active moiety) indicates that the CYP2D6 polymorphism may be of limited importance for the clinical outcome of the treatment (Scordo et al., 1999).

The plasma level of 9-hydroxy-risperidone is around 20 times higher in CYP2D6 EM patients than that of risperidone (Aravagiri et al. 1998). In patients receiving a risperidone dosage of 3 mg b.i.d., under steady state conditions the through plasma concentrations of risperidone were about 10 ng/ml and those of the active moiety were about 45 ng/ml, i.e. four to five times higher (Heykants et al. 1994).

Risperidone therapy with *serotonin-specific reuptake inhibitor* (SSRI) comedication was associated with 4.6-fold increase in the plasma concentrations of risperidone compared to patients without comedication (Balant-Gorgia et al., 1999). This fact supports the previously
described inhibitory effect of SSRI drugs on CYP2D6 enzyme activity and, consequently, the decrease in the formation of 9-hydroxy-risperidone from risperidone.

De Leon and Bork (1997) described the case of a patient who was receiving risperidone and carbamazepine concomitantly (an inducer of CYP3A4) and whose serum levels of 9-hydroxy-risperidone doubled after the discontinuation of carbamazepine, suggesting the involvement of CYP3A4 in the metabolism of risperidone.

5. CLINICAL IMPLICATIONS

The clinical importance of polymorphic drug metabolizing CYP enzymes is increasingly recognized. The implication of pharmacogenetic factors in the therapeutic effect and in the occurrence of side-effects or drug interactions is of utmost importance, however, research into this field is still lagging behind.

At present our knowledge of the clinical importance is mainly obtained from in vitro and animal studies, and from human trials with healthy volunteers. The animal models frequently used in the receptor studies to elucidate the pharmacodynamic action of the antipsychotic drugs are less valuable in the research of drug metabolizing enzymes. The reason for that is the striking interspecies differences in the content and activity of the CYP enzymes. Therefore the result from animal studies will not reflect the disposition of these drugs in humans.

In the everyday clinical situation psychiatric drugs usually exert their effect when steady-state conditions are reached, thus the direct interpretation of in vitro data is also not possible. 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 (LLerena et al., 1996, Fuhr, 1998).
b. a dose dependent inhibition of the drug on the cytochrome enzymes (LLerena et al., 1993b).
c. an inhibition due to drug interactions (inhibitors or competitive substrates) (Inaba et al., 1986b; Brøsen et al., 1987).

Andreasen et al. (1997) found that the frequency of CYP2D6 PM subjects was 3 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. Several other authors have also noted the higher incidence of extrapyramidal side-effects among PM patients (Arthur et al., 1995; Armstrong et al., 1997; Vandel et al., 1999).

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 (Poulsen et al., 1996). 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 (Tyndale et al., 1997; Romach et al., 2000).

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 (Kalow and Tang, 1991a; Schrenk et al., 1998).
b. autoinduction when a drug accelerates its own metabolism by induction of the cytochrome enzyme involved in its disposition (e.g. carbamazepine) (Tomson et al., 1989).
c. use of concomitant inducers (e.g. carbamazepine), which diminish the plasma concentration of the active drug (Spina et al., 1996).

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 (Bertilsson et al., 1993; Dalen et al., 1997).
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.

5.1. 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 (Haverkamp et al., 2000). It has been claimed that the risk is substantially higher in patients treated with thioridazine (Reilly et al., 2000). Thioridazine is a phenothiazine antipsychotic drug extensively used world-wide (1.900.000 prescriptions annually in UK, 35% of all antipsychotic drugs). In the United States, the Food and Drug Administration and in the United Kingdom, the Medicines Control Agency restricted the use of thioridazine due to the potential cardiac toxicity of the substance. The drug remains in use only as a second-line treatment for schizophrenia.

The cardiotoxicity of thioridazine has been proved in both animal and in vitro studies (Hale and Poklis, 1986; Haverkamp et al., 2000). Although the mechanism of this side-effect has not been fully clarified, data from these studies have suggested the possible role of different cardiac ionic channels (Haverkamp et al., 2000; Tristani-Firouzi et al., 2001). 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 (Buckley et al., 1995). 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 mesoridazine dose and QTc interval was shown in nine patients (Dillenkoff et al., 1972). A similar relationship between thioridazine dose and QTc has been
found in a large population study (Reilly et al., 2000). 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 (Hartigan-Go et al., 1996). The cardiac side-effect of thioridazine seems to be dose related. Thus, plasma concentration increase due to overdose and/or due to drug interactions at the cytochrome P450 enzymes, which produce elevations in thioridazine plasma levels (Carillo et al., 1999) may increase QTc interval and the risk of ventricular arrhythmias.

The activity of the 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.
5.2 Clozapine withdrawal symptoms

Atypical antipsychotics have an improved side-effect profile and seem to be effective on both positive and negative symptoms of schizophrenia. The first antipsychotic drug of this class was clozapine, which has been followed in the last few years by several new atypical antipsychotic drugs of different chemical structure (e.g. risperidone, olanzapine, sertindole, quetiapine, etc.). However, the well-known side-effects of clozapine, of which the most important is agranulocytosis appearing in about 1% of the patients (Alvir and Lieberman, 1994), prompts psychiatrists to switch to a newer atypical antipsychotic drug in order to be able to avoid the regular haematological control of the patients. With the introduction of these new antipsychotics in the treatment of schizophrenia the mode of switching to a new antipsychotic medication has become extremely important.

Withdrawal symptoms for typical antipsychotics are generally mild and self-limited (Dixon et al., 1993). However, following withdrawal of clozapine serious symptoms with rapid onset have been repeatedly reported (Parsha et al., 1993; Baldessarini et al., 1995; Meltzer et al., 1995). 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 (Meltzer et al., 1995). However, the origin of these symptoms is still not known and the involvement of pharmacokinetic factors can not be ruled out.
II. AIMS
1. JUSTIFICATION OF THE RESEARCH

In clinical practice in general, and in psychiatry particularly, the variability of clinical efficacy and appearance of side effects among patients treated with the same doses is frequently observed. One of the main reasons for this interindividual variability of antipsychotic drug response can be due to differences in plasma concentration of drug and/or metabolites. One of the major factors influencing drug elimination and thus plasma concentrations is the activity of the cytochrome P450 enzymes. It has been shown that the genetic polymorphism of the CYP2D6 enzyme plays a major role in the elimination of several antipsychotic drugs including haloperidol, thioridazine and risperidone. Supported in previous in vitro studies, it has been shown in healthy volunteers studies that the plasma concentration/dose of the antipsychotic drugs and metabolites is substantially different in poor metabolizers of CYP2D6 (5-10% of Caucasian population) compared to extensive metabolizers.

In a previous study (De la Rubia, 1997) we found among patients receiving thioridazine a dose-dependent inhibition of CYP2D6 (debrisoquine MR) activity, and a relevant influence of CYP2D6 enzyme activity and smoking on plasma levels.

The present studies will evaluate the hypothesis that due to genetic, or environmental factors poor metabolizers have higher plasma concentrations of parent drug than extensive metabolizers, and also if environmental factors like smoking or drug inhibition due to concomitant drug treatment will effect the disposition of the antipsychotic drugs studied.

The clinical implication of this fact will be related to the relevance of drug or metabolite concentrations in therapeutic activity and/or in side-effects. The relevance of CYP enzyme phenotyping and genotyping in the prediction of side-effects will be evaluated. The possible use of therapeutical drug monitoring to predict drug metabolizing enzyme activity by the help of using different drug metabolite ratios and thus therapeutic efficacy and/or side-effects will be also investigated.
2. **GENERAL AIM OF THE STUDIES**

To determine the implications of the CYP2D6 (debrisoquine hydroxylase) 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).

3. **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.
III. MATERIALS AND METHODS
1. GENERAL CONSIDERATIONS

1. General design of the studies

Three antipsychotic drugs used extensively world-wide, viz. thioridazine, haloperidol, and risperidone, were studied. These drugs were selected partly on the basis of their frequent use and also because previous studies had suggested important implications of pharmacogenetic factors in their metabolism. 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.
1.2. Ethical considerations

The pharmacogenetic studies were carried out in patients staying in general clinical settings. The patients were informed about the aim and protocol of the study and they - or in cases of incapacitation (*non compos mentis*), their legal guardians – gave their prior consent to the participation.

The study was performed according to the norms laid down by the Helsinki Declaration adopted by the 18th World Medical Association (WMA; www.wma.net) General Assembly (Helsinki, Finland, June 1964) and amended by the 29th WMA General Assembly (Tokyo, Japan, October 1975), the 35th WMA General Assembly (Venice, Italy, October 1983), the 41st WMA General Assembly (Hong Kong, September 1989), the 48th WMA General Assembly (Somerset West, Republic of South Africa, October 1996) and the 52nd WMA General Assembly (Edinburgh, Scotland, October 2000).

The research protocols were approved by the Local Ethics Committee of the corresponding institute at the University of Extremadura, Spain and the University of Debrecen, Hungary.

1.3. Patient study protocols

The research protocols for the studies contained the following information:

a. General data: identification of the patient by initials, identification number, types of probes, clinical case number, and date of examination.

b. Clinical data: demographic and anamnestic data, familial and personal antecedents (including previous treatment) and diagnosis according to Diagnostic Manual and Statistic for Mental Disorders of the American Psychiatric Association (DSM-IV, 1994).

c. Personal habits, such as caffeine and alcohol consumption, tobacco smoking.

d. Treatment records: the present medications of the patient including all comediations besides the drug of interest. In the protocols the following data were recorded: starting dates of drug treatment, doses, dose changes, daily administration schedules.
1.4. Debrisoquine hydroxylation phenotyping

1.4.1. Study protocol

The metabolic phenotype was determined by the administration of the test drug, debrisoquine (Declinax, Hoffman-La Roche, Switzerland), according to the protocol introduced by Mahgoub et al. (1977), and modified to a routine procedure (Cobaleda, 1988; LLerena, 1988). After emptying their bladder, the subjects took a single oral dose of 10 mg debrisoquine sulphate at around 9.00 a.m. After taking the drug, the subjects refrained from eating for 1 hour. After the ingestion of debrisoquine, the urine was collected over the next eight hours. This interval was considered to be sufficient, since after six hours of collection no variability in debrisoquine MR was observed (Philip et al., 1987).

The urine was collected in containers of 2 litres and was stored at 4° C until further processing. After the measurement of the urine volume, two samples of 20 ml each were prepared and stored at –20 C until measurement. According to the literature no alteration of the debrisoquine or 4-hydroxy-debrisoquine concentration in the sample occurs during storage (Steiner, 1987; Cobaleda; 1988).

1.4.2. Determination of debrisoquine and 4-hydroxy-debrisoquine in the urine

Debrisoquine and its metabolite, 4-hydroxy-debrisoquine, were analyzed with the method developed originally by Lennard et al. (1977) and adapted for a routine laboratory protocol. (Cobaleda, 1988; LLerena, 1988). Samples were derivatized by acetyl-acetone and the derived pyrimidines were extracted and analyzed by gas chromatography with hydrogen flame ionization.

The extraction method consisted of the following steps:
To 1 ml of urine in crystal tubes, 0.5 ml saturated sodium bicarbonate, 0.5 ml of methanol, 75 μl of guanoxan as internal standard (IS) and 0.5 ml of acetyl-acetone were added. The tubes were placed into an agitation bath at 50° C for 16 hours. To separate the pyrimidine compounds, 6 ml of diethyl-ether was added after the incubation and the tube was vortexed vertically. Then the ether phase was collected and transferred to crystal tubes. To the samples 0.3 ml M hydrochloric acid (4M) was added. The aqueous layer then was transferred to
crystal tubes and put in warm bath at 56° C for 15 minutes to evaporate the remaining ether. To neutralize the pH, 0.4 ml sodium hydroxide (4M) was added. Finally 40µl of carbon disulfide was added, and the injected onto the column. The gas chromatograph was a Varian (Palo Alto, CA, USA) 2440-10 model with hydrogen flame ionization detector. The glass column was 2 m long with an inside diameter of 3 mm. The column was filled with 3% OV 225 on Chromosorb W HP grade support (Varian, Palo Alto, CA, USA). The temperature of the column and detector was set at 250° C. The gas flow at a constant rate of 60 ml/min for hydrogen (pressure: 4 bar), 50 ml/min for nitrogen (pressure: 4 bar) and 240 ml/min for pure synthetic air (pressure: 3 bar) was maintained.

The retention times were 1.2 minutes for debrisoquine, 2.8 minutes for guanoxan, and 3.2 minutes for 4-hydroxy-debrisoquine. To calculate the amount of the compound, the area under the curve (AUC) of the corresponding peaks was measured by Spectra-Physics integrator, Model SP-4290. The AUC of debrisoquine and 4-hydroxy-debrisoquine was divided by the AUC of the IS in order to correct for possible variations due to the technique. From a standard curve with known amounts, the concentration of debrisoquine and 4-hydroxy-debrisoquine was calculated. The detection limit of the method is 0.5 µmol/l for both debrisoquine and 4-hydroxy-debrisoquine.

1.4.3. Calculation of debrisoquine urinary metabolic ratio (MR)

Since the administered Declinax tablets contain the equivalent of 57.1 µmol of debrisoquine base, the measured debrisoquine concentration is multiplied by the urine volume and divided by 57.1, and the result is the percentage of the elimination of debrisoquine (% elimination of debrisoquine). The amount eliminated as the major metabolite is calculated similarly (% elimination of 4-hydroxy-debrisoquine). The debrisoquine oxidation phenotype is determined by calculation of the urinary MR, which is the ratio between percentages of doses eliminated as debrisoquine and its principle metabolite, 4-hydroxy-debrisoquine (Mahgoub et al., 1977).

The urinary MR is a stable and reproducible parameter for determining the metabolic phenotype, furthermore, its value is not influenced by urine volume or pH (Silas et al., 1978, Sloan et al., 1983).
1.4.4. Calculation of the frequency of phenotypes

In order to classify subjects as extensive and poor metabolizers, individuals with a debrisoquine MR over 12.6 were considered as PMs. The value for discriminating EMs from PMs has been validated previously in Caucasians, including Spaniards. (Evans et al., 1980; Benítez et al., 1988; LLerena et al., 1996).

1.5. 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® DNA Mini Kit (Quiagen, Hilden, Germany). The AmpliTaq Gold™ System (Perkin-Elmer Inc., Wellesley, USA) was used to amplify the CYP2D6*3, CYP2D6*4 and CYP2D6*6 alleles by tetra-primer PCR, whereas Expand™ Long Template PCR System (Roche Diagnostics, Basel, Switzerland) was used to amplify the CYP2D6*5 allele by multiplex PCR. Amplifications were performed with a PTC-100™ thermocycler (MJ Research, Inc. Watertown, USA). Oligonucleotide sequences and PCR conditions for the detection of the CYP2D6*3, *4, *5 and *6 alleles have been previously described (Marez et al., 1997; Gaedik A et al., 1999, Hersberger et al., 2000). PCR products were separated by electrophoresis in agarose gels and were visualized by staining with ethidium bromide.

1.6. 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.

1.7. 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 (glucose, urea, creatinine, AST, ALT, gammaGT), haematological (RBC, WBC, platelet count, haematocrit, haemoglobin).

c. On the day of the blood sampling side effects were determined with the clinically validated Utvalg for Kliniske Undersogelser (UKU) Side Effect Scale (Lingjaerde et al., 1987).

d. Routine ECG was performed with an automated equipment in each patient on the day of the blood-sampling.

The compliance of patients was assured because all the patients were hospitalized in a clinical setting and because the investigated drugs were administered under the supervision of the unit staff.

1.8. Statistical analyses

Results are expressed as means and S.D. or means and 95% confidence intervals (95% CI). Statistical calculations were carried out using the GraphPad Prism 3.01 software for Windows 95 and NT (GraphPad Software Inc., www.graphpad.com). Spearman’s non-parametric test for the correlation of different variables was used. Different groups were compared with Mann-Whitney two-tailed, non-parametric t-test. p<0.05 was considered as statistically significant.
2. DEVELOPMENT OF METHODS FOR THE DETERMINATION OF PLASMA LEVELS OF ANTIPSYCHOTICS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

2.1. General conditions

2.1.1. Chemicals

Acetic acid, ammonia solution, butanol, diethyl ether, dimethyloctalamine, hydrochloric acid, hexane, heptane, isoamyl-alcohol, perchloric acid, phosphoric acid, potassium dihydrogen phosphate, potassium hydroxide, triethylamin, HPLC grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Diisopropyl-ether and sodium hydroxide were from Panreac (Castellar del Valles, Spain). Water was Milli-Q quality and was obtained by a Millipore water purification system (Millipore, Bedford, MA, USA). Thioridazine, sulforidazine and mesoridazine were kindly supplied by Novartis (Basel, Switzerland). Risperidone, 9-OH-risperidone and metoxi-risperidone were donated by Janssen Research Products (Lammerdries, Belgium). Promethazine, clozapine, N-desmethylclozapine and protriptyline were supplied by RBI (Natick, MA, USA). Haloperidol and haloperidol chloride were obtained from Sigma (St. Louis, MI, USA). The drugs tested for possible interference were prepared from commercial formulations.

2.1.2. Instrumentation

High-performance liquid chromatography (HPLC) assay was performed using a Beckman HPLC chromatograph (Beckman, Fullerton, CA, USA) equipped with a solvent pump (Module 110B) and a programmable detector (Module 166). Peaks were detected by ultraviolet absorption. Data were collected and processed by an IBM compatible PC and the Beckman System Gold Programme (version 7.11).

2.1.3. Calculation of plasma concentrations

The peak heights or in case of thioridazine the peak areas (area under the curve - AUC) obtained in the chromatograms were calculated automatically by the System Gold
programme. The peak height or AUC of the different identified peaks were divided by the peak of the corresponding internal standard in order to correct for possible variations due to the technique. From a standard curve with known amounts of the substance, the concentration of different compounds was calculated.

2.2. Measurement of plasma concentration of thioridazine and its metabolites

2.2.1. Extraction procedure

To 500 µl of plasma, 25 µl of promethazine (internal standard, IS), 2.5 ml of ammonia solution (0.5 mol/litre) and 400µl of diisopropyl-ether were added. Tubes were gently vortexed vertically for 30 minutes and then centrifuged at 3500 rpm for 15 min at 15° C temperature. Subsequently, 200 µl of the supernatant was pipetted to Eppendorf tubes and centrifuged at 1500 rpm for 5 minutes. Then, 120 µl of the supernatant was transferred again to Eppendorf tubes and the centrifugation with the same conditions was repeated. After this centrifugation, 80 µl of the supernatant was pipetted again into Eppendorf tubes and the samples were evaporated to dryness by heating them at 50° C for an hour. The residues were then resuspended in mobile phase and 20 µl was injected onto the HPLC column.

2.2.2. Chromatographic conditions

All chromatographic separations were performed at ambient temperature on a straight-phase 150x4.6 mm, 3µm particle size, spherisorb silica column (Sugelabor S.A., Madrid, Spain). The injector was a Rheodyne 20 µl-loop. The mobile phase consisted of 72.2% acetonitrile, 27.4 % methanol, 0.4% ammonia solution (25%). The pH of the mobile phase was 10.7. The mobile phase was filtered through a 0.22 µm GS-filter (Millipore, Bedford, MA, USA) and degassed by ultrasound before use. The flow rate was maintained at 2 ml/min. Peaks were detected by ultraviolet absorption at 267 nm.
2.3. Measurement of plasma concentration of haloperidol

Plasma concentrations of haloperidol were measured in duplicates by high-performance liquid chromatography (HPLC) with UV detection according to a previously published method (Llerena et al., 1992a) with some minor modification.

2.3.1. Extraction procedure

2 ml of plasma was pipetted into 10 ml polypropylene tubes, in duplicates. Then, 200 µl of the haloperidol chloride (IS), 1 ml sodium hydroxide solution (1M) and 4 ml of hexane-butanol (95%-5%) were added to each tube. The tubes were gently vortexed vertically for 20 minutes and then centrifuged at 3000 rpm for 10 min at 15°C temperature. 3.7 ml of the supernatant was pipetted to 10 ml polypropylene tubes and 150 µl of phosphate buffer was added, then vortexed manually and centrifuged at 3000 rpm for 5 minutes. The upper phase was decanted and 1 ml of diethyl ether was added and the centrifugation with the same conditions was repeated. The diethyl ether phase was decanted again, and the rest of the diethyl ether phase of the samples were evaporated by drying them at 40°C for 20 minutes. 100 µl of the residues was injected onto the column.

2.3.2. Chromatographic conditions

All chromatographic separations were performed at ambient temperature on a reverse-phase Ultrasphere column, 75x4.6 mm, particle size 3µm (Beckman S.A., Madrid, Spain). The injector was a Rheodyne 100 µl-loop. The mobile phase consisted of 710 ml of water, 280 ml of acetonitrile, 3 ml of triethylamin and 1.6 ml of phosphoric acid. The pH of the mobile phase was 2.8. The flow rate was maintained at 1.5 ml/min. Peaks were detected by ultraviolet absorbance at 254 nm.

2.4. Measurement of plasma concentration of risperidone and its metabolite

The plasma levels of risperidone and 9-OH-risperidone were determined by high performance liquid chromatography based on a method developed at Huddinge Hospital, Sweden
(Svensson JO, personal communication), with some modifications. This method is similar to
another one published recently (Avenoso et al., 2000).

2.4.1. Extraction procedure

To 1 ml of plasma in 10 ml centrifuge tubes, 40 μl of the internal standard (metoxi-
risperidone, IS) solution (5 μg/ml), 1 ml of 0.5 M sodium hydroxide buffer and 4 ml of
isoamyl-alcohol in diisopropyl-ether (DlPE) were added. The tube was briefly shaken on a
vortex-mixer for 10 minutes. After centrifugation at 3000 rpm for 10 min, the aqueous layer
was collected in a 10 ml centrifuge tube, then 175 μl of acetic acid (25 mM) were added and
the contents mixed for 5 min. The mixture was centrifuged (5 min, 3000 rpm) and the organic
layer was discarded. Then, 500 μl of heptane were added. The heptane layer was aspirated
and evaporated in a gentle stream of nitrogen. 30 μl of the aliquot was injected into the
chromatograph.

2.4.2. Chromatographic conditions

The analytical column (250 mm x 4.6 mm I.D) was packed with Hypersil ODS (3 μm particle
size) coated with C18 groups (Sugelabor, Madrid, Spain). The mobile phase was 72 % water,
28% acetonitrile, 5.44 g of KH₂PO₄, and 400 μl of dimethyloctalamine (DMOA). The flow-
rate was 0.8 ml/min and detection wavelength at 278 nm. Peaks were detected by ultraviolet
absorbance at 254 nm.

2.5. Measurement of plasma concentration of clozapine and its metabolite

2.5.1. Extraction procedure

Sample preparation was carried out by liquid-liquid extraction. 2 ml of human plasma was
pipetted into a 10 ml polypropylene tube and 10 to 100 μl of 0.01 mg/ml clozapine and N-
desmethyloclozapine, 100 μl of 0.005 mg/ml protriptyline (IS) and 200 μl of 2 M sodium
hydroxide were added. The plasma was mixed with a hand vortexer and then 5 ml of the
hexane:isoamyl-alcohol (98.5:1.5 v/v) was added to the tubes, which were then capped and
placed into a shaker for 20 minutes. After shaking, the tubes were centrifuged at 3600 rpm for 5 minutes. The organic phase was drawn off and put into a 10 ml polypropylene tube, to which 120 µl of 0.1 M hydrochloric acid was added. This tube was vortexed and centrifuged at 3600 rpm for 5 min. The organic layer was drawn off and evaporated at 40° C under N₂ atmosphere for 2 hours. 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.

2.5.2. Chromatographic conditions

The mobile phase was a mixture of 5.0 g of potassium dihydrogene phosphate, 320 ml of acetonitrile, 190 µl of perchloric acid, and 100 µl of phosphoric acid completed to 1 litre with water. Before analysis the mobile phase was filtered through a 0.22 µm filter (Millipore, Ireland). Separation was carried out at room temperature using an ODS Beckman Partisil (5µm; 150x4.6 mm ID) column. The flow rate was set at 1 ml/min and detection wavelength at 230 nm.
3. CLINICAL IMPLICATION OF CYP2D6 HYDROXYLATION CAPACITY IN PSYCHIATRIC PATIENTS

The overview of the studies involved in the Thesis is given in Table 5.

<table>
<thead>
<tr>
<th>Study Number</th>
<th>Drug</th>
<th>No of patients</th>
<th>Plasma concentration</th>
<th>CYP enzyme activity</th>
<th>Other parameters</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>thioridazine</td>
<td>65</td>
<td>thioridazine and its metabolites</td>
<td>debrisoquine MR</td>
<td>smoking habits; UKU Scale</td>
<td>Ref.: De La Rubia, 1997</td>
</tr>
<tr>
<td>II.</td>
<td>thioridazine</td>
<td>16</td>
<td>thioridazine and its metabolites</td>
<td>debrisoquine MR; genotype</td>
<td></td>
<td>subset of study I.</td>
</tr>
<tr>
<td>III.</td>
<td>thioridazine</td>
<td>27</td>
<td>thioridazine and its metabolites</td>
<td>debrisoquine MR</td>
<td></td>
<td>subset of study I.</td>
</tr>
<tr>
<td>IV.</td>
<td>haloperidol</td>
<td>27</td>
<td>haloperidol</td>
<td>debrisoquine MR</td>
<td>smoking habits; UKU Scale</td>
<td></td>
</tr>
<tr>
<td>V.</td>
<td>risperidone</td>
<td>43</td>
<td>risperidone and its metabolite</td>
<td>debrisoquine MR</td>
<td>smoking habits, concomitant drug treatment; UKU Scale</td>
<td></td>
</tr>
<tr>
<td>VI.</td>
<td>thioridazine</td>
<td>65</td>
<td>thioridazine and its metabolites</td>
<td>debrisoquine MR</td>
<td>ECG</td>
<td>Study I population</td>
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<tr>
<td>VII.</td>
<td>clozapine</td>
<td>1</td>
<td>clozapine and its metabolite</td>
<td></td>
<td>BPRS</td>
<td>case report</td>
</tr>
</tbody>
</table>

Table 5. Overview of the studies described in the present Thesis

3.1. Pharmacogenetics of thioridazine (Study I-III)

3.1.1. Implications of CYP2D6 enzyme activity for the metabolism of thioridazine (Study I)

The pharmacogenetics of thioridazine was studied in 65 Caucasian, chronic psychiatric patients without any relevant organic disease. They were hospitalized at Mérida Psychiatric Hospital (Extremadura, Spain). The patients were on continuous oral antipsychotic monotherapy with thioridazine (Melleril, Novartis, Switzerland) for at least 15 days before the study in order to assure steady-state conditions. The administered dose range was 20 to 500 mg/day and the average dose was 140±93 mg/day (mean±S.D.). As concomitant treatment four patients received antiparkinsonian drugs, six benzodiazepines, and one carbamazepine. The average duration of thioridazine treatment was 818±1053 (range: 15-3650) days. The mean age was 55±13 years (range: 27-80). Of the study population, 59 (91%)
were males and 47 (72%) 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.

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

In this study the effects of thioridazine dose changes were evaluated. 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 dose changes were determined by the physician according to the clinical status of the patient. The mean age of the patients in this subset was 59.5±12.6 (range: 37 to 80). The initial dose range was 20 to 300 mg/day, the average dose was 126±68.5 mg/day. 12 patients had two dose reductions and 4 patients had one. Finally, 10 patients became completely drug free (Table 10). The debrisoquine MR and the plasma levels of thioridazine were determined after a minimum period of seven days following the dose change. The CYP2D genotypes and phenotypes were determined as described before. The debrisoquine MR was determined as described previously.

3.1.3. 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. There were 23 males and 4 females in the group, aged between 37 to 80 years (mean: 61.2±10.2). Their mean body weight was 73.7 ± 16.9 kg. The patients were on a continuous oral thioridazine (Melleril, Novartis) 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.
3.2. The effect of CYP2D6 enzyme activity and smoking on the plasma concentrations of haloperidol (Study IV)

Twenty-seven Spanish, Caucasian psychiatric patients without any relevant organic disease were studied. They were hospitalized at Mérida Psychiatric Hospital (Extremadura, Spain). The patients were on continuous oral neuroleptic monotherapy with haloperidol (Haloperidol®, Syntex Latino) and they were receiving drug for at least 14 days in order to assure steady-state conditions. The dose range was 1.5 to 30 mg/day and the average dose was 7±5 mg/day (mean±S.D.). The mean age of the patients was 47±15 years (range:23-77). Of the 27 enrolled patients, 22 (81%) were males and 20 (74%) were tobacco smokers, defined as smoking regularly more than 5 cigarettes per day. The plasma levels of haloperidol and debrisoquine MR were determined as described previously.

3.3. 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 diagnosed according to DSM-IV criteria and hospitalized at the Psychiatric Hospital of Mérida (Extremadura, Spain) and University of Debrecen, Department of Psychiatry (Debrecen, Hungary). The mean age of the patients was 47.5±16 years (range: 28-77). The patients had received at least 7 days of continuous oral risperidone (Risperdal, Janssen, Belgium) therapy to reach steady-state conditions with an average daily dose of 4.6±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 incidence of extrapyramidal side effects was low, and comedication with an antiparkinsonian drug was needed only in one patient (biperiden, 2.5%). The plasma levels of risperidone, its metabolite and CYP2D6 phenotypes were determined as described previously.
3.4. 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 (Schouten et al., 1991). The cut-off value for limit of risk of arrhythmia was set at 456 ms (Reilly et al., 2000).

3.5. 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 hospitalized for the fifth time after the reappearance of mild anxiety, social isolation, lack of interest in daily routine, dysphoria, and dysthymia. In recent years he had almost continuously a mild preoccupation with somatic disorders and coenestopathic hallucinations. He had been on clozapine treatment for more than five years and his maintenance dose was 300 mg clozapine and 4 mg clonazepam daily during the previous twelve months. At admission the patient expressed his firm decision to stop taking clozapine in the future because of the regular haematological control. He gave written consent to his participation in an ongoing research project with sertindole, therefore, the protocol of tapering off his clozapine dose and introducing sertindole after 48 hour washout period was initiated.

From day 1 to day 6 of his stay in hospital the clozapine dose was gradually reduced and then the administration of the drug was stopped. After a 48 hour washout period, on day 8 sertindole 4 mg/day was started. The dose of sertindole was increased according to the prescribing information up to 20 mg reached by day 23. During this period (from day 16) the patient started to complain about anxiety, nervousness, tension fears and auditory hallucinations. Due to the extreme anxiety from day 19 comedication with an increased dose
of clonazepam, valproic acid, and alprazolam was necessary. From day 20, in addition, 100 mg of zuclopenthixol acetate had to be administered for 3 days because of extreme fear of dying, marked impairment in daily routine functioning, poor attention and persistently severe anxiety. He had profuse sweating, nausea and diarrhoea as well. Since the anxiety and fear did not decrease and also dysarthria developed owing to the high doses of benzodiazepines (6 mg/day alprazolam and 6 mg/day clonazepam), 100 mg/day clozapine was reinitiated on day 24 and the severe symptoms of the patient disappeared in two days (day 26). During the next seven days this clozapine dose was maintained with 20 mg/day sertindole. On day 31 the dose of clozapine was decreased again to 75 mg/day, but severe anxiety reappeared within hours. After increasing the dose of clozapine to 100 mg/day on day 34, anxiety disappeared again. Thus, it was decided to keep the patient on clozapine therapy and sertindole was gradually decreased and then stopped on day 46. After 56 days the patient was discharged from hospital with 175 mg/day clozapine and 900 mg/day valproic acid in a good general condition.

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.
IV. RESULTS
1. DETERMINATION OF PLASMA CONCENTRATIONS OF ANTIPSYCHOTIC DRUGS

1.1. Determination of plasma concentrations of thioridazine and its metabolites

1.1.1. System performance

Retention times for sulforidazine, thioridazine, internal standard (IS) and mesoridazine were 2.8, 3.4, 4.1, and 5.9 minutes, respectively. A calibration curve was made for each assay. The linear regression parameters were calculated for 3 independent calibration curves using 6 concentrations between 0.5 and 3 μmol/l. The calibration curves were linear over the established concentration range ($r^2>0.99$) for the detection of thioridazine and each metabolite. The absolute recoveries of thioridazine and its metabolites were calculated by dividing the peak areas of extracted plasma samples by those obtained for standards directly injected onto the column. The recovery was 95.1% for sulforidazine, 73% for thioridazine and 59% for mesoridazine. The limit of detection, as defined by the minimum signal-to-noise ratio of 4, was 100 nmol/l for sulforidazine, 75 nmol/l for thioridazine and 180 nmol/l for mesoridazine. Plasma concentration of thioridazine and its metabolites were quantifiable in 100% of the samples.

1.1.2. Precision and accuracy

The within-day precision was determined by measuring the spiked plasma concentrations for each calibration sample (six calibration points at concentrations: 0.5-3 μmol/l) from its corresponding calibration curve. The precision was calculated by the coefficients of variation (%CV). The %CV of the calibration points was under 10% for all substances. The inter-day and within-day precision was also expressed by the coefficients of variation. The values were 4.3% for sulforidazine, 10.8% for thioridazine and 5.5% for mesoridazine. Reference chromatogram is shown in Fig. 5.
Figure 5. Sample HPLC chromatograms of (A) extracted human plasma spiked with IS, (B) human plasma extract spiked with standard solutions (1 µmol/L) of thioridazine, mesoridazine, and sulforidazine, and (C) plasma extract of a patient receiving 100 mg thioridazine orally per day. Peaks A and B are unidentified metabolites of thioridazine.
1.1.3. Chromatographic interferences

In total 49 psychotropic drugs - including 12 antipsychotics, 11 antidepressants, 17 benzodiazepines, and 9 other drugs - were tested for possible chromatographic interference (Table 6).

<table>
<thead>
<tr>
<th>Drug</th>
<th>retention time (min)</th>
<th>Drug</th>
<th>retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>midazolam</td>
<td>1.15</td>
<td>flurazepam</td>
<td>1.21</td>
</tr>
<tr>
<td>periciazine</td>
<td>1.23</td>
<td>clotiapine</td>
<td>1.26</td>
</tr>
<tr>
<td>haloperidol</td>
<td>1.30</td>
<td>clomethiazol</td>
<td>1.63</td>
</tr>
<tr>
<td>trimipramine</td>
<td>1.65</td>
<td>flurazepam</td>
<td>1.71</td>
</tr>
<tr>
<td>fluvoxamine</td>
<td>1.86</td>
<td>phenobarbital</td>
<td>1.89</td>
</tr>
<tr>
<td>promethazine</td>
<td>1.91</td>
<td>biperiden</td>
<td>1.94</td>
</tr>
<tr>
<td>tiapridal</td>
<td>2.03</td>
<td>amitriptyline</td>
<td>2.31</td>
</tr>
<tr>
<td>clomipramine</td>
<td>2.35</td>
<td>sulphiride</td>
<td>2.49</td>
</tr>
<tr>
<td>chlorpromazine</td>
<td>2.69</td>
<td>sulforidazine</td>
<td>2.69</td>
</tr>
<tr>
<td>imipramine</td>
<td>2.91</td>
<td>thioridazine</td>
<td>3.13</td>
</tr>
<tr>
<td>lormetazepam</td>
<td>3.92</td>
<td>fluoxetine</td>
<td>4.63</td>
</tr>
<tr>
<td>mesoridazine</td>
<td>5.54</td>
<td>diazepam</td>
<td>7.53</td>
</tr>
</tbody>
</table>

No peaks were detected with the following substrates: amineptin, maprotiline, moclobemide, trazodone, lefopramine, perphenazine, pimoazid, levomepromazine, trifluoperazine, tiopropazine, pinizepam, bentazepam, alprazolam, lorazepam, halazapam, loprazolam, trizolam, zopiclone, zolpidem, bromazepam, cazarlazepam, chlorazepam, ketazolam, valproic acid, lithium, phenytoin, and carbamazepine.

Table 6. Retention times of drugs tested for potential interference with thioridazine determination by HPLC

1.2. Determination of plasma concentration of haloperidol in plasma

The method was the adaptation of a previously published method (LLerena et al 1992a). The chromatographic parameters were the following in the present study:

The intra-day and inter-day precisions expressed by the coefficient of variations (%CVs) were below 15%. The limit of detection defined as the minimum signal-to-noise ratio of 4 was 0.17 ng/ml for haloperidol, and the recovery was 85.3%. Plasma concentration of haloperidol was quantifiable in 100 % of the samples.
1.3. Determination of plasma concentration of risperidone and its metabolite

1.3.1. System performance

After the solvent front no interfering peaks were detected, reference sample from plasma spiked with 9-hydroxy-risperidone, risperidone and metoxi-risperidone (IS) is shown in Fig. 6.

![Figure 6. HPLC chromatogram of human plasma spiked with 10 nmol/L of 9-OH-risperidone, risperidone and metoxi-risperidone (IS)](image)

Retention times were 3.0, 4.2, and 6.8 min for 9-OH-risperidone, risperidone, and metoxi-risperidone (IS), respectively. The mean recoveries for risperidone and 9-hydroxy-risperidone calculated at three different concentrations (20, 40 and 80 nmol/l) were 100.5 and 102.6%, respectively (Table 7). 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.
### Table 7. Extraction recovery of risperidone and 9-OH-risperidone from plasma

<table>
<thead>
<tr>
<th>analyte</th>
<th>concentration added (nmol/l)</th>
<th>recovery (mean, n=3) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>risperidone</td>
<td>20</td>
<td>107.7</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>94.5</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>99.3</td>
</tr>
<tr>
<td>9-OH-risperidone</td>
<td>20</td>
<td>111.9</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>97.9</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>98.0</td>
</tr>
</tbody>
</table>

#### 1.3.2. Precision and accuracy

The intra-day and inter-day precision and accuracy were evaluated by analyzing blank plasma spiked with different amounts of 9-hydroxy-risperidone and risperidone. The results given in Table 8 show that the day-to-day variations for both compounds were less than 2%.

<table>
<thead>
<tr>
<th>Concentration (nmol/l)</th>
<th>n</th>
<th>%CV</th>
<th>Intra-day Accuracy (%)</th>
<th>Inter-day Accuracy(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>risperidone</td>
<td>20</td>
<td>3</td>
<td>0.46</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>3</td>
<td>0.49</td>
<td>95, 0.49, 95</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>3</td>
<td>1.40</td>
<td>100</td>
</tr>
<tr>
<td>9-OH-risperidone</td>
<td>20</td>
<td>3</td>
<td>1.84</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>3</td>
<td>1.34</td>
<td>95, 1.33, 95</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>3</td>
<td>0.65</td>
<td>99, 0.65, 99</td>
</tr>
</tbody>
</table>

#### Table 8. Precision and accuracy of the determination of risperidone and 9-OH-risperidone in spiked plasma

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%.

#### 1.3.3 Chromatographic interferences

The specificity of the method was tested by injecting 42 commonly used psychiatric drugs (Table 9). An amount of each was injected into the HPLC system and, after extraction, it was
found that the only one, which interfered with the assay, was levopromazine. Numerous antidepressants, anxiolytics and neuroleptics did not interfere.

Drugs were determined by analyzing 1 ml of plasma sample spiked with an amount of each compound.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Retention time (min)</th>
<th>Drug</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>amineptin</td>
<td>n.d.</td>
<td>imipramine</td>
<td>3.61</td>
</tr>
<tr>
<td>maprotiline</td>
<td>7.39</td>
<td>clomipramine</td>
<td>5.90</td>
</tr>
<tr>
<td>trimipramine</td>
<td>n.d.</td>
<td>fluoxetine</td>
<td>4.20</td>
</tr>
<tr>
<td>trazodone</td>
<td>4.21 and 7.36</td>
<td>lofepramine</td>
<td>4.23</td>
</tr>
<tr>
<td>amitryptiline</td>
<td>5.12</td>
<td>perphenazine</td>
<td>n.d.</td>
</tr>
<tr>
<td>pimozid</td>
<td>n.d.</td>
<td>haloperidol</td>
<td>n.d.</td>
</tr>
<tr>
<td>thioridazine</td>
<td>6.36</td>
<td>sulpiride</td>
<td>n.d.</td>
</tr>
<tr>
<td>levopromazine</td>
<td>3.54 and 3.92</td>
<td>trifluoperazine</td>
<td>n.d.</td>
</tr>
<tr>
<td>tioproperazine</td>
<td>n.d.</td>
<td>peryziacyne</td>
<td>n.d.</td>
</tr>
<tr>
<td>tiapridal</td>
<td>n.d.</td>
<td>halazepam</td>
<td>n.d.</td>
</tr>
<tr>
<td>midazolam</td>
<td>2.64</td>
<td>zopiclone</td>
<td>3.76</td>
</tr>
<tr>
<td>clorazepate</td>
<td>n.d.</td>
<td>bromazepam</td>
<td>7.10</td>
</tr>
<tr>
<td>flurazepam</td>
<td>n.d.</td>
<td>metazolame</td>
<td>n.d.</td>
</tr>
<tr>
<td>valpromide</td>
<td>n.d.</td>
<td>chlometiazol</td>
<td>3.89</td>
</tr>
<tr>
<td>phenobarbital</td>
<td>n.d.</td>
<td>pinazepam</td>
<td>n.d.</td>
</tr>
<tr>
<td>betazepam</td>
<td>n.d.</td>
<td>alprazolam</td>
<td>2.59 and 4.85</td>
</tr>
<tr>
<td>lorazolam</td>
<td>n.d.</td>
<td>lorazepam</td>
<td>2.65</td>
</tr>
<tr>
<td>triazolam</td>
<td>n.d.</td>
<td>zolpidem</td>
<td>3.61 and 6.63</td>
</tr>
<tr>
<td>lazepam</td>
<td>n.d.</td>
<td>lormetazepam</td>
<td>n.d.</td>
</tr>
<tr>
<td>chlorpromazine</td>
<td>n.d.</td>
<td>phrometazine</td>
<td>4.76</td>
</tr>
<tr>
<td>lithium</td>
<td>n.d.</td>
<td>fluvoxamine</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

n.d.: not detectable

**Table 9.** Chromatographic interferences with risperidone: retention times of selected drugs

1.4. Determination of plasma concentration of clozapine and its metabolite

1.4.1. System performance

A chromatogram of plasma from a healthy, drug-free donor spiked with 100 ng/ml clozapine and N-desmethylclozapine and a sample chromatogram of a schizophrenic patient treated with clozapine for more than one month is shown Fig. 7. The retention times for N-desmethylclozapine, clozapine and protriptylline were 3.9, 5.1, and 12.4 minutes, respectively.
The absolute recoveries of clozapine and N-desmethylclozapine were 92% and 37%, respectively. Plasma concentrations of clozapine and its metabolite were quantifiable in 100% of the samples.
1.4.2. Precision and accuracy

The within-day precision was determined by measuring the spiked plasma concentrations for each calibration sample from its corresponding calibration curve. At each concentration level samples were analyzed three times a day. The %CV was under 3.2% for clozapine and under 2.6% for N-desmethylclozapine at all calibration points. At each concentration level samples were analyzed on four different days in order to determine the inter-day precision. The %CV was less than 1.0% for both substances.

Blank plasma samples were spiked with clozapine and N-desmethylclozapine in the range 50-500 ng/ml and at each concentration level (n=5) these samples were analyzed in duplicates. Standard curves for clozapine and N-desmethylclozapine were linear in each case with correlation coefficients of 0.996 and 0.994, respectively. The limit of detection - defined as a signal-to-noise ratio of 4 - was 3 ng/ml for clozapine and 5 ng/ml for N-desmethylclozapine. The limit of quantification determined in triplicates was 25 ng/ml for both substances.

1.4.3. Chromatographic interferences

In total 47 psychotropic drugs - including 12 antipsychotics, 10 antidepressants, 16 benzodiazepines, 9 other drugs - were tested for possible chromatographic interference. The testing of standard solutions containing other psychotropic drugs that may be used in combination with clozapine, revealed no interferences for any of the commonly used drugs. The retention times of the most important psychotropic drugs are given in Table 10.

<table>
<thead>
<tr>
<th>Drug</th>
<th>t&lt;sub&gt;R&lt;/sub&gt; (min)</th>
<th>Drug</th>
<th>t&lt;sub&gt;R&lt;/sub&gt; (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>clozapine</td>
<td>-7.2</td>
<td>chlorpromazine</td>
<td>-4.5</td>
</tr>
<tr>
<td>N-desmethylclozapine</td>
<td>-8.5</td>
<td>haloperidol</td>
<td>-2.4</td>
</tr>
<tr>
<td>protriptyline (IS)</td>
<td>0</td>
<td>perphenazine</td>
<td>n.d.</td>
</tr>
<tr>
<td>trimipramine</td>
<td>-3.2</td>
<td>phrometazine</td>
<td>-6.8</td>
</tr>
<tr>
<td>amitriptyline</td>
<td>-3.5</td>
<td>alprazolam</td>
<td>1.2</td>
</tr>
<tr>
<td>imipramine</td>
<td>1.5</td>
<td>diazepam</td>
<td>7.1</td>
</tr>
<tr>
<td>maprotiline</td>
<td>2.6</td>
<td>lorazepam</td>
<td>-1.3</td>
</tr>
<tr>
<td>fluoxetine</td>
<td>n.d.</td>
<td>pinazepam</td>
<td>-6.5</td>
</tr>
<tr>
<td>fluvoxamin</td>
<td>n.d.</td>
<td>bentazepam</td>
<td>-8.3</td>
</tr>
</tbody>
</table>

n.d.: not detectable

Table 10. Relative retention times (t<sub>R</sub>) from internal standard (protriptyline 12.4) of some important psychotropic drugs that could be used in combination with clozapine (out of 49 tested drugs)
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. To compare with CYP2D6 phenotypes in healthy subjects, a control group of 336 Spanish, healthy volunteers was used. Of this population of healthy volunteers, 18 subjects were classified as PMs, while the rest (318 subjects, 94.6%) as EMs (Fig. 8, LLerena, 1988, De La Rubia, 1997). Of the 65 patients on thioridazine monotherapy, 53 had debrisoquine log MR>1.1 and thus were phenotypically PMs of CYP2D6 (De La Rubia, 1997). In the haloperidol patient group consisting of 27 patients, 4 were phenotypically PMs of CYP2D6 as shown in Fig. 9. 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 (Fig. 9).

![Healthy volunteers](image)

**Figure 8.** The histogram of the distribution of debrisoquine MR in Spanish healthy volunteers (n=336) control group (from De La Rubia 1997 with permission)
Figure 9. The histogram of the distribution of log debrisoquine MR in patients on (A) haloperidol monotherapy (n=27) and (B) risperidone monotherapy (n=27), compared to the previous study of a group of patients treated on (C) thioridazine monotherapy (n=65) (De La Rubia, 1997, with permission)
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 ($r=0.47$, $p<0.001$) (Fig. 10). In patients receiving risperidone monotherapy ($n=27$) the administered dose did not correlate with debrisoquine MR (Fig. 10). Previously, we found in patients treated with thioridazine a correlation of debrisoquine MR with the dose of thioridazine ($p<0.001$, De la Rubia, 1997) (Fig. 11).

Figure 10. Correlation of the debrisoquine MR with the daily dose of haloperidol (A) and risperidone (B)
3.1. The effect of thioridazine dose changes on the debrisoquine Metabolic Ratio (MR)

A new study was designed to analyze the previously found correlation of debrisoquine MR and thioridazine dose (Fig. 11, De la Rubia, 1997). Thioridazine, mesoridazine and sulforidazine plasma levels, the CYP2D6 genotype and the debrisoquine MR of the patients are listed in Table 11. 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 (Table 11).

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 (Fig. 12).

<table>
<thead>
<tr>
<th>Patient</th>
<th>CYP2D6 genotype</th>
<th>Thioridazine dose</th>
<th>Debrisoquine MR</th>
<th>Thioridazine plasma levels</th>
<th>Mesoridazine plasma levels</th>
<th>Sulforidazine plasma levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>wt/wt</td>
<td>100</td>
<td>29.3</td>
<td>0.64</td>
<td>1.12</td>
<td>0.31</td>
</tr>
<tr>
<td>1-R1</td>
<td></td>
<td>50</td>
<td>27.4</td>
<td>0.19</td>
<td>0.31</td>
<td>0.11</td>
</tr>
<tr>
<td>1-R2</td>
<td></td>
<td>0</td>
<td>1.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>wt/wt</td>
<td>100</td>
<td>10.0</td>
<td>0.34</td>
<td>1.67</td>
<td>0.31</td>
</tr>
<tr>
<td>2-R1</td>
<td></td>
<td>50</td>
<td>5.3</td>
<td>0.20</td>
<td>1.18</td>
<td>0.21</td>
</tr>
<tr>
<td>2-R2</td>
<td></td>
<td>0</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>wt/wt</td>
<td>150</td>
<td>67.9</td>
<td>1.90</td>
<td>2.40</td>
<td>0.63</td>
</tr>
<tr>
<td>3-R1</td>
<td></td>
<td>50</td>
<td>45.4</td>
<td>0.62</td>
<td>2.85</td>
<td>0.16</td>
</tr>
<tr>
<td>3-R2</td>
<td></td>
<td>0</td>
<td>3.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>wt/wt</td>
<td>50</td>
<td>40.8</td>
<td>1.03</td>
<td>1.74</td>
<td>0.41</td>
</tr>
<tr>
<td>4-R1</td>
<td></td>
<td>0</td>
<td>2.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>wt/wt</td>
<td>100</td>
<td>8.4</td>
<td>0.11</td>
<td>0.82</td>
<td>0.14</td>
</tr>
<tr>
<td>5-R1</td>
<td></td>
<td>0</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>wt/wt</td>
<td>100</td>
<td>34.1</td>
<td>0.82</td>
<td>1.86</td>
<td>0.22</td>
</tr>
<tr>
<td>6-R1</td>
<td></td>
<td>50</td>
<td>26.4</td>
<td>0.66</td>
<td>0.78</td>
<td>0.11</td>
</tr>
<tr>
<td>6-R2</td>
<td></td>
<td>0</td>
<td>11.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>wt/wt</td>
<td>150</td>
<td>46.5</td>
<td>0.62</td>
<td>1.02</td>
<td>0.31</td>
</tr>
<tr>
<td>7-R1</td>
<td></td>
<td>0</td>
<td>11.1</td>
<td>-</td>
<td>-</td>
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<td>wt/wt</td>
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<td>30.1</td>
<td>0.84</td>
<td>1.59</td>
<td>0.45</td>
</tr>
<tr>
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<td></td>
<td>150</td>
<td>20.3</td>
<td>0.29</td>
<td>0.91</td>
<td>0.23</td>
</tr>
<tr>
<td>9</td>
<td>wt/wt</td>
<td>200</td>
<td>21.6</td>
<td>0.84</td>
<td>1.58</td>
<td>0.40</td>
</tr>
<tr>
<td>9-R1</td>
<td></td>
<td>100</td>
<td>16.3</td>
<td>0.40</td>
<td>1.31</td>
<td>0.21</td>
</tr>
<tr>
<td>9-R2</td>
<td></td>
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<td>7.5</td>
<td>0.39</td>
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</tr>
<tr>
<td>10</td>
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<td>0.94</td>
<td>0.39</td>
</tr>
<tr>
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</tr>
<tr>
<td>11</td>
<td>wt/wt</td>
<td>200</td>
<td>35.5</td>
<td>0.49</td>
<td>1.18</td>
<td>0.33</td>
</tr>
<tr>
<td>11-R1</td>
<td></td>
<td>100</td>
<td>27.7</td>
<td>0.31</td>
<td>0.60</td>
<td>0.16</td>
</tr>
<tr>
<td>12</td>
<td>wt/*4</td>
<td>50</td>
<td>18.5</td>
<td>0.41</td>
<td>1.35</td>
<td>0.19</td>
</tr>
<tr>
<td>12-R1</td>
<td></td>
<td>0</td>
<td>4.1</td>
<td>-</td>
<td>-</td>
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<td>wt/*4</td>
<td>150</td>
<td>81.7</td>
<td>1.67</td>
<td>1.86</td>
<td>0.40</td>
</tr>
<tr>
<td>13-R1</td>
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<td>50</td>
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<td>0.40</td>
<td>0.65</td>
<td>0.10</td>
</tr>
<tr>
<td>14</td>
<td>wt/*4</td>
<td>100</td>
<td>18.1</td>
<td>0.28</td>
<td>1.11</td>
<td>0.19</td>
</tr>
<tr>
<td>14-R1</td>
<td></td>
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<td>18.3</td>
<td>0.20</td>
<td>0.66</td>
<td>0.12</td>
</tr>
<tr>
<td>15</td>
<td>*4/*4</td>
<td>20</td>
<td>33.7</td>
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<td>-</td>
</tr>
<tr>
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<td>21.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>*4/*4</td>
<td>100</td>
<td>20.9</td>
<td>1.52</td>
<td>1.32</td>
<td>0.22</td>
</tr>
<tr>
<td>16-R1</td>
<td></td>
<td>0</td>
<td>20.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 11.** The CYP2D6 genotype, thioridazine daily dose (mg/day), debrisoquine metabolic ratio (MR) and plasma concentration of thioridazine and its metabolites (μmol/L) in patients (n=16) receiving thioridazine monotherapy at an initial dose level and after dose changes (R1 - first dose change, R2 - second dose change)
Figure 12. Correlation of the debrisoquine metabolic ratio (MR in log scale) with the thioridazine dose in different genotype groups (CYP2D6 EM homozygotes, heterozygotes, and PM monozygotes)
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) (De la Rubia, 1997).

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 µg/ml/mg).

The dose corrected mean plasma concentration (C/D) was 2.97±4.04 ng/ml/mg (range: 0.22-17.38) for risperidone and 12.04±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±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 (De la Rubia, 1997) (Fig. 13).

The plasma levels of haloperidol and the daily dose correlated significantly (r=0.8, p<0.001) (Fig. 14).

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.
**Figure 13.** Correlation of thioridazine plasma concentration with the administered daily dose (De la Rubia, 1997, with permission)

**Figure 14.** Correlation of haloperidol plasma concentration with the administered daily dose
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 (De la Rubia, 1997), while mesoridazine or sulforidazine did not. The correlation between the debrisoquine MR and the plasma levels of haloperidol was lower, but significant (r=0.38, p<0.05) (Fig. 15). 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 (Fig. 16).

Figure 15. The correlation between the debrisoquine MR and the dose-corrected plasma concentrations (C/D) of haloperidol

Figure 16. The correlation between the debrisoquine MR to the dose-corrected plasma concentrations (C/D) of risperidone
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 (Fig. 17). 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 (Fig. 18).

**Figure 17.** The correlation between the debrisoquine MR and the plasma ratio of thioridazine/mesoridazine in 27 patients receiving thioridazine monotherapy

**Figure 18.** The relationship between the debrisoquine MR and the risperidone/9-OH-risperidone ratio (CYP2D6 activity) among forty patients treated with risperidone ($r=0.79$, $p<0.001$)
6. THE EFFECTS OF SMOKING ON THE ELIMINATION OF ANTIPSYCHOTIC DRUGS

As we have shown previously (De la Rubia, 1997) smokers received higher doses of thioridazine (mean dose±S.D.: 150±101 mg/day) than non-smokers (116±63 mg/day). 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±0.21 versus 0.45±0.30 ng/ml/mg). However the log debrisoquine MR (mean±S.D.) was significantly (p<0.05) higher in non-smokers (1.0±0.9), than in smokers (0.2±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 (Table 12 and 13). Among the patients receiving concurrently strong inhibitors of CYP2D6, the debrisoquine MR (p<0.01) and the risperidone/9-OH-risperidone ratio were increased (p<0.01). The risperidone plasma concentration/dose ratio was almost six times higher in comedicated patients than in the group treated with monotherapy (p<0.01) (Table 12).

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>No. of patients</th>
<th>Concomitant drug treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monotherapy</td>
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<td>-</td>
</tr>
<tr>
<td>Coadministered drug:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>strong CYP2D6 inhibitor</td>
<td>2</td>
<td>20 mg/day paroxetine</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>20 mg/day citalopram</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>200 mg/3 weeks zuclopenthixol depot</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>250 mg fluphenazine/3 weeks</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>100 mg/day chlorpromazine + 200 mg/3 weeks zuclopenthixol depot</td>
</tr>
<tr>
<td></td>
<td>1 (8 in total)</td>
<td>50 mg/day thioridazine</td>
</tr>
<tr>
<td>Coadministered drug:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>weak/no CYP2D6 inhibition</td>
<td>2</td>
<td>75 mg/day clozapine</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>300 mg/day clozapine</td>
</tr>
<tr>
<td></td>
<td>1 (5 in total)</td>
<td>3 mg/day biperiden</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

Table 12. Concomitant treatments in patients receiving risperidone treatment

<table>
<thead>
<tr>
<th></th>
<th>all patients</th>
<th>risperidone monotherapy</th>
<th>risperidone and strong CYP2D6 inhibitors</th>
<th>risperidone and other drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of patients</td>
<td>40</td>
<td>27</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>average dose risperidone</td>
<td>4.53±2.34</td>
<td>4.19±2.19</td>
<td>5.43±3.0</td>
<td>5.25±2.12</td>
</tr>
<tr>
<td>9-OH-risperidone</td>
<td>2.96±4.05</td>
<td>1.5±2.38</td>
<td>7.0±3.87*</td>
<td>3.88±5.86</td>
</tr>
<tr>
<td>R/9-OH-R ratio (log)</td>
<td>-0.77±0.53</td>
<td>-0.94±0.45</td>
<td>-0.25±0.25*</td>
<td>-0.71±0.66</td>
</tr>
<tr>
<td>debrisoquine MR (log)</td>
<td>-0.35±0.69</td>
<td>-0.69±0.48</td>
<td>0.52±0.29*</td>
<td>-0.07±0.69</td>
</tr>
</tbody>
</table>

* R/9-OH-R ratio: risperidone/9-OH-risperidone ratio
* statistically different form monotherapy group, p<0.01

Table 13. The dose-corrected (C/D) plasma concentrations of risperidone and 9-OH-risperidone in different subsets (Table 11) of patients receiving risperidone under steady state conditions
8. ANTIPSYCHOTIC TREATMENT AND ITS SIDE-EFFECTS

8.1. Extrapyramidal side effects

Each symptom characteristic of the drug-induced extrapyramidal syndrome (akinesia, rigidity and tremor) was rated on the three-point UKU scale. These points were added (maximum: 9) and patients reaching a score of four or more points were designated as having 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 µmol/liter, 95% CI 0.3-1.06 versus 0.41 µmol/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 (1.24, PM) and risperidone/9-OH-risperidone ratio (6.95).

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 (Fig. 19). The mean dose of thioridazine among patients with QTc>420 msec (n=35, 54%) was significantly higher (167±106 [95%CI: 130-203] versus 109±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, Table 13). 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 (Fig. 20). Also, all but one patient with QTc higher than 456 msec were PMs. QTc interval between 420 and 456 msec was found in 30 patients, and 90% of them were PMs.

<table>
<thead>
<tr>
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<th>Correlation coefficient</th>
<th>95% CI</th>
<th>Significance</th>
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<tr>
<td>Thioridazine dose (mg/day)</td>
<td>r=0.27</td>
<td>[0.016 – 0.484]</td>
<td>p=0.033</td>
</tr>
<tr>
<td>Thioridazine plasma concentration</td>
<td>r=0.27</td>
<td>[0.015 – 0.484]</td>
<td>p=0.033</td>
</tr>
<tr>
<td>Debrisoquine MR (log)</td>
<td>r=0.31</td>
<td>[0.069 – 0.524]</td>
<td>p=0.01</td>
</tr>
<tr>
<td>Thioridazine/mesoridazine ratio</td>
<td>r=0.27</td>
<td>[0.015 – 0.483]</td>
<td>p=0.033</td>
</tr>
</tbody>
</table>

*Table 13. Correlation between the corrected QT interval (QTc) and the thioridazine dose, plasma concentration and thioridazine/mesoridazine ratios*

*Figure 19. Correlation between QTc interval and thioridazine dose in patients receiving thioridazine antipsychotic monotherapy (n=65). The cut-off value for limit of risk of arrhythmia at 456 ms (dotted line); upper normal limit for QTc interval at 420 ms (solid line).*
Figure 20. Correlation between the QTc interval and the debrisoquine MR (log) in patients receiving thioridazine antipsychotic monotherapy (n=65).
### 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 main results are shown in Table 15.

<table>
<thead>
<tr>
<th>Days</th>
<th>1-5</th>
<th>6-7</th>
<th>8-11</th>
<th>12-17</th>
<th>18-23</th>
<th>24-30</th>
<th>31-37</th>
<th>38-56</th>
</tr>
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<tbody>
<tr>
<td><strong>Drugs (mg/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>clozapine</td>
<td>300→0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>75→100</td>
<td>100→175</td>
<td></td>
</tr>
<tr>
<td>sertindole</td>
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<td>4</td>
<td>8</td>
<td>12→20</td>
<td>20</td>
<td>20→12</td>
<td>12→0</td>
</tr>
<tr>
<td>valproic acid</td>
<td>-</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>900→1800</td>
<td>1800→900</td>
<td>900</td>
<td>900</td>
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<tr>
<td>clonazepam</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4→6</td>
<td>3→0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>zuclopenthixol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3x100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>alprazolam</td>
<td>0→6</td>
<td>6</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**BPRS (days: 8, 16, 23, 30)**

<table>
<thead>
<tr>
<th>Days</th>
<th>32</th>
<th>59</th>
<th>56</th>
<th>39</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma levels of clozapine (ng/ml, days: 16, 30, 52)</strong></td>
<td>0</td>
<td>76</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma levels of N-desmethylclozapine (ng/ml, days: 16, 30, 52)</strong></td>
<td>0</td>
<td>56</td>
<td>78</td>
<td></td>
</tr>
</tbody>
</table>

**Table 15.** The drug treatment of the patient, the corresponding Brief Psychiatry Rating Scale (BPRS) values (on days 8, 16, 23, 30), and the plasma concentrations (ng/ml) of clozapine and its main metabolite N-desmethyl clozapine (on days 16, 30, 52)
V. 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 therapeutical doses. The CYP2D6 activity affects the plasma levels of the thioridazine, haloperidol and risperidone and, therefore, may influence the therapeutical 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.

The present HPLC method developed for the determination of thioridazine and its metabolites allows a rapid, sensitive and reliable measurement of the plasma levels of thioridazine, mesoridazine and sulforidazine in human plasma comparing to previously published methods (Stoll et al., 1984; Allender, 1985; Whelpton et al., 1988; Svensson et al., 1990). Chlorpromazine was the only drug that could potentially interfere with the determination of thioridazine and its metabolites. Therefore, caution should be taken when analyzing thioridazine samples with the present method from patients comedicated with chlorpromazine. However, this combination is not frequently used in clinical practice.

The plasma levels of risperidone and 9-OH-risperidone were determined by high performance liquid chromatography based on a method originally developed at Huddinge
Hospital, Sweden (Svensson JO, personal communication), with modifications. The method was aimed to be a simple and specific method for the quantitative determination of risperidone and 9-OH-risperidone in plasma samples. The method was designed to be rapid, simple, specific and easy to perform. The principal advantage of the method presented here relative to other published methods (Price and Hoffman, 1997) is the use of liquid-liquid sample preparation. Liquid-liquid extraction is less expensive than solid-phase sample preparation. The intra-day and inter-day variations of risperidone and 9-hydroxyrisperidone (<2%) is comparable with previous result (Woestenborgs et al., 1992; Balant-Gorgia et al., 1999). This method is able to quantify the two substances with a good precision in the range 10-160 nmol/l and is well adapted for the monitoring of plasma levels in psychiatric patients. The plasma concentration of risperidone was below the detection limit in almost 33% of the samples. This result agrees with a former report (Aravagiri et al., 1998) where 23% of samples had undetectable amounts of the drug. According to these data in 1/3 of the patients taking risperidone the pharmacological effect might be attributed only to 9-OH-risperidone.

A simple HPLC method suitable for simultaneous determination of clozapine and its major metabolite, N-desmethylclozapine in human plasma samples was developed. This method was aimed to be used with easy to handle equipments for the routine monitoring of plasma levels in a general psychiatric department or unit. Therefore the method was developed to be rapid, simple, specific, easy to perform and inexpensive. Protriptyline was regarded as an acceptable internal standard because it exhibits similar linear extraction properties, i.e., hydrophobicity, basic pKₐ, and chromatographs proximate like the two analyte peaks. Baseline separation could be obtained by HPLC for all compounds to be determined within less than 15 minutes. The procedure enabled the determination of both clozapine and N-desmethylclozapine using liquid-liquid extraction method and HPLC with ultraviolet detection. Lovdahl et al. (1991) found the extraction recoveries of 84% for clozapine and 28% for N-desmethylclozapine with a similar extraction method. The present method proved to be more sufficient with extraction recoveries of 92% for clozapine and 37% for N-desmethylclozapine. The intra-day and inter-day variations of clozapine and N-desmethylclozapine were found to be better than in most of other published methods, <1% versus 4% for intra-day and <3.2% versus 6% for inter-day variations (Freeman et al., 1996; Weigmann et al., 1997; Guitton et al., 1997; Akerman, 1997; Avenoso et al., 1998). Clozapine and its metabolite could be simultaneously quantified at concentrations as low as 25 ng/ml. Plasma levels of these drugs at therapeutically effective doses (100-800 ng/ml) are far above the detection limit of this method (Liu et al., 1996).
Since the method was aimed for routine plasma level measurement of clozapine, the range of reliable response was determined in order to cover the usual plasma concentration levels of patients taking clozapine. With the HPLC system described here, no other important psychiatric drug showed any interference with CZP, its metabolite or the internal standard.

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. In our study the ratio of patients with debrisoquine MR>12.6 was 81.5% (De la Rubia, 1997). Thioridazine has been found to be a strong inhibitor of CYP2D6 both \textit{in vitro} (von Bahr et al. 1985) and in patients (Benítez et al., 1989, Spina et al., 1991, Baumann et al., 1992). 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 (LLerena et al., 1993b).

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 \textit{CYP2D6*4} allele (Table 11). 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 (Fig. 12). The inhibition of the enzyme activity was related to the parent compound thioridazine, since neither mesoridazine, nor sulforidazine had any considerable effect on the debrisoquine MR.
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 (Wilens and Stern, 1990; Maynard and Soni, 1996).

The results presented here 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 (Spina et al., 1991; LLerena et al., 1987; LLerena et al., 1993b), it can be stated that after this period no therapeutical consequences of the former CYP2D6 enzyme inhibition are to be expected in clinical practice.

Thioridazine is a phenothiazine antipsychotic drug, which is frequently used in the elderly population (Schneider, 1993). This population is usually taking several drugs concomitantly, and this increases the risk of drug-drug interactions. Due to the observed CYP2D6 enzyme inhibition by thioridazine, there is a certain risk of undesired drug-drug interactions if thioridazine is coadministered with agents that are also substrates of this enzyme (such as tricyclic antidepressants, beta-blockers, or other antipsychotics). In support of this hypothesis, potentially dangerous pharmacokinetic interactions with thioridazine have been reported in recent years (Wilens and Stern, 1990; Maynard and Soni, 1996).

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%) (LLerena et al., 1996). The present study confirms the inhibition of CYP2D6 by haloperidol under steady-state conditions in patients (LLerena et al., 1993b).

In the present study no inhibition of the enzyme activity was observed with risperidone among patients, which supports the in vitro data from the literature, where Shin at al. (1999) found that the estimated $K_i$ values for CYP2D6-catalyzing dextrorphan formation (marker of enzyme activity) were higher for risperidone (21.9 nmol/litre) than for thioridazine (1.4 nmol/litre), chlorpromazine (6.4 nmol/litre) or haloperidol (7.2 nmol/litre). 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 occur (Wilens and Stern, 1990; Maynard and Soni, 1996). 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 dose-corrected plasma levels of thioridazine and its metabolites, mesoridazine and sulforidazine showed large interindividual variability (De la Rubia, 1997). 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 (Von Bahr et al., 1991). To our knowledge, no data concerning the relationship between the CYP2D6 genotype and thioridazine metabolism under steady-state conditions in patients receiving monotherapy has been published so far. 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 (Eap at al., 1996).

The results of the present study also 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 (Huang et al., 1993; Mannens et al., 1993). The present data agree with the previously reported association between the CYP2D6 PM genotype and increase of the risperidone/9-OH-risperidone ratio (Scordo et al., 1999), since the ratio of risperidone/9-OH-risperidone correlated strongly with the debrisoquine MR (Fig. 18). In an in vitro study (Fang et al., 1999), as well as in reports from clinical settings (Bork et al., 1999; Spina et al., 2000b) CYP3A4 was also suggested to be involved in the metabolic conversion of risperidone to 9-OH-risperidone, although at therapeutical doses CYP2D6 may be the main enzyme of biotransformation. The contribution of the CYP3A4 enzyme may be dominant in the CYP2D6 PM patients or when the CYP2D6 enzyme activity is inhibited by concomitant drug treatment (Bork et al., 1999; Spina et al., 2000b).

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 (Scordo et al., 1999). Similarly, no correlation was found between the active moiety and the debrisoquine MR in the present study. In in vitro (animal brain tissue and cloned human receptors) and ex vivo (quantitative receptor autoradiography in animal brain sections) studies, risperidone and 9-hydroxy-risperidone were reported to exert similar pharmacological effects on brain 5-HT_2 and D_2 receptors (Leysen et al., 1994; Megens et al., 1994). Furthermore, positron emission tomography (PET) data in healthy volunteers showed that the receptor occupancies after a single 1 mg dose of risperidone were similar in CYP2D6 EM and PM patients (Nyberg et al., 1995). However, according to a recent study CYP2D6 poor metabolizers, who were enzyme deficient, did not appear to tolerate risperidone well (Bork et al., 1999).

In the present study also the only patients who needed antiparkinsonian comedication with biperiden had the highest log debrisoquine MR (1.24, PM) 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.

5. CORRELATION BETWEEN THE DEBRISOQUINE METABOLIC RATIO AND THE DRUG/METABOLITE RATIO

One of the findings of the study was that the plasma concentration ratio of thioridazine/mesoridazine correlated significantly with the debrisoquine MR (LLerena et al., 2000). 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 risperidon/9-OH-risperidone ratio may allow us to assess the CYP2D6 enzyme capacity by monitoring the therapeutic drug levels of risperidone and its metabolites. Being aware of the actual activity of the enzyme might be useful in clinical practice to estimate the possible risk of drug interactions that might be caused by the impaired CYP2D6 enzyme activity. 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. This is an important finding, since 72% of the patients were smokers. A significant difference was also found in the thioridazine/mesoridazine ratio between the groups of smokers and non-smokers (De la Rubia, 1997). 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. This enzyme is probably not CYP2D6, since its activity is only slightly, if at all, influenced by smoking (LLerena et al., 1996). In addition, there was no difference in the debrisoquine MR between smokers and non-smokers in this study, which supports the involvement of an enzyme different from CYP2D6. We have previously described that the concomitant use of fluvoxamine increased thioridazine plasma levels markedly (Carillo et al., 1999). Since fluvoxamine is a potent inhibitor of the CYP1A2 activity and CYP1A2 can be induced by smoking (Bock et al., 1994), we suggest that CYP1A2, in addition to CYP2D6, is involved in the metabolism of thioridazine.

It can be useful to know the CYP2D6 activity (the debrisoquine MR and the CYP2D6 genotype) in patients treated with thioridazine, because high plasma levels of thioridazine may result in side-effects (Hale and Poklis, 1986; Drolet et al., 1999). The influence of environmental factors must be taken into account as well. 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, since in our sample the mean daily dose of haloperidol was even lower (0.1±0.11 mg/kg body weight), but no difference was found between smokers and non-smokers. Several authors have reported that smokers have significantly lower plasma levels of haloperidol than non-smokers (Jann et al., 1986, Shimoda et al., 1999), and that the clearance of haloperidol is higher in smokers (Miller et al., 1990). However, none of these studies offered an explanation for all interindividual differences, even in case of statistically significant differences between the haloperidol plasma levels of smokers and non-smokers. Shimoda et al. (1999) found that the difference between smokers and non-smokers was statistically significant only in the low-dose haloperidol group (with an arbitrary cut-off point of 0.2 mg/kg body weight daily dose). The interethnic differences in the CYP2D6 polymorphism might explain the observed discrepancy (Lou, 1990). However, the debrisoquine MR was significantly higher in non-
among smokers the CYP2D6 activity was higher, which may suggest that in this patient group an alternative metabolic pathway (i.e. CYP1A2) were induced by smoking and the competitive inhibition decreased at the CYP2D6 enzyme. The coadministration of fluvoxamine, a potent CYP1A2 inhibitor, has been reported to increase the haloperidol plasma levels (Vandel et al., 1995), which also supports the involvement of CYP1A2 in the metabolism of haloperidol. 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 (Balant-Gorgia et al., 1999).

7. DRUG INTERACTIONS AND THE CYP2D6 ENZYME ACTIVITY

The importance of pharmacokinetic drug interactions that involve cytochrome enzymes has been increasingly emphasized (LLerena and Kiivet, 1994), as CYP2D6 plays an important role in the metabolism of several important psychotropic agents and other drugs (i.e. beta-blockers, antidepressants, etc.) (LLerena et al., 1996). 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 (Table 12 and 13). 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. SIDE-EFFECTS

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 found to be related to a higher risk of extrapyramidal symptoms (Vandel et al., 1999; Scordo et al., 2000). 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. According to a recent study, CYP2D6 poor metabolizers, who were enzyme deficient, did not appear to tolerate risperidone well (Bork et al., 1999). The obtained results show that patients with impaired CYP2D6 enzyme activity due to genetic factors or enzyme inhibition have six times higher plasma concentrations of risperidone than EM patients, a fact which may influence the occurrence of side-effects. Thus, the differences in the clinical efficacy and side-effect profile of risperidone and 9-OH-risperidone in humans need further investigation.

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 (Schouten et al., 1991); therefore, a QTc prolongation greater than 456 msec indicates a high risk of arrhythmias and sudden deaths (Reilly et al., 2000). According to the present data, 50% of the patients were at risk of cardiac side-effects. Nevertheless, they were treated at the clinically recommended doses (average 140 mg/day).

The QTc interval correlated with the thioridazine dose, which confirms the results of previous studies investigating the dose-dependent QTc prolongation caused by thioridazine (Hartigen-Go et al., 1996; Reilly et al., 2000). Additionally, the present study also shows that at a dose
of 150 mg/day or higher the risk of potential cardiac side-effects is increased, since all patients with QTc over the upper normal limit fell in that dose range (Fig. 19). 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 results indicate that the plasma concentration of thioridazine is related to the lengthening of QTc interval among psychiatric patients receiving antipsychotic treatment with thioridazine. A unique study evaluating the plasma concentration–effect found no correlation in healthy volunteers (Hartigan-Go et al., 1996). The reason for this discrepancy may be the small number of subjects involved in that study and/or the low doses used (10 and 50 mg). These doses were much lower than the ones prescribed in the present study.

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 (Hale and Poklis, 1986). 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. The rate of PMs was very high (82%) as compared to healthy volunteers (Benítez et al., 1988) and support the idea of CYP2D6 inhibition by thioridazine treatment. Thus, according to the present data, PMs are prone to the risk of thioridazine cardiotoxicity (Fig. 20).

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 (LLerena et al., 2000) or from concomitant medications which are inhibitors or substrates of CYP2D6 (Carillo et al., 1999). Considering that thioridazine is frequently used among elderly patients (Schneider, 1993), 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 (Llerena et al., 2000) 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 11 days of 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 (Perry et al., 1991; Liu et al., 1996; Spina et al., 2000a). 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.

9. CLINICAL USE OF PHARMACOGENETIC DATA

The results of the present research work have confirmed the importance of genetical and environmental factors in the metabolism of antipsychotic drugs, and thus in the therapeutical outcome and the risk of side-effects. It is to be noted, however, that the involvement of different factors shows substantial differences among the drugs investigated, therefore, a drug specific analysis of these factors is needed. In clinical practice, the influence of the CYP
enzyme activity can be determined by genotyping or phenotyping, and the overall influence is reflected by the plasma concentration of the administered drugs and their metabolites.

In the view of the present data, assessment of the CYP2D6 status might be a useful aid for clinical psychiatrists to predict interindividual variability in the plasma concentration of antipsychotic drugs and to tailor therapeutic regimens to the individual patient. The cost/benefit ratio of monitoring the plasma levels of antipsychotic drugs and CYP enzyme phenotyping and genotyping is the focus of thorough research at present (De Leon et al., 1998; Van der Weide and Steijns, 1999; Chou et al., 2000).

The present data suggest that the genetically inherited activity is modified by different environmental factors (such as drug inhibition, concomitant drug treatment, and smoking), therefore, genotyping gives only limited information on the actual activity of the enzyme. The suggested new use of therapeutical drug monitoring to calculate parent drug/metabolite ratios may provide a mean to predict the enzyme activity without phenotyping. Since the phenotyping procedure (giving probe drug and collect urine) may result difficult in psychiatric patients, these ratios may be a valuable help to the physician to evaluate possible pharmacokinetic interactions or unpredictable adverse effects when doses are changed or a new drug is added to the therapeutical regimen of the patient.

Nevertheless, at present in clinical practice if unexpected side-effects occur or therapeutic failure is observed with antipsychotic drugs, the possible involvement of pharmacogenetic factors should also be considered and the adequate examinations (plasma concentrations of drug and metabolite, CYP2D6 phenotyping and genotyping) be carried out.
VI. CONCLUSIONS
1. GENERAL CONCLUSION

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.

2. SPECIFIC CONCLUSION

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 clinical 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. The clinical consequences of this inhibition can be drug interactions or side-effects (e.g. cardiotoxicity) due to higher than expected plasma levels, since the inhibition may lead to drug interactions and also to the occurrence of side-effects.

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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Figure 4. The main metabolic pathways of risperidone disposition

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Figure 8. The histogram of the distribution of debrisoquine MR in Spanish healthy volunteers (n=336) control group.

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Figure 20. Correlation between the QTc interval and the debrisoquine MR (log) in patients receiving thioridazine antipsychotic monotherapy (n=65)
ANNEX II.

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