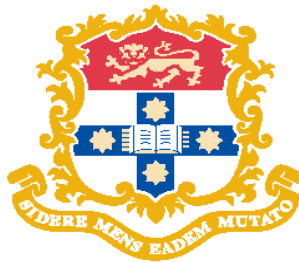


A Comparison of the Effect of Omeprazole and Rabeprazole on Clozapine Serum Concentrations

Naghmeh Jabarizadekivi

A thesis submitted in fulfillment of the
requirements for the degree of
Master of Philosophy



Faculty of Pharmacy
The University of Sydney

November 2007

*Apart from the assistance which is acknowledged herein, this thesis
represents the original work of the author.*

Naghmeh Jabarizadekivi

Preface:

Clozapine is a drug of choice for treatment of refractory schizophrenia, which is primarily metabolized by Cytochrome P450 1A2 (CYP1A2). Norclozapine is its main metabolite. There are reports of wide ranging gastrointestinal side effects associated with clozapine therapy, that result in concomitant administration of proton pump inhibitors to treat acid-related disorders. Omeprazole is an established CYP1A2 inducer, while an *in vitro* study has shown that rabeprazole is much less potent in this regard. There is no available information about the impact of rabeprazole on CYP1A2 activity in patients.

Firstly, this information is essential when prescriptions are changed from omeprazole to rabeprazole to reduce medication costs. Therefore, the aim of this study was to compare the effects of rabeprazole and omeprazole on CYP1A2-mediated clearance (CL/F) of clozapine.

Secondly, the effective dosage of clozapine varies widely among patients, making it necessary to individualize drug therapy with clozapine. The reason for dosage variation could be due to the influence of patient-related variables on clozapine plasma concentrations. Therefore, another aim of this study was to investigate the relationship between patient variables, such as age, gender, cigarette smoke, weight and body mass index and clozapine clearance (CL/F).

A cross-over study design was used for this study. Twenty patients from Macquarie hospital who were receiving clozapine and rabeprazole (with no other interacting medications) were recruited in this study. Blood samples were taken at 30 min, 1 hr, 2 hr and 12 hr after a dose of clozapine. Rabeprazole was then replaced with omeprazole. After at least 1 month blood samples were again collected at the above corresponding intervals after clozapine. The plasma concentrations of clozapine and norclozapine were determined by high performance liquid chromatography. Abbottbase Pharmacokinetic Systems Software, which utilizes Bayesian forecasting, was used to estimate pharmacokinetic parameters of clozapine. The ratio of plasma norclozapine/clozapine concentrations at trough level was used to reflect CYP1A2 activity.

No difference was observed in clozapine clearance (CL/F) and CYP1A2 activity during concurrent therapy with either rabeprazole or omeprazole.

According to some studies CYP1A2 induction by omeprazole is dose dependent. Furthermore, since rabeprazole is a weak CYP1A2 inducer *in vitro*, we conclude that omeprazole and rabeprazole may not induce CYP1A2 activity when used at conventional therapeutic dosage (<40 mg/day). Hence, replacement of omeprazole with rabeprazole at conventional therapeutic dosages (20 or 40 mg daily) offers no advantages in the management of patients with schizophrenia on clozapine and no dose adjustment is required.

Consistent with previous studies, clozapine concentrations were found to be significantly lower in cigarette smokers due to CYP1A2 induction.

No relationship was found between age, gender, or weight and clozapine clearance (CL/F). However, body mass index showed a significant negative correlation with clozapine clearance (CL/F).

Since weight gain and lipid accumulation are common side effects of clozapine they may be associated with a reduction of CYP1A2 activity and clozapine clearance (CL/F). Moreover, high lipoprotein levels may decrease the unbound fraction of clozapine and decrease the availability of clozapine for oxidation by cytochrome P450 enzymes.

Therefore, it is concluded that omeprazole and rabeprazole may not induce CYP1A2 activity when used at conventional therapeutic dosage (<40mg/day). Hence, replacement of omeprazole with rabeprazole does not require the dose of clozapine to be adjusted. Moreover, the negative correlation between clozapine clearance (CL/F) and BMI is informative. Further studies are now required to clarify the relationship between BMI, lipoprotein levels and clozapine clearance in patients with schizophrenia.

Acknowledgements

My deepest gratitude to Professor Michael Murray who kindly accepted to be my continuing supervisor after Dr Mano Chetty departed. I would like to thank him for his scientific guidance, philosophical structure and debate behind the science presented here. My thanks to Dr Mano Chetty for all her support and advice. Thank you again to Michael and Mano for their encouragement, leadership and inspiration over my study.

I would like to thank the clinical collaborators at Macquarie hospital who made this research possible, particularly John Glen, Dr Glenys Dore, Dr Seema Sharma, and Zvijezdana Stankovic.

Thank you to Andrew Ellis and Bruce Tattam for help with analytical assays to quantitate clozapine and norclozapine concentrations.

Thank you to my lab mates, Vivian, Susan, Fabrizio, and Noelia for their brilliant cooperation and friendship.

To my husband, Mahan, you have been the center of my life. Thank you for your incredible support. All my love.

To my parents, the kindest parents in the world and my lovely sister.

Presentations:

1. N Jabarizadekivi, B Tattam, J Glen, G Dore, S Sharma, Z Stankovic, M Murray and M Chetty. A comparison of the effect of omeprazole and rabeprazole on clozapine serum concentrations. 5th Research Conference 2006 “From Cell to Society 5”, Sydney, Australia.
2. N Jabarizadekivi, B Tattam, J Glen, G Dore, S Sharma, Z Stankovic, M Murray and M Chetty. A comparison of the effect of omeprazole and rabeprazole on clozapine serum concentrations. Postgraduate Research Conferences, Faculty of Pharmacy, University of Sydney, 2006.
3. N Jabarizadekivi, A Ellis, J Glen, G Dore, S Sharma, Z Stankovic, M Chetty and M Murray. A comparison of the effect of omeprazole and rabeprazole on clozapine serum concentrations. Final presentation, Faculty of Pharmacy, University of Sydney, 2007.
4. N Jabarizadekivi, A Ellis, J Glen, G Dore, S Sharma, Z Stankovic, M Chetty and M Murray. A comparison of the effect of omeprazole and rabeprazole on clozapine serum concentrations. The Australian Pharmaceutical Science Association Annual Conferences, 2007, Manly, Australia.

Abbreviations

ALT	Alanine Transaminase
AST	Aspartate Transaminase
AUC	Area Under the Concentration-Time Curve
AUC ₀₋₁₂	Area Under the Curve from Zero to Twelve Hours after the Dose
AUC _{0-∞}	Area Under the Curve from Zero to infinity Hours after the Dose
BMI	Body Mass Index
CL	Clearance
CL/F	Apparent Clearance
CL _h	Hepatic Clearance
CL _{int}	Intrinsic Enzyme Activity
C _{max}	Maximum Serum Concentration
CYP	Cytochrome P450 enzyme
EM	Extensive Metabolizer
FMO	Flavin-containing monooxygenase-3
F _u	Unbound Fraction of the Drug
GERD	Gastroesophageal Reflux Diseases
GGT	γ-glutamyl Transpeptidase
HPLC	High Performance Liquid Chromatography
hr	Hour
IM	Intermediate Metabolizers
K _i	Inhibition Constant
LFT	Liver Function Test
ME	Mean Prediction Error
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
PKS	Abbottbase Pharmacokinetic System
PM	Poor Metabolizer
PPIs	Proton Pump Inhibitors
RMSE	Root Mean Square Error
SNP	Single Nucleotide Polymorphism

SSRIs	Selective Serotonin Reuptake Inhibitors
T _{max}	Time to Reach Maximum Plasma Concentration
t _{1/2}	Half Life
UM	Ultrarapid Metabolizers
V _d	Volume of Distribution
V _d /F	Apparent Volume of Distribution

Table of Content

Preface	iii
Acknowledgments	vi
Presentations	vii
Abbreviations	viii
CHAPTER ONE: LITERATURE REVIEW	1
1.1. Cytochrome P450 and the clearance of xenobiotics	2
1.1.1 Drug Metabolism	2
1.1.2. Overview of the CYP System	3
1.1.3. Drug-Drug Interactions	15
1.1.4. Metabolic Drug-Drug Interactions	15
1.1.5. CYP Pharmacogenetics and Metabolic Interactions in Psychiatry	18
1.2. Schizophrenia and Drug Treatment	20
1.2.1. Typical Antipsychotics	22
1.2.2. Atypical Antipsychotics	23
1.2.2.1. Clozapine	25
1.2.2.1.1.Pharmacodynamics of Clozapine	25
1.2.2.1.2.Pharmacokinetics of Clozapine	26
1.2.2.1.3.Metabolism of Clozapine	29
1.2.2.1.4.Pharmacokinetic Drug Interactions of Clozapine	32
1.2.2.2. Other Atypical Antipsychotics	38
1.3. Proton Pump Inhibitors (PPIs): Pharmacology and Pharmacokinetic Interactions	41
1.3.1. Physiochemical Properties	41
1.3.2. Indications	42
1.3.3. Pharmacodynamics	42
1.3.4. Pharmacokinetics: Role of CYP2C19 Genotype	47
1.3.4.1. Metabolism of Omeprazole and Esomeprazole	49
1.3.4.2. Metabolism of Rabeprazole	50
1.3.4.3. Metabolism of Lansoprazole and Pantoprazole	52
1.3.5. Interaction Profile of PPIs	53
1.3.5.1. Modulation of Gastric pH	53
1.3.5.2. Interaction with P-glycoprotein Transporters	54
1.3.5.3. Interaction with CYP Enzymes	55
1.3.5.3.1. Inhibition of CYP-mediated Drug Oxidation by Omeprazole	55
1.3.5.3.2. Induction of CYP Genes by Omeprazole	57

1.3.5.3.3.	Inhibition of CYP Enzymes by Rabeprazole	59
1.3.5.3.4.	Induction of CYP Genes by Rabeprazole	59
1.4.	Aims of the Thesis	61
	CHAPTER TWO: MATERIALS AND METHODS	62
2.1.	Study Subjects	63
2.2.	Study Design	64
2.3.	Analytical Method	66
2.4.	Estimation of Clozapine Clearance	67
2.4.1.	Bayesian Model for Estimate of Clozapine Clearance Using Abbottbase Pharmacokinetic System (PKS)	67
2.5.	Statistical Analysis	70
	CHAPTER THREE: RESULTS	71
3.1.	Subjects	73
3.2.	Relationship Between Norclozapine/Clozapine Ratio in Plasma and Clozapine Clearance	75
3.3.	Comparison of the Effects of Omeprazole and Rabeprazole on Clozapine Clearance	79
3.3.1.	Influence of Smoking on Clozapine Concentrations	85
3.4.	Impact of Other Covariates on Clozapine Clearance	88
3.4.1.	Age	88
3.4.2.	Gender	80
3.4.3.	Weight	90
3.4.4.	Body Mass Index (BMI)	91
3.5.	Evaluation of the Model Obtained With the Abbottbase Bayesian PKS Program	94
3.5.1.	Internal Evaluation of PKS	94
3.5.2.	External Evaluation of PKS	98
	CHAPTER FOUR: DISCUSSION	100
	Appendix I: Patients' Co-mediations	111
	Appendix II: Participant Information Sheet	112
	Appendix III: Consent form to Participate in a research Project	118
	References	123

CHAPTER ONE: LITERATURE REVIEW

1.1. Cytochrome P450 and the Clearance of Xenobiotics

We are continually exposed to foreign chemicals from a number of different sources. The different sources of xenobiotics include the diet, food additives, cosmetic products and environmental pollutants. In addition, drugs are another important source of xenobiotics. Generally, xenobiotics are lipophilic chemicals, which are metabolized by complex enzymatic pathways in order to be cleared from the body. Cytochrome P450 enzymes (CYPs) are a major group of heme-containing microsomal enzymes involved in the metabolism of drugs. This enzymatic pathway is also responsible for the biosynthesis and degradation of endogenous compounds such as steroids, lipids, and vitamins (Gonzalez & Tukey 2004; Wilkinson 2005).

1.1.1. Drug Metabolism

Many drugs are hydrophobic which allows them to enter cells through lipid bilayers so that they may interact with intra-cellular receptors and other targets. This hydrophobic feature of drugs makes them difficult to be eliminated from the body. With a few exceptions, most drugs undergo phase 1 and phase 2 metabolism by different isoenzymes of CYP to increase their water solubility and promote their elimination in urine or bile.

In phase 1, drugs undergo oxidation, reduction or hydrolytic reactions. These chemical reactions occur mainly through the action of CYPs. During phase 1 some functional groups such as -OH, -COOH, and -O- are added to substrate in order to increase water solubility and which usually results in an inactive metabolite. However

in some cases, inactive drugs (pro drugs) are converted to active drugs by phase 1 metabolism.

Phase 1 is followed by phase 2 metabolism in which further enzymes form polar conjugates of the phase 1 metabolites. Phase 2 includes glucuronidation, sulfation, acetylation, or methylation and increases the elimination of drug from tissues (Gonzalez & Tukey 2004).

1.1.2. Overview of the CYP System

CYPs are a group of hemoproteins composed of a constant coenzyme (iron-protoporphyrin IX) and a variable protein (Figure 1.1). The substrate specificity of different CYPs is due to these differences in the protein portion (Testa & Kramer 2007). Beside liver, which is the major site of CYP expression, the epithelium of the small intestine, lungs, brain and skin are other important sites of expression (Wilkinson 2005).

The CYPs are membrane-bound enzymes. They are embedded in the lipid bilayer of the smooth endoplasmic reticulum membrane, although steroid metabolizing CYPs are located in mitochondria (Tanaka 1998; Testa & Kramer 2007). CYPs are connected to the membrane bilayer by their N-terminal region and to the cytosol via their C-termini (Testa & Kramer 2007). CYPs interact with flavoprotein NADPH and NADH cytochrome P450 oxidoreductases which transfer electrons to the CYPs. Figure 1.2 illustrates the interactions between CYPs and NADPH-CYP reductase within the membrane.

Figure 1.1. The overall fold of CYP1A2 (Lozano et al 1997)

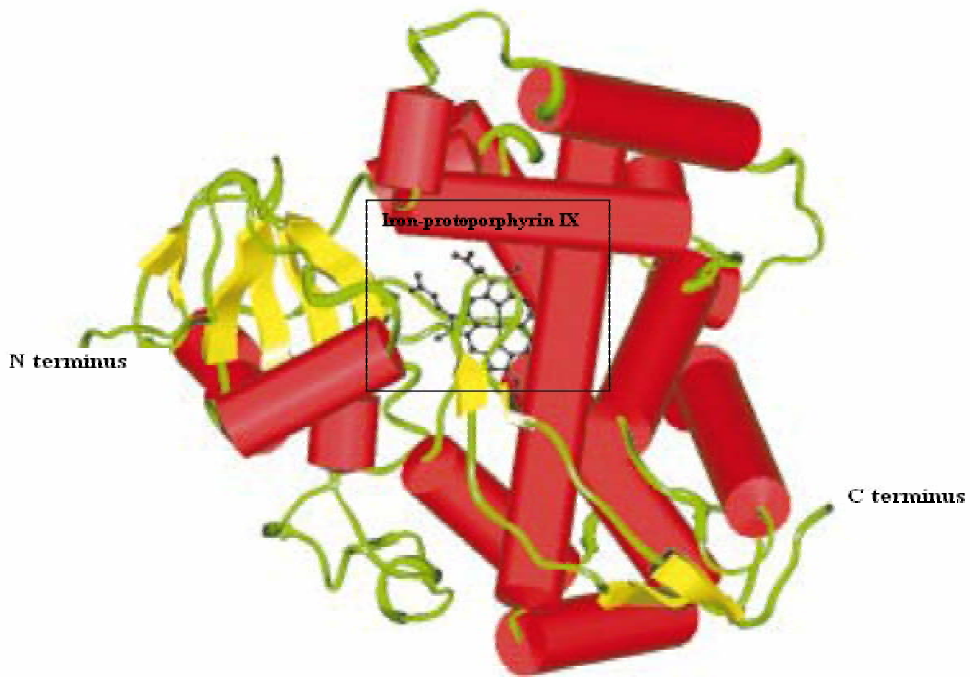
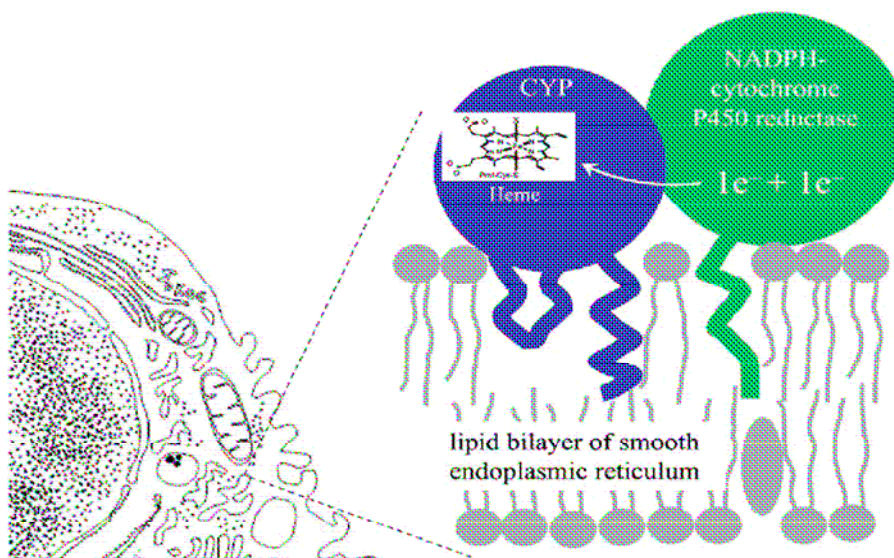


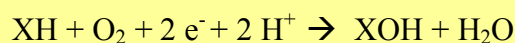
Figure 1.2. The membrane location of CYP (Testa & Kramer 2007)

The membrane location of cytochrome P450

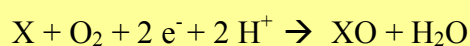


The main function of the CYP system is monooxygenation. Iron-protoporphyrin IX binds O₂ in the CYP active site. CYP accepts O₂ and an electron from NADPH, reduces and cleaves molecular oxygen, which allows one O-atom to be transferred to the substrate (Testa & Kramer 2007). Among the reactions carried out by CYPs are *N*-dealkylation, *O*-dealkylation, aromatic hydroxylation, *N*-Oxidation, *S*-oxidation, deamination, and dehalogenation as shown below, where X = substrate and XO = oxidized metabolite

(Gonzalez & Tukey 2004).



Or



CYPs are classified by their amino acid similarity. Cytochromes with 40% or greater sequence homology are placed in the same family, and those with 55% or greater homology are placed in the same subfamily (Nelson et al 1996). For instance, based on this nomenclature, CYP2C19 belongs to the subfamily C within family 2.

The nomenclature of CYP genes

CYP (root)

2 (family)

C (sub-family)

19 (individual gene)

To the present, 57 CYP genes have been identified in humans. Only a few of them are associated with the metabolism of drugs, mainly in the CYP1, CYP2 and CYP3 families (Wilkinson 2005). It has been estimated that six enzymes, CYP1A2, 2C9, 2C19, 2D6, 2E1 and 3A4/5, are responsible for oxidation of 90% of human drugs (Tanaka 1998). Individual CYP forms show affinity toward structurally unrelated compounds. Thus, more than one CYP may contribute to the metabolism of a particular drug (Gonzalez 1988). This unusual overlap of CYP activities is one of the main reasons for drug-drug interactions. Furthermore, the activity of CYPs may vary because of individual differences in expression (Roots et al 2004). Mutations in CYP genes can also contribute to inter or intra-individual differences in metabolism (Nebert & Russell 2002).

CYP1 family:

The members of this family include CYP1A1 and CYP1A2. CYP1A1 is located mainly in extrahepatic tissue, while CYP1A2 is expressed in liver and accounts for about 15% of the total hepatic CYP content (Raunio et al 1995). The CYP1 family is mainly responsible for the metabolism of planar polyaromatic/heterocyclic amines and amides, polycyclic aromatic hydrocarbon (PAHs), and polyhalogenated aromatic hydrocarbons (PHAHs). PAHs and PHAHs are potent inducers of the CYP1 family due to their strong affinities for the aryl hydrocarbon receptor (AHR), which is a member of the helix-loop-helix group of transcription factors (Nebert & Dalton 2006). CYP1A2 also metabolizes about 24 drugs, while CYP1A1 and CYP1B1 are less involved in drug metabolism (Nebert & Dalton 2006). Caffeine, a drug which is

highly dependent on CYP1A2 for its metabolism, can be used as a specific probe for *in vivo* CYP1A2 phenotyping. Thus, the paraxanthine/caffeine concentration ratio in plasma or urine reflects CYP1A2 activity (Doude van Troostwijk et al 2003b). Some commonly used drugs have been shown to induce and inhibit CYP1A2 activity. For example fluvoxamine is associated with CYP1A2 inhibition (Rasmussen et al 1995). Table 1.1 shows the main substrates, inducers and inhibitors of CYP1A2.

Some single nucleotide polymorphisms (SNPs) of the *CYP1A2* gene have been identified in the 5' flanking region and in intron 1 of *CYP1A2* (www.imm.ki.se/CYPalleles/cyp1a2.htm). Some of these are associated with the inducibility of CYP1A2 expression in smokers. For example, a study of 185 healthy Caucasian non-smokers and 51 smokers identified a -164C→A SNP in intron 1 of the *CYP1A2* gene (the *CYP1A2*1F* allele). In this study, smokers with the A/A genotype showed a 60 % to 70% higher CYP1A2 activity than those with either A/C or C/C genotype. It appears that the *CYP1A2*1F* allele which is observed in one-third of Caucasians could represent a highly inducible genotype of CYP1A2 (Sachse et al 1999). Another SNP identified in CYP1A2 is the -360 C→A polymorphism (*CYP1A2*1C* allele), which shows a lower activity in smokers and non-smokers (Han et al 2001). Despite these recent findings, Catteau et al have suggested that genetic factors are probably negligible in overall CYP1A2 activity (Catteau et al 1995). This is compatible with the very low incidence of SNPs in the coding region of CYP1A2 that produce altered polypeptide sequences.

Table 1.1. Some Important Substrates, Inhibitors and Inducers of CYP1A2
(www.medicine.iupui.edu/flockhart/table.htm; 27/10/2006)

CYP1A2 substrates	CYP1A2 inhibitors	CYP1A2 inducers
Antidepressants Amitriptyline Clomipramine Imipramine Fluvoxamine Antipsychotics Haloperidol Clozapine Olanzapine Methylxanthine/s Theophylline Caffeine Others Paracetamol Phenacetin Tacrine	Amiodarone Cimetidine Ciprofloxacin Fluoroquinolones Fluvoxamine Interferon Methoxsalen Mibefradil	Smoking Omeprazole Rifampicin Barbiturates Phenytoin Carbamazepine Insulin

CYP2C subfamily:

It has been suggested that the CYP2Cs have the most complex drug metabolizing properties of any subfamily (Rettie et al 2000). The CYP2C subfamily includes CYP2C8, CYP2C9, CYP2C18 and CYP2C19 and accounts for about 20% of total hepatic CYPs (Rettie et al 2000). The CYP2C subfamily is also associated with extensive pharmacogenetic variation. For example, 2% to 5% of Caucasians and 13% to 23% of Asians are poor metabolizers (PMs) for CYP2C19, and the remainders are extensive metabolizers (EMs) (Rettie et al 2000). Approximately 95% of the population exhibits two particular variant alleles of CYP2C19 (CYP2C19*2 and CYP2C19*3) that have been shown to result in the PM phenotype (Wedlund 2000). Diazepam is predominantly metabolized by CYP2C19. Thus, the plasma

concentrations of diazepam are higher in PM, which may result in prolongation of sedation in individuals exhibiting this phenotype (Rettie et al 2000). Proton pump inhibitors (PPIs) are also major substrates for CYP2C19. Studies have shown that the rate of healing for both gastric and duodenal ulcers with PPI therapy can be affected by CYP2C19 genotype and is more rapid in PMs (Furuta et al 1998; Klotz 2006). Hydroxylation of S-mephenytoin and omeprazole, which are prototype reactions for this enzyme, may be used for the determination of CYP2C19 activity (Testa & Kramer 2007). Table 1.2 shows major substrates, inducers and inhibitors of CYP2C19.

Genetic polymorphism is also exhibited by CYP2C9, the most abundant member of the CYP2C subfamily (Rettie et al 2000). A comprehensive review by Daly and King suggests that the maintenance dose of warfarin, which is primarily metabolized by CYP2C9, is dependent on CYP2C9 genotype (Daly & King 2003). For instance, patients who are heterozygous for CYP2C9*2/*3 may require lower doses than those who are homozygous or heterozygous for the wild-type enzyme (Daly & King 2003). Hydroxylation of diclofenac and tolbutamide are further reactions that can be attributed to CYP2C9 (Testa & Kramer 2007). Table 1.3 shows the principal substrates, inducers and inhibitors of CYP2C9. The available evidence suggests that the clinical importance of CYP2C8 and CYP2C18 is lower than other CYPs 2C.

Table 1.2. Some Important Substrates, Inhibitors and Inducers of CYP2C19
 (www.medicine.iupui.edu/flockhart/table.htm; 27/10/2006)

CYP2C19 substrates	CYP2C19 inhibitors	CYP2C19 inducers
<p>Proton Pump Inhibitors omeprazole Lansoprazole Pantoprazole Rabeprazole</p> <p>Anticonvulsants Diazepam Phenytoin Primidone Phenobarbitone</p> <p>Antidepressants Amitriptyline Clomipramine Imipramine Moclobemide</p> <p>Others Citalopram Indomethacin Propranolol Cyclophosphamide</p>	<p>Chloramphenicol Cimetidine Fluoxetine Fluvoxamine Indomethacin Ketoconazole Lansoprazole Omeprazole Probenecid Ticlopidine Topiramate</p>	<p>Carbamazepine Prednisone Rifampicin</p>

Table 1.3. Some Important Substrates, Inhibitors and Inducers of CYP2C9
 (www.medicine.iupui.edu/flockhart/table.htm; 27/10/2006)

CYP2C9 substrates	CYP2C9 inhibitors	CYP2C9 inducers
NSAIDs Diclofenac Ibuprofen Meloxicam S-naproxen Piroxicam Oral Hypoglycemic Agents Tolbutamide Glyburide Glibenclamide Glipizide Angiotensin II Antagonists Losartan Irbesartan Others Amitriptyline Fluoxetine Phenytoin Tamoxifen Torsemide S-warfarin	Amiodarone Fluconazole Fluvastatin Fluvoxamine Isoniazid Lovastatin Sertraline Zafirlukast	Rifampin Secobarbital

CYP2D subfamily:

The CYP2D gene is located on human chromosome 22 and is a CYP that has clinical importance in the metabolism of xenobiotics (Heim & Meyer 1992). The CYP2D6 enzyme is responsible for the metabolism of 25% of known drugs, and among them antipsychotic and antidepressant agents are major substrates (de Leon et al 2006). Table 1.4 shows some important substrates, and inhibitors of CYP2D6; although certain studies have suggested that CYP2D6 is inducible, the majority of studies do

not suggest this. CYP2D6 is highly polymorphic and more than 80 different alleles have been recognized (Heim & Meyer 1992). CYP2D6 expresses four different phenotypes in man. The ultra-rapid metabolizers (UMs) have three or more copies of active alleles. 1% to 10% of Caucasians and up to 2% of Asians are UMs. The extensive metabolizers (EMs) are normal subjects. This group has two active alleles; although there are a number of possible active alleles. Intermediate metabolizers (IMs) have one nonfunctional allele so that CYP2D6 activity is lower than in EMs. The poor metabolizers (PMs) do not have functional alleles. 5% to 10% of Caucasians and 1% of Asians are PMs (de Leon et al 2006). The PM phenotype is clinically important because of the likelihood of adverse reactions caused by increased plasma concentrations. In addition, lack of drug efficacy may be observed in UM subjects because of their capacity to clear CYP2D6 substrates (Wilkinson 2005). The antidepressant desipramine is metabolized by CYP2D6. It is recommended that patients with the PM phenotype should take 30% of the standard dose and UMs need a higher than average dose of desipramine (Thuerauf & Lunkenheimer 2006). This study suggests that, because CYP2D6 is the predominant pathway for the metabolism of antidepressants, and CYP2D6 is highly polymorphic, routine phenotyping in psychiatry could be helpful in directing the dosage of antidepressants. Hydroxylation of debrisoquine and demethylation of dextromethorphan are two drug oxidations that are diagnostic for CYP2D6 activity (Testa & Kramer 2007).

Table 1.4. Some Important Substrates, and Inhibitors of CYP2D6 (Wilkinson 2005)

CYP2D6 Substrates	CYP2D6 Inhibitors
Beta Blockers Alprenolol Bufuralol Carvedilol S-metoprolol Propranolol Timolol Tricyclic Antidepressant Amitriptyline Clomipramine Imipramine Nortriptyline Antiarrhythmic agents Flecainide Mexillettine Antipsychotic agents and SSRIs Fluoxetine Haloperidol Paroxetine Perphenazine Venlafaxine Opioids Codeine Dextromethorphan	Tricyclic Antidepressant Clomipramine Antiarrhythmic agents Quinidine Antipsychotic agents and SSRIs Fluoxetine Haloperidol Paroxetine

CYP3A subfamily:

The CYP3As are responsible for the disposition of more than half of the known drugs. Table 1.5 shows some important substrates, inhibitors and inducers of CYP3A4. This enzyme is primarily expressed in the liver and the intestinal epithelium (Wilkinson 2005). The two principal enzymes of this subfamily - CYP3A4 and CYP3A5 - have very similar substrates specificities which makes it difficult to distinguish them. The activities of other CYP3A enzymes, such as CYP3A43, are very low compared to the other CYP3As, and CYP3A7 is primarily a fetal enzyme (Wilkinson 2005).

Table 1.5. Some Important Substrates, Inhibitors and Inducers of CYP3A (Wilkinson 2005)

CYP3A Substrate	CYP3A Inhibitors	CYP3A Inducers
Calcium Channel Blockers Diltiazem Felodipine Nifedipine Verapamil Immunosuppressant agents Cyclosporine Tacrolimus Benzodiazepine Alprazolam Midazolam Triazolam Statins Atorvastatin Lovastatin Macrolide Antibiotics Clarithromycin Erythromycin Anti-HIV agents Indinavir Nelfinavir Ritonavir Saquinavir	Calcium Channel Blockers Diltiazem Verapamil Azole Antifungal agents Itraconazole Ketoconazole Macrolide Antibiotics Clarithromycin Erythromycin Troleandomycin Anti-HIV agents Ritonavir Saquinavir Indinavir Delaviridine Others Grapefruit Juice (Bergamottin) Mifepristone Nefazodone	Rifamycins Rifabutin Rifampin Rifapentine Anticonvulsant agents Carbamazepine Phenobarbital Phenytoin Anti-HIV agents Efavirenz Nevirapine Others St. John`s wort

1.1.3. Drug-Drug Interactions

A drug-drug interaction occurs when the effectiveness or toxicity of a drug is altered by the concomitant administration of another drug. Multiple drug therapy is common in some disorders and diseases and also in elderly patients. Therefore, multiple drug therapy may increase the possibility of drug interactions (Hemeryck and Belpaire 2003). In general, drug interactions can be attributed to pharmacodynamic and pharmacokinetic interactions (Reynolds 1990).

Pharmacodynamic interactions: Pharmacodynamic interactions occur when two drugs act at the same receptor sites, resulting in additive, synergistic or antagonistic effects (Reynolds 1990).

Pharmacokinetic interactions: Pharmacokinetic interactions are attributed to the changes that occur in the absorption, distribution, metabolism or excretion of a drug and /or its metabolites when another drug or chemical agent is added to the treatment. Most commonly this occurs when one CYP is involved in the metabolism of both drugs (Reynolds 1990).

1.1.4. Metabolic Drug-Drug Interactions

Since hepatic CYPs are responsible for the metabolism of many drugs and they participate in metabolic pathways for several drugs, there is considerable potential for drug interactions. Most interactions occur when a particular CYP is involved in the metabolism of two different drugs that are co-administered (e. g. fluvoxamine and clozapine, which are both substrates for CYP1A2). Therefore, since most of the interactions occur with CYP enzymes, it is important to be aware of the CYPs that are

involved in the metabolism of particular drugs, and to avoid combination of medications that may interact with that CYP.

Pharmacokinetic interactions caused by inhibition or induction of CYP activity are among the most common causes of drug interactions that may occur during polypharmacy. Metabolic interactions not only affect the plasma concentrations of drugs but also influence their efficacy and may also increase toxicity (Hemeryck & Belpaire 2003). Moreover, some drugs can induce their own oxidative metabolism and that of co-administered medications (e.g. certain steroid hormones and herbal products such as St. John's wort increase the metabolism of orally administered drugs by increasing CYP3A4 levels (Madabushi et al 2006).

Pharmacokinetic interactions caused by inhibition of CYP activity may occur when there are direct interactions between drugs that compete for the CYP. There are two basic types of inhibition mechanisms: reversible and irreversible. During irreversible inhibition, formation of a stable enzyme-drug complex leads to the loss of a CYP's activity. The formation of this complex prevents the access of other drugs to the CYP enzyme. In this case, new enzyme must be synthesized for the restoration of CYP activity. The inhibitions of CYP3A4 by erythromycin and CYP2D6 by paroxetine are examples of this type of interaction (Tanaka 1998; Prior and Baker 2003). However, in reversible inhibition the activity of CYPs would be restored by clearance of the inhibitory agents (Prior and Baker 2003; Dailly et al 2002). This type of interaction is seen on co-administration of diazepam and omeprazole (Tanaka 1998).

The effect of enzyme induction is to increase CYP gene expression resulting in increased oxidation and clearance of a drug (Dailly et al 2002; Tanaka 1998). Since the activity of a particular CYP may be increased by exposure to an inducer drug, it may result in difficulty to maintain appropriate therapeutic effects without a dose increase (Tanaka 1998).

The inhibition of CYPs in enterocytes of the intestinal epithelium may decrease the pre-systemic metabolism of some drugs. The activity of CYP3A4 in the intestine can influence drug bioavailability (Blume 2006). For example, felodipine is a CYP3A4 substrate and is absorbed into the enterocytes of intestinal epithelium after oral administration. However, CYP3A4 in enterocytes metabolizes felodipine efficiently and only about 30% of a drug dose enters the portal vein. CYP3A4 in liver subsequently further metabolizes felodipine so that only 15% of a drug dose is actively available and can reach the systemic circulation. Thus, the inhibition of CYP3A4 in enterocytes may have significant effect on drug bioavailability (Wilkinson 2005). It is now established that bergamottin in grapefruit juice is a potent inhibitor of intestinal CYP3A4 (Wilkinson 2005).

1.1.5. CYP Pharmacogenetics and Metabolic Drug Interactions in Psychiatry

Since monotherapy for psychiatric illness is rarely adequate, combination therapy is frequent but may increase the incidence of drug interactions (Strain et al 2004; Karow & Lambert 2003). Long term treatment for psychiatric illness also increases the potential for drug-drug interactions (Fleming & Chetty 2005). It has been reported that up to 4 medications may be used concurrently in the treatment of schizophrenic patients. Among the comedications, antidepressants, anxiolytics, and anticonvulsants are commonly prescribed (Rittmannsberger et al 1999). Some of the antidepressants are potent inhibitors and certain anticonvulsants are potent inducers of CYPs. Understanding the potential interactions is important so that clinicians may avoid toxicity or treatment failure.

Furthermore, wide inter-individual differences due to polymorphic expression of the CYPs that are responsible for the disposition of antipsychotic agents influence both the efficacy and adverse effects of drug therapy in psychiatry (Vandel et al 2007). Differential expression of CYP alleles may influence efficacy and toxicity in a particular population. For example, expression of inactive variant alleles of CYP2D6 is particularly important in psychiatric patients, due to its involvement in the metabolism of typical antipsychotics (such as, thioridazine and chlorpromazine), certain atypical antipsychotics (such as, risperidone) and the tricyclic antidepressants (Murray 2006). Moreover, CYP2D6 contributes to the metabolism of some endogenous compounds, such as neurotransmitters, which indeed may contribute to psychiatric and neurologic illnesses. However, more studies are now required to confirm this possibility (Vandel et al et al 2007).

Table 1.6 indicates the principal allelic variant of CYPs that are involved in the metabolism of antipsychotic agents, and which may influence response to drug therapy.

Table 1.6. Important genetic polymorphism of CYPs associated with clinical variations of response (Pirmohamed & Park 2003)

P450 enzymes	Variant	Frequencies in Caucasians	Clinical significance
CYP1A2	CYP1A2*1F	68%	High enzyme inducibility
CYP2C9	CYP2C9*2 CYP2C9*3	8 to 13% 7 to 9%	Reduced enzyme activity Reduced enzyme activity
CYP2C19	CYP2C19*2 CYP2C19*3	13% 0%	Reduced enzyme activity Reduced enzyme activity
CYP2D6	CYP2D6*4 CYP2D6*5 CYP2D6*10 CYP2D6*17	12 to 21% 4 to 6% 1 to 2 % 0%	Reduced enzyme activity§ Reduced enzyme activity§ Reduced enzyme activity# Reduced enzyme activity#

§, In Caucasians: CYP2D6 PMs are much more frequent. Four alleles *3, *4, *5, and *6 account for most (98%) inactive alleles (Bradford 2002).

#, *10, which is frequent in Asians and *17, which is more frequent in blacks may have different activity for different drugs (de Leon et al 2006).

1.2. Schizophrenia and Drug Treatment

About 1% of the world population is affected by schizophrenia, a severe psychiatric disorder (Kessler et al 1994). The pathogenesis of schizophrenia has not been fully explained. However, the dopamine hypothesis may contribute to the pathogenesis of schizophrenia as suggested by: I) Most antipsychotic agents potently antagonise dopaminergic receptors, particularly the D2 receptor. II) Drugs that increase dopamine activity such as amphetamine, levodopa, and apomorphine may exacerbate schizophrenia. III) The density of dopaminergic receptors in the brains of postmortem untreated schizophrenics has been shown to be increased. IV) Positron emission tomography shows increased density of dopaminergic receptors in both treated and untreated schizophrenic patients compared to normal populations. V) The concentration of homovanillic acid (HVA), a metabolite of dopamine, is lower in patients who have been successfully treated for schizophrenia (Potter & Hollister 2004).

Schizophrenics exhibit positive and negative symptoms. The positive symptoms are associated with hallucination, delusion, agitation, and incoherent thought and speech. The negative symptoms include emotional withdrawal, apathy, attention impairment and anhedonia. Other symptoms such as anxiety, depression and cognitive impairment are also involved with schizophrenia (Chetty & Murray 2007).

Antipsychotic and neuroleptic drugs are used to treat schizophrenia and some other psychoses and agitated states. Antipsychotic agents are classified into two general classes: the typical and atypical agents.

A. Typical agents include phenothiazine derivatives (e.g. chlorpromazine, thioridazine), thioxanthene derivatives (e.g. thiothixene), and butyrophenone derivatives (e.g. haloperidol).

B. Atypical antipsychotics include clozapine, olanzapine, quetiapine, risperidone, ziprasidone and aripiperazole (Potter & Hollister 2004).

Chlorpromazine was introduced in the 1950s for the treatment of schizophrenia (Chetty and Murray 2007). After its introduction this agent decreased the morbidity and mortality due to schizophrenia (Chetty & Murray 2007). However, over the past decade the pharmacologic management of schizophrenia has changed so that now the atypical or second generation antipsychotics have become the first line of treatment because the neurological side effects are lower than those produced by the typical or first generation antipsychotics, such as phenothiazine, thioxanthene, and butyrophenone derivatives (Spina & de Leon 2007). The first generation of antipsychotics are extremely potent and produce a high incidence of extrapyramidal symptoms, such as tardive dyskinesia and dystonia. These may lead to the development of parkinsonian symptoms, such as tremor, rigidity and akathisia (Murray 2006). From a clinical perspective, the new generation of antipsychotics tend to have fewer extrapyramidal side effects and are broadly effective in treatment of both negative and positive symptoms of schizophrenia, while the first generation drugs are mainly effective against the positive symptoms of schizophrenia (Murray 2006).

Treatment with atypical antipsychotics is associated with increased risk of cardiovascular disease and metabolic disorders such as weight gain, hyperglycemia and lipid dysregulation. Different observations suggest that the increased risk of insulin resistance, dyslipidemia and hyperglycemia in patients treated with atypical antipsychotics is due to the weight gain during pharmacotherapy with these agents. However, limited controlled studies suggested that glucose dysregulation is a direct effect of clozapine and olanzapine treatment and independent of adiposity (Newcomer 2005). Moreover, elevation of serum concentrations of liver enzymes has been reported in patients who have been treated with clozapine (Newcomer 2005).

1.2.1. Typical Antipsychotics

Chlorpromazine and haloperidol are among the major typical antipsychotics used for the treatment of schizophrenia. Chlorpromazine has a complex metabolic pathway and generates a range of metabolites. CYP3A4 is mainly responsible for oxidation of the heterocyclic sulphur of chlorpromazine (Cashman et al 1993). However, other CYPs such as 2D6 and 1A2 may also be involved in 7-hydroxylation and N-oxidation of chlorpromazine (Cashman et al 1993). An *in vitro* study showed that CYP2D6 is inhibited by chlorpromazine (Dayer et al 1992). The clearance of propranolol and debrisoquine were also impaired by chlorpromazine (Miller & Rampling 1982; Syvahlahti et al 1986). These findings support a role for CYP2D6 in chlorpromazine elimination.

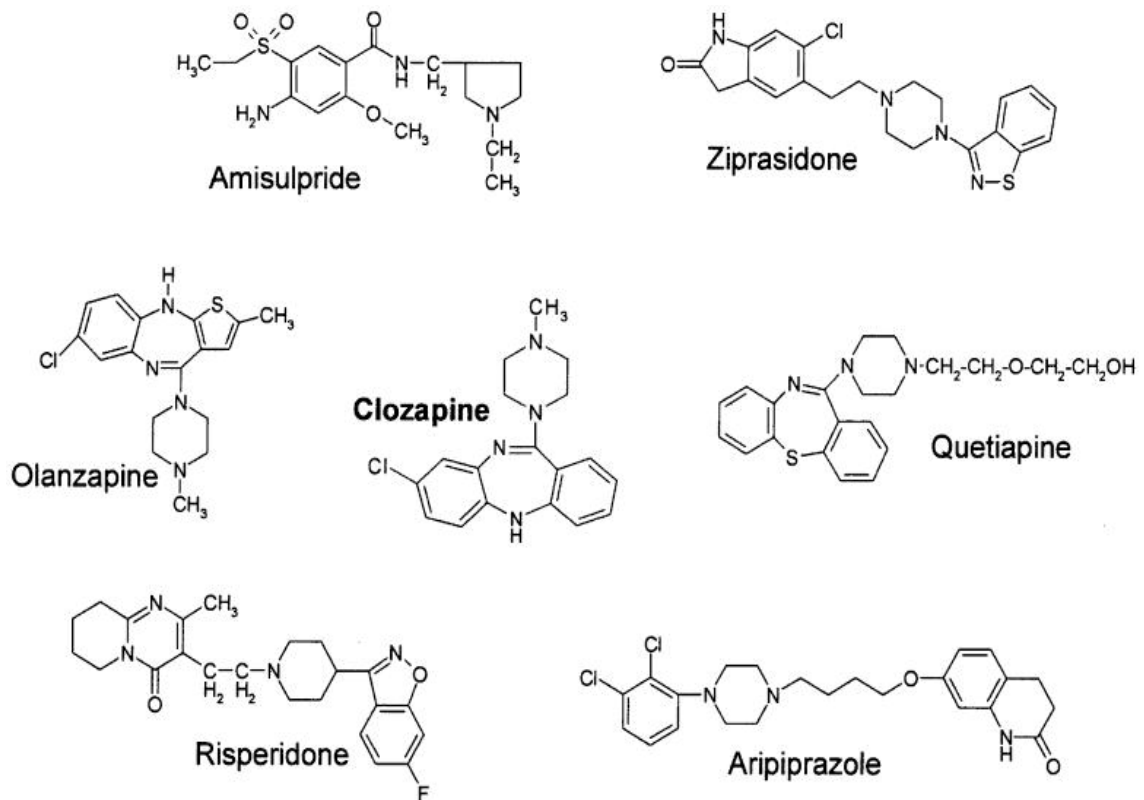
Haloperidol is mainly metabolized by CYP3A4 and CYP2D6 to the *N*-dealkylated metabolite (Fang et al 1997). Co-administration of the antiepileptic agents phenytoin, phenobarbital and carbamazepine decreased plasma concentrations of haloperidol (Linnoila et al 1980). This is consistent with induction of CYP3A4 by the antiepileptic agents. Combined therapy with haloperidol and chlorpromazine has also been used recently in schizophrenic patients (Murray 2006), which may complicate the use of both drugs. Thus, the combination of chlorpromazine and haloperidol increased the serum concentrations of the latter, which was most likely due to the inhibition of CYP2D6 by chlorpromazine. Suzuki et al reported that the plasma concentrations of haloperidol were higher in homozygous CYP2D6*1/*1 individuals than in those carrying the CYP2D6*5, PM genotype (Suzuki et al 2001). Thus, in addition to potential drug interactions, CYP2D6 genotype may also influence therapy with typical antipsychotics.

1.2.2. Atypical Antipsychotics

A major problem with typical antipsychotics is the failure of therapy in a substantial proportion of patients (termed treatment resistance). These drugs may be effective in the treatment of positive symptoms (such as hallucination and delusion), but in some cases they may worsen the negative symptoms (such as social withdrawal and avolition) (Karow and Naber 2002). Clozapine, olanzapine, quetiapine, risperidone, ziprasidone and aripiperazole are among the new generation of antipsychotic agents. However, despite the introduction of a number of different atypical agents, clozapine

is still the most effective treatment for refractory schizophrenia (Chetty & Murray 2007). Figure 1.3 shows the chemical structure of atypical antipsychotics.

Figure 1.3. Chemical structures of atypical antipsychotics (Murray 2006)



1.2.2.1. Clozapine

Clozapine, the first atypical antipsychotic agent, is a piperidine derivative of the benzodiazepine family, which is mainly used in refractory schizophrenic patients and in patients who cannot tolerate the adverse effects of traditional antipsychotic treatment. Clozapine has many unique clinical advantages over the conventional antipsychotic agents. For instance, clozapine has a lower incidence of extrapyramidal side effects, such as tardive dyskinesia and dystonias. In addition, atypical antipsychotics such as clozapine are effective in both negative (such as social withdrawal and avolition) and positive (such as hallucination and delusion) signs of schizophrenia (Murray et al 2006). Agranulocytosis is one of the rare but serious side effects of clozapine that can lead to discontinuation of clozapine therapy (Baldessarini & Frankenburg 1991). Agranulocytosis was noted soon after the introduction of the drug and limited its usage for some time. However, the incidence of agranulocytosis was decreased from 1% to 0.38%, as a result of compulsory homologous monitoring via the Clozaril National Registry (Lieberman 1998). Despite the potential for agranulocytosis and other side effects such as orthostatic hypotension, sedation, seizure, and weight gain, the therapeutic advantages of clozapine outweigh its risks (Masellis et al 2000).

1.2.2.1.1. Pharmacodynamics of Clozapine

Most antipsychotic agents are antagonists for dopaminergic receptors, but also may have some affinity for other receptors such as α_1 adrenoceptors, serotonin 5-HT₂,

muscarinic, and H₁ histaminic receptors. The clozapine antagonist affinity for different receptors is reportedly D₄ = α₁ > 5-HT₂ > D₂ = D₁ (Potter & Hollister 2004).

The relationship between clozapine concentration and therapeutic effect has been evaluated in numerous studies (Chetty & Murray 2007). There is a consensus that the minimum therapeutic plasma level of clozapine is 350-420 ng/mL (Chetty & Murray 2007). Freeman & Oyewumi suggested a therapeutic range of about 400 ng/mL (1.2 μM) to 1000 ng/mL (3.0 μM) for serum clozapine concentrations (Freeman & Oyewumi 1997). Some side effects such as weight gain, nausea, constipation, sialorrhea, hepatotoxicity, eosinophilia and akathisia appear to be unrelated to plasma concentrations of the drug, but other side effects appear to occur at plasma concentrations that exceed 1000 ng/mL (Chetty & Murray 2007). It has been reported that the risk of drowsiness, sedation, hypotension and seizure increases with increased clozapine plasma concentrations. It has been recommended that plasma concentrations should not exceed 1000 ng/mL (Chetty & Murray 2007).

1.2.2.1.2. Pharmacokinetics of Clozapine

Clozapine exhibits linear pharmacokinetics at therapeutic levels (Perry et al 1998; Doude van Troostwijk et al 2003a). The lipophilic clozapine is well absorbed after oral administrations and its variable bioavailability is not affected by food (Chetty & Murray 2007). Clozapine reaches maximum plasma concentrations (C_{max}) after 1 to 3.6 hours, elimination half-life (t_{1/2}) is variable in the range 5.8 to 33 hours, total

plasma clearance is 37 to 55 L/h, and the volume distribution is 5 to 7 L/kg (Cheng et al 1998). Oral bioavailability of clozapine varies between 12 to 81% (Spina & de Leon 2007) and its high protein-binding exceeds 90 % (Cheng et al 1998). A study of 15 psychiatric patients showed that the unbound fraction of clozapine in serum is about 5.5% and its renal clearance is 11% of the creatinine clearance (Schaber e al 1998).

The pharmacokinetic behavior of clozapine is subject to wide inter- and intraindividual variation. Some important reasons for this variability include diet, age, disease, and CYP pharmacogenetics as well as the presence of exogenous inhibitors or inducers of CYPs that are involved in the metabolism of clozapine (Murray 2006; Dailly et al 2002). Table 1.7 compares different pharmacokinetic parameters of atypical antipsychotics.

Table 1.7. Pharmacokinetic parameters of atypical antipsychotic agents (Spina & de Leon 2007)

	Bioavailability (%)	Protein Binding (%)	Half-life (Hours)	Time to reach steady-state (days)	Enzymes responsible for biotransformation	Active metabolite(s)
Clozapine	12 to 81	>90	6 to 33	4 to 8	CYP1A2 , CYP2C19 CYP3A4, CYP2D6 FMO	Norclozapine -
Risperidone	68	90	3 to 24	4 to 6	CYP2D6 , CYP3A4	9-hydroxyrisperidone
Olanzapine	60 to 80	93	20 to 70	5 to 7	CYP1A2 , CYP2D6 UGT1A4, FMO	-
Quetiapine	NA	83	5 to 8	2 to 3	CYP3A4	-
Sertindole	75	99	85 to 99	15 to 20	CYP2D6 , CYP3A4	-
Ziprasidone	60	>99	4 to 10	2 to 3	CYP3A4, aldehyde oxidase	-
Aripiprazole	NA	>99	48 to 68	14	CYP3A4 , CYP2D6	Dehydro-aripiprazole
Amisulpride	43 to 48	17	12	2 to 3	Not clinically relevant	-

UGT, UDP-glucuronosyltransferases; FMO, flavin-containing monooxygenase-3 system. In bold the most likely to have clinical relevance.

1.2.2.1.3. Metabolism of Clozapine

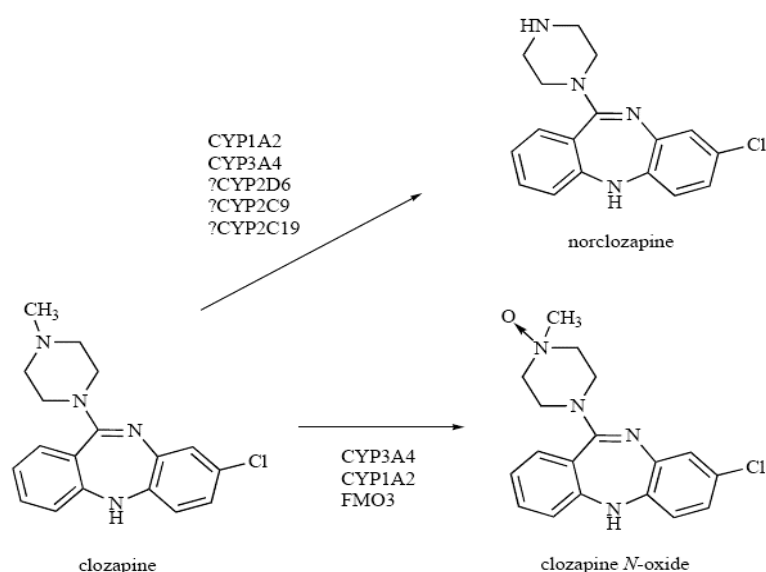
In man clozapine is extensively metabolized by *N*-oxidation to clozapine-*N*-oxide and *N*-demethylation to *N*-desmethylclozapine (norclozapine) (Dain et al 1997). Figure 1.4 shows the major metabolic pathways of clozapine. An *in vitro* study has determined the relative contributions of CYP1A2, 3A4, 2C9, 2C19, and 2D6 to the *N*-demethylation of clozapine at high and low (50 and 5 μ M) concentrations of the drug in relation to the clinically relevant range for the concentrations found in liver (Olesen & Linnet 2001). The results indicated that CYP1A2 was the major isoform involved in the formation of norclozapine at low concentration and made a 30% contribution to overall metabolism. CYP2C19 and CYP3A4 contribute 20% to 25%. However, at the higher concentration (50 μ M), CYP3A4 could be more important to norclozapine formation (37%). The contribution of CYP1A2 was 22%, which was still higher than the other CYPs. Importantly, however these studies were done in recombinant systems expressing single CYPs, which can significantly affect the findings. Study of CYPs in isolation (recombinant systems) is done with caution because findings in combination with other CYPs (e.g. in microsomes) can differ. This is due to the catalytic versatility of the enzymes, particularly in the absence of other competing CYPs.

Several further studies have shown that CYP1A2 is the major enzyme involved in formation of norclozapine in microsomal fractions from human liver, while CYP3A4 mainly contributes to formation of clozapine-*N*-oxide (Linnet & Olesen 1997; Pirmohamed et al 1995; Eiermann et al 1997; Tugnait et al 1999).

Studies in microsomes, rather than recombinant systems, probably provide a more accurate reflection of the situation that occurs *in vivo*.

According to Bertilsson et al CYP1A2 activity is responsible for about 70% of the variation in oral clearance of clozapine (Bertilsson et al 1994). Variable response to antipsychotics such as clozapine could be explained by differential expression of CYP1A2 in schizophrenic patients (Ozdemir et al 2001). Caffeine is also a CYP1A2 substrate and its conversion to paraxanthine may be used as a safe and convenient phenotypic marker for CYP1A2 activity. The correlation between caffeine and clozapine clearance indicates the importance of CYP1A2 to the metabolism of clozapine (Taylor 1997; Bertilsson et al 1994). Moreover, it has been shown that the clozapine dose (mg/kg) is linearly related to the serum concentration of paraxanthine/caffeine (Doude van Troostwijk et al 2003a). In summary, *in vitro* and *in vivo* studies have established that CYP1A2 activity is an important factor in clozapine efficacy (Doude van Troostwijk et al 2003b).

Figure 1.4. Metabolic pathway of Clozapine (Chetty and Murray 2007)



The evidence shows that CYP1A2 pharmacogenetics may affect clozapine metabolism. It has been shown that individuals who are homozygous for the CYP1A2 allele that contains a C→A SNP in intron 1 (CYP1A2*1F) exhibit ultrarapid activity. These patients are unresponsive to clozapine because of the failure to maintain adequate serum concentrations (Chin et al 2004; Ozdemir et al 2001). Further studies have shown that the efficacy of clozapine in ultrarapid metabolizers could be retrieved by concurrent treatment with fluvoxamine (50mg/d), which markedly inhibited CYP1A2 activity (Ozdemir et al. 2001).

Carrillo et al have shown that the ratio of norclozapine/clozapine in plasma correlates with CYP1A2 activity as determined by urinary caffeine test in that study (Carrillo et al 1998). It was suggested that this ratio shows not only the importance of CYP1A2 activity in clozapine clearance, but could also be a valuable estimate of CYP1A2 activity in schizophrenic patients. Dailly et al used this ratio for determination of CYP1A2 activity and their findings were consistent with those of Carrillo et al (Dailly et al 2002). Thus, clozapine clearance and CYP1A2 activity, reflected by the norclozapine/clozapine ratio, were linearly related. This ratio could provide insight into the inter- and intra-individual variation of clozapine plasma concentrations, as it relates to CYP1A2 activity (Dailly et al 2002).

1.2.2.1.4. Pharmacokinetic Drug Interactions of Clozapine

Treatment of refractory schizophrenia is long term and likely to include co-administration of a number of other medications. Some of these medications are used to improve the clinical efficacy of clozapine or to reduce its adverse effects. Thus, concurrent therapy includes antidepressants, mood stabilizers, laxatives and proton pump inhibitors to prevent acid stomach. The risk of interaction increases during polypharmacy in schizophrenic patients (Chetty & Murray 2007).

Clozapine: Interactions with CYP Inhibitors

Fluvoxamine, a selective serotonin reuptake inhibitor (SSRI), has been reported to significantly impair clozapine clearance. The plasma concentrations of both clozapine and noreclozapine increase during co-administration of fluvoxamine (Hiemke et al. 1994; DuMortier et al 1996). Fluvoxamine increased the elimination half-life of clozapine from 17.3 to 50.6 hours (Wetzel et al 1998). Furthermore, patients may experience greater sedation or extrapyramidal side effects after addition of fluvoxamine to clozapine therapy (Kuo et al 1998). Fluoxetine, another widely used SSRI, also reportedly increased plasma clozapine concentrations up to 70% (Spina et al 1998).

Although SSRI, and particularly fluvoxamine, may increase clozapine concentrations in serum to toxic levels, it has been suggested that the use of fluvoxamine could enable lower dose therapy with clozapine (Lammers et al 1999). In addition, the combination with fluvoxamine may improve the neuroleptic effectiveness of clozapine in clozapine-resistant schizophrenia (Silver et al 1996).

Ciprofloxacin and fluoroquinolone antibiotics increase clozapine plasma concentrations, by inhibiting CYP1A2 activity (Batty et al 1995; Raaska & Neuvonen 2000). Similarly, the CYP1A2 substrate caffeine also inhibits CYP1A2- dependent clozapine oxidation (Carrillo & Benitez 2000). Since schizophrenic patients have the habit of high coffee intake, it is important to determine the clinical significance of interactions between caffeine and clozapine. Evidence shows that caffeine increases clozapine concentration (Carrillo et al 1998). Hägg et al have shown that the oral clearance of clozapine decreases by 14% and its mean AUC (0, ∞) increases by 19% in presence of caffeine (Hägg et al 2000). An increasing number of reports of extrapyramidal side effects of clozapine due to ingestion of coffee or cola drinking is consistent with the suggestion that replacement with decaffeinated beverages could minimize such side effects (Vainer & Chouinard 1994).

Valproate, a mood stabilizer agent, is usually administered to control seizures in schizophrenic patients who are on high doses of clozapine (Toth & Frankenburg 1994).

Findings of the effect of valproate on serum clozapine concentrations are controversial. While some studies indicate that valproate decreases clozapine concentration by 51% or 15% (Finley & Warner 1994; Longo & Salzman 1995), others have reported increases of up to 39% (Centorrino et al 1994), or no effect (Facciola et al 1999).

Clozapine: Interactions with CYP Inducers

Carbamazepine, an anticonvulsant that is sometimes co-prescribed with clozapine to control seizures, decreased serum concentrations of the drug up to 68% (Jerling et al 1994). A case report showed that discontinuation of carbamazepine in two patients led to increases in serum concentrations of clozapine by 70%-100% (Raitasuo et al 1993). Apart from these pharmacokinetic interactions, the combination of carbamazepine and clozapine is not recommended due to an increased risk of neutropenia (Junghan et al 1993).

Lane et al have reported that after discontinuing phenobarbital serum clozapine concentrations increased by 75% (Lane et al 1998). Another report indicated that inclusion of phenytoin in the medication profile of two patients who were receiving clozapine decreased plasma concentrations of the latter by 65 to 85% (Miller 1991). Similarly, the evidence shows that serum clozapine concentrations are lower in patients who are co-medicated with rifampicin (Wietholtz et al 1995). Supporting this evidence, one forensic report indicated that clozapine concentrations were decreased in the presence of rifampicin. Replacement of rifampicin with ciprofloxacin increased serum concentrations of clozapine (Joos et al 1998). This may be due to the combined effect of removing CYP1A2 inducer and introducing a CYP inhibitor into therapy.

The average schizophrenic patient smokes 22.4 cigarettes per day, which is higher than the general population (Herran et al 2000). Several studies have indicated that the serum levels of clozapine in non-smokers are higher than in smokers (Seppala et al 1999; van der Weide et al 2003).

de Leon performed a comprehensive study on the effect of smoking and caffeine on atypical antipsychotic dosing and suggested that the induction effect of smoking on CYP1A2 persists for at least two to four weeks (de Leon 2004). The separate effects of smoking and caffeine intake on clozapine therapy are summarized in Table 1.8 (de Leon 2004). Haslemo et al suggested that smoking of 7 to 12 cigarettes daily is sufficient to achieve maximal metabolism of clozapine and olanzapine metabolism. They also recommended that the dose of clozapine should be 50% higher in smokers in order to optimize therapy (Haslemo et al 2006).

Sachse et al have suggested that the SNP (C→A) in intron 1 of the CYP1A2*1F gene variant increases the inducibility of the gene (Sachse et al 1999). This study showed that smokers who possessed the homozygous genotype for the variant allele had approximately 1.6-fold higher CYP1A2 activity than either heterozygotes or individuals who were homozygous wild-type. However, in non-smokers, no significant differences in CYP1A2 activity were observed among the different genotypes (Sachse et al 1999). Cessation of smoking in a patient who was homozygous for the CYP1A2*1F allele strongly increased serum clozapine levels up to 3004 ng/mL (Bondolfi et al 2005). It was also reported in that study that replacement of omeprazole, another established CYP1A2 inducer, by pantoprazole resulted in a very high plasma clozapine concentrations (Bondolfi et al 2005). In contrast, another study suggested that this variant of CYP1A2 has a lesser impact than smoking on clozapine clearance (van der Weide et al 2003). The ratio of clozapine plasma concentration/clozapine daily dose (C/D) was measured in homozygous and heterozygous patients carrying the *1F allele and in smoker and

non-smoker groups: the ratio of C/D for smokers with or without the A/A genotype were not significantly different (van der Weide et al 2003).

Table 1.8. Recommended correction factors for smoking and using caffeine with clozapine (de Leon 2004)

Study	Side Effects	Recommended correction factor *
Smoking with clozapine	seizure; Antimuscarinic and sexual side effects	1.5
Using caffeine with clozapine	Super ventricular tachycardia; Sedation	0.6

* Standard clozapine doses are multiplied by the recommended correction factor.

Clozapine and omeprazole interactions:

It has been suggested that psychiatric illness could be associated with abnormalities of esophageal function (Clouse & Lustman 1983) and this necessitates treatment with a range of drugs. Recent studies have also suggested that clozapine therapy is associated with a number of gastrointestinal disorders, such as abnormal esophageal motility (McCarthy et al 1994), gastric reflux and gastro-oesophageal reflux (Laker & Cookson 1997). Therefore, concomitant use of clozapine and medication for treatment of gastrointestinal symptoms are frequently needed. Two case studies showed that in schizophrenic patients who responded poorly to conventional therapy, treatment with clozapine resulted in gastric discomfort. Endoscopy revealed moderate to severe (grade 3) reflux oesophagitis with erosions. Symptoms abated after lowering dosage or stopping clozapine therapy altogether, but recurred after completing the course of omeprazole treatment (Laker & Cookson 1997).

It has been reported that during omeprazole therapy with 40 and 60 mg/day in two schizoaffective patients, plasma clozapine concentrations were decreased by 41.9% and 44.7%, respectively (Frick et al 2003). It was suggested that induction of CYP1A2 activity by omeprazole led to the decrease in serum clozapine concentrations (Frick et al 2003). Mookhoek et al showed that replacement of omeprazole by pantoprazole (a weaker CYP1A2 inducer) in 13 patients resulted in an increase in the mean clozapine and norclozapine levels in 3 non-smokers by 134 ng/mL and 117 ng/mL, respectively (Mookhoek & Loonen 2004). However, the small number of patients recruited in this study prevented a recommended dosage adjustment in cases where omeprazole was to be replaced by pantoprazole.

Table 1.9 summarizes pharmacokinetic interactions associated with drugs that are used in combination with clozapine.

Table 1.9. Summary of pharmacokinetic interactions involving clozapine

	Interacting medications	Proposed mechanisms	
Clozapine	Carbamazepine	Induction of CYP1A2, CYP3A4 and UGT	Jerling et al 1994; Junghan et al 1993
	Phenobarbital	Induction of CYP1A2, CYP3A4 and UGT	Lane et al 1998
	Phenytoin	Induction of CYP3A4	Miller 1991
	Rifampicin	Induction of CYP3A4	Wietholtz et al 1995
	Omeprazole	Induction of CYP1A2	Frick et al 2003; Mookhoek & Loonen 2004
	Tobacco smoke	Induction of CYP1A2	deLoen 2004
	Fluvoxamine	Inhibition of CYP1A2 and, to a lesser extent, CYP2C19 and CYP3A4	Hiemke et al 1994; DuMortier et al 1996
	Paroxetine	Inhibition of CYP2D6	Spina et al 1998
	Fluoxetine	Inhibition of various CYP isoforms (CYP2D6, CYP2C19 and CYP3A4)	Spina et al 1998
	Ciprofloxacin	Inhibition of CYP1A2	Raaska & Neuvonen 2000
	Valproate (controversial results)	Enzyme inhibition?	Centorrino et al 1994; Facciola et al 1999
	Caffeine	inhibition of CYP1A2	Carrillo & Benitez 2000

1.2.2.2. Other Atypical Antipsychotics

Olanzapine is an atypical antipsychotic agent that is structurally similar to clozapine in which one of the carbocyclic systems of clozapine has been replaced by a thiophene ring (Figure 4) (Murray 2006). The major metabolic pathways of olanzapine are *N*-glucuronidation mediated by UGT1A4 and *N*-demethylation mediated by CYP1A2 (Callaghan et al 1999). To a lesser extent, CYP2D6 and the flavin containing monooxygenase-3 are also involved in the metabolism of olanzapine (Callaghan et al 1999). Higher clearance rate and lower serum concentrations of olanzapine have been reported in smokers, which is consistent with an important role

for CYP1A2 (Carillo et al 2003). Concomitant use of olanzapine and fluvoxamine increased the serum concentrations of olanzapine and decreased in the clearance of the drug (Weigmann et al 2001). Because fluvoxamine inhibits CYP1A2, the clinical findings are also consistent with a role for the enzyme in olanzapine biotransformation.

Risperidone undergoes approximately 70% first pass hepatic extraction by CYPs. (Byerly & DeVane 1996). Risperidone is primarily metabolized by CYP2D6 to its active metabolite 9-hydroxyrisperidone; CYP3A4 may have a lesser role (Fang et al 1999). It is reported that the affinity of 9-hydroxyrisperidone for the D2-dopamine receptor is similar to that of the parent drug (Spina & de Leon 2007). The genetic polymorphism of CYP2D6 may influence the therapeutic effects or the risk of side effects of risperidone. De Leon has suggested that the incidence of side effects leading to discontinuation of treatment with risperidone is greater in CYP2D6 PM subjects (de Leon et al 2005), while low serum concentrations of risperidone in UM subjects for CYP2D6, which may lead to treatment failure (Guzey et al 2000). Spina et al showed that cotherapy with the SSRI paroxetine, an inhibitor of CYP2D6, increased the serum concentrations of risperidone (Spina et al 2001). Long term combination treatment of risperidone and fluoxetine, another SSRI that inhibits CYP2D6 and to lesser extent CYP3A4, decreased risperidone clearance and increased the incidence of parkinsonism, an adverse effect of risperidone (Spina et al 2002).

The metabolism of **Ziprasidone** is complex. The major pathway is oxidation at sulfur to form ziprasidone-sulphoxide, ziprasidone-sulphone, and methyl-dihydroziprasidone (Prakash et al 2000). About two-thirds of ziprasidone metabolism is mediated by the

cytosolic aldehyde oxidase while CYP3A4, but not other CYPs, makes a minor contribution (Prakash et al 2000). Co-administration of the CYP3A4 inhibitor ketoconazole, decreased the clearance of ziprasidone and increased its serum concentrations (Miceli et al 2000). Ziprasidone also inhibits CYP2D6 and CYP3A4 *in vitro* (Wilner et al 2000).

Quetiapine is primarily metabolized by CYP3A4, whereas CYP2D6 has a minor role (Murray 2006; DeVane & Nemeroff 2001). In a multiple dose study, concomitant administration of the CYP3A4 inhibitor ketoconazole increased quetiapine serum concentrations (Dev & Raniwalla 2000). Dose adjustments have been suggested during concomitant use of quetiapine and the CYP3A4 inducer phenytoin (Wong et al 2001).

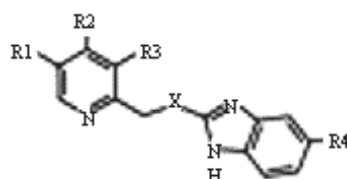
1.3. Proton Pump Inhibitors (PPIs): Pharmacology and pharmacokinetic Interactions

Proton pump inhibitors (PPIs) are considered the most potent drugs of choice for the treatment of acid related disorders. Omeprazole, lansoprazole, pantoprazole, rabeprazole and esomeprazole are included in this class. From a clinical point of view, the newer PPIs (rabeprazole and esomeprazole) inhibit acid secretion with a faster onset that persists for longer than the older drugs in this class (omeprazole, pantoprazole and lansoprazole) (Robinson 2001).

1.3.1. Physiochemical Properties

Generally, PPIs are weak lipophilic bases that contain the pyridylmethylsulfinyl benzimidazole nucleus. Different substitutions on the pyridine or benzimidazole rings result in a small differences in their activity (Huang & Hunt 2001). Figure 1.5 shows the different chemical structures of PPIs.

Figure 1.5. Chemical structure of PPIs (Li et al 2004)



	R1	R2	R3	R4	X
Omeprazole	CH ₃	OCH ₃	CH ₃	OCH ₃	
Lansoprazole	H	OCH ₂ CF ₃	CH ₃	H	
Pantoprazole	H	OCH ₃	OCH ₃	OCHF ₂	
Rabeprazole	H	OCH ₂ CH ₂ CH ₂ OCH ₃	CH ₃	H	

1.3.2. Indications

The proton pump inhibitors, omeprazole, esomeprazole, lansoprazole, pantoprazole and rabeprazole, are the most effective antisecretory agents available for treatment of gastric acid related diseases. It has been established that PPIs are superior to earlier treatments for acid related disorders, such as antacid therapy, anticholinergic agents and H₂ receptor antagonists (Robinson 2004). PPIs are currently used for treatment of Peptic Ulcer Disease, Gastroesophageal reflux disease, Dyspepsia, Zollinger-Ellison Syndrome and Scleroderma oesophagus. The combination of antibiotics and PPIs is the key treatment for the eradication of *H. Pylori*. PPIs are also prescribed for acute upper gastrointestinal bleeding and prevention of acid aspiration (Kazung and Trevor 2005). Furthermore, PPIs are used for prophylaxis of stress or peptic ulcer induced by Non-Steroidal Anti- Inflammatory Drugs (NSAIDs) (Blume et al 2006).

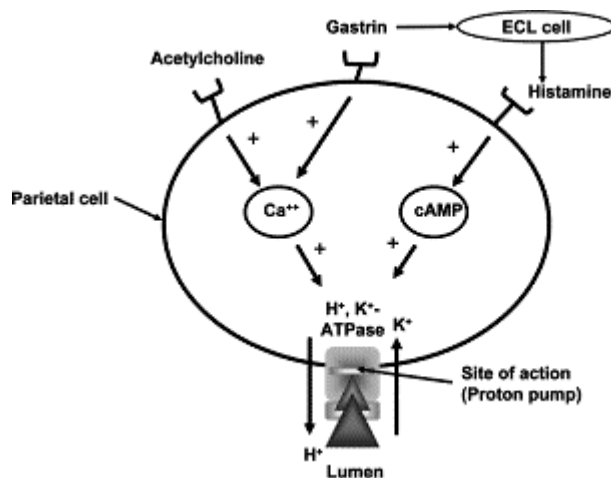
1.3.3. Pharmacodynamics

At least three types of receptors (histamine, gastrin and acetylcholine) on the parietal cell are involved in gastric acid secretion. Activation of any of these receptors can stimulate the H⁺, K⁺-ATPase (acid pump) which increases acid secretion. The gastric proton pump H⁺, K⁺-ATPase has a sulfhydryl group near the potassium-binding site on the luminal side of the canalicular membrane. The drugs are present as the ionized and non-ionized form in solutions, in ratios that are dependent on the pK_a of drug and the pH of the solution. The neutral form of drug may pass through lipophilic membranes and enter cells. From the Henderson-Hasselbach equation ($\text{pH} = \text{pK}_a + \log \frac{[\text{base}]}{[\text{acid}]}$), the differences between drug pK_a and intracellular pH determines the

extent of the lipophilic neutral form of the drug and its accumulation within the cell (Horn 2006). Once PPIs cross the parietal cell membrane into the highly acidic (pH 1) parietal cell canaliculus, they are protonated and therefore no longer lipophilic.

Figure 1.6 shows the site of action of PPIs (Blume et al 2006; Williams & Pounder 1999). The active forms of omeprazole and other PPIs are the stable cyclic sulphenamides that bind covalently with sulfhydryl groups of the acid pump and irreversibly inhibit pump activity. The rate of conversion of the parent PPI drug to the sulphenamide at pH~1 is rapid and takes only 1.3-4.6 minutes. However, at a higher pH the rate of conversion is different for PPIs and ranges from about 7.2 minutes for rabeprazole to 1.4 hour for omeprazole (Kromer et al 1998). The higher pKa of rabeprazole of ~5 compared to omeprazole (~4) explains its more rapid onset and greater acid suppression. The extent and duration of intragastric acid inhibition associated with sulphenamide formation correlate with the healing rates of peptic ulcer and erosive oesophagitis and the control of acid related symptoms (Huang & hunt, 2001). A meta-analysis has suggested that the rate of duodenal ulcer healing would be almost complete after 4 weeks if the pH was maintained at greater than 3 for 18 hours (Burget et al 1990).

Figure 1.6. The site of action of PPIs (Blume et al 2006).



Rabeprazole: rabeprazole has the same mode of action as the other PPIs, but due to different pyridine and benzimidazole substitution (Figure 1.5) it has a higher pKa. Therefore it undergoes a more rapid conversion to the sulphenamide form and also inhibits the H⁺, K⁺-ATPase more rapidly (Williams et al 1998). In addition, rabeprazole appears to dissociate more rapidly and completely from the H⁺, K⁺-ATPase than either omeprazole or lansoprazole, which suggests that pump inhibition may be partially reversible (Huang & Hunt, 2001) although this remains to be established. Evidence indicates that rabeprazole achieves complete H⁺, K⁺-ATPase inhibition after 5 minutes, whereas 30 minutes is reported for omeprazole and lansoprazole, and pantoprazole achieves 50% inhibition after 45 minutes (Besancon et al 1997).

According to a study in healthy Chinese volunteers, after the first dose of rabeprazole (20 mg) the mean AUC of rabeprazole was 80% of that measured after repeated dosing for 8 days (Hu et al 2005). This pharmacokinetic feature of rabeprazole

suggests that the maximum acid inhibitory efficacy of rabeprazole (pharmacodynamic effect) appears after one dose of the drug. A comparative study of the antisecretory effects of rabeprazole and omeprazole (20 mg dose) in *H. pylori*-negative subjects showed that, after single doses, the 24-h intragastric pH decrease with rabeprazole was more pronounced than with omeprazole. The results of this study have been summarized in Table 1.10 (Williams et al 1998). Another recent study reported that rabeprazole (20 mg) increased intragastric pH to ~ 3 to 4 for a longer period (24 hours) than pantoprazole (40 mg) in patients with gastroesophageal reflux disease (GERD) (Warrington et al 2005). *In vivo* studies have shown that rabeprazole is more potent against *H. pylori* activity than omeprazole and lansoprazole. Thus, MIC₅₀ and MIC₉₀ of rabeprazole against eight strains of *H. pylori* were lower than those values for omeprazole and lansoprazole (Fujiyama et al 1994).

It is recommended that some PPIs should to be taken before meal in order to inhibit proton pumps before they undergo activation by food (Horn 2006). Table 1.11 shows the impact of food on the pharmacokinetics of PPIs (Horn 2006).

Table 1.10. Comparison of intragastric acidity/ pH and plasma gastrin on day 1 and day 8 of dosing with rabeprazole 20 mg and omeprazole 20 mg, once daily, in healthy H. pylori-negative male volunteers (n=23) (Williams et al 1998)

	Day 1 Rabeprazole 20 mg	Omeprazole 20 mg	Day 8 Rabeprazole 20 mg	Omeprazole 20 mg
24-h intragastric acidity (mmol.h/L)	331*	640	160	218
24-h plasma gastrin (pmol.h/L)	N/A	N/A	1687 *	1085
Median 24-h intragastric pH	3.2 *	2	4.7	4.2
% time 24-h intragastric pH > 3	55 §	37	69 ‡	60
% time 24-h intragastric pH > 4	44 §	25	59 ‡	51

* ≤ 0.001 , § < 0.01 , ‡ < 0.05 vs. omeprazole

Table 1.11. Effect of food on the pharmacokinetics of PPI drugs (Horn 2006)

PPI	Cmax	Tmax	AUC	Recommendation
Esomeprazole	--	--	↓43-53%	1 h before food
Lansoprazole	↓50-70%	--	↓50-70%	Before food
Omeprazole	↓25%	↑	↔	Before food
Pantoprazole	↔	↑	↔	with or without food
Rabeprazole	↔	↑	↔	with or without food

↔ no effects, ↑ increase, ↓ decrease

1.3.4. Pharmacokinetics: Role of CYP2C19 Genotype

PPIs have similar pharmacokinetic features, including rate of absorption, maximum plasma concentration, and total drug absorption (Li et al 2004). Table 1.12 summarizes the pharmacokinetic parameters of common PPI drugs.

All PPIs undergo hepatic oxidation usually mediated by CYP2C19 and CYP3A4. Figure 1.7 shows the major metabolic pathways of PPIs.

The pharmacokinetics and pharmacodynamics of PPIs are related to CYP2C19 genotype (Desta et al 2002). As mentioned in section 1.2, CYP2C19 (S-mephenytoin 4'-hydroxylase) exhibits a large number of different polymorphic forms. Those who are deficient in the ability to metabolize S-mephenytoin are also deficient in PPI oxidation (Wedlund 2000). Moreover, recently it has been reported that CYP2C19 is responsible for the metabolism of about 80% of the PPIs omeprazole, lansoprazole and pantoprazole in EMs, while CYP3A4 may be more important in PMs (Desta et al 2002). The available evidence suggests that PMs recover better from gastric disorders compared with EM patients, presumably because of their lower capacity to clear PPIs (Furuta et al 2005).

Figure 1.7. The metabolic pathways of different PPIs (Ishizaki & Horai 1999)

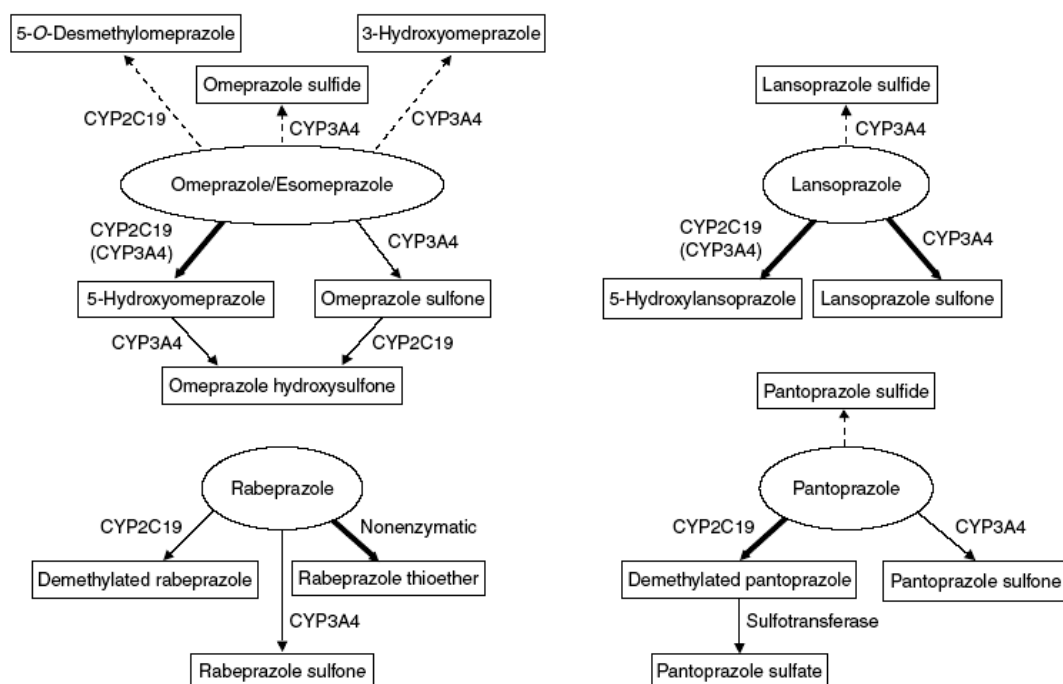


Table 1.12. Pharmacokinetic parameters of PPI drugs (Stedman & Barclay 2000)

Pharmacokinetic parameters	Omeprazole 20 mg	Pantoprazole 40 mg	Lansoprazole 30 mg	Rabeprazole 20 mg
AUC ($\mu\text{g}\cdot\text{h}/\text{mL}$)	0.2 - 1.2	2 - 5	1.7 - 5	0.8
C_{max} ($\mu\text{g}/\text{mL}$)	0.08 - 8	1.1 - 3.3	0.6 - 1.2	0.41
T_{max} (h)	1 - 3	2 - 4	1.3 - 2.3 *	3.1§
$t_{1/2}$ (h)	0.6 - 1	0.9 - 1.9	0.9 - 1.6	1
CL (L.h/kg)	0.45	0.08 - 0.13	0.2 - 0.28	0.5
Vd (L/kg)	0.31 - 0.34	0.14 - 0.17	0.39 - 0.46	
Bioavailability (%)	Variable 35-> 65 (with repeated dose)	Constant 57 - 100	Constant 80 -91	52 #
Protein binding (%)	95	98	97 - 99	95 - 98
Dose Linearity	non-linear	linear	linear ‡	linear

* Delayed to 3.5-3.7 with food, § delayed by 1.7 h with food, ‡ non-linear in some studies for doses < 20 mg and intravenous administration. #, Sclar et al 1994.

AUC, area under the concentration-time curve; C_{max} , maximum serum concentrations; T_{max} , time to maximum serum concentration; $t_{1/2}$, elimination half life; CL, drug clearance; V_d , apparent volume of distribution.

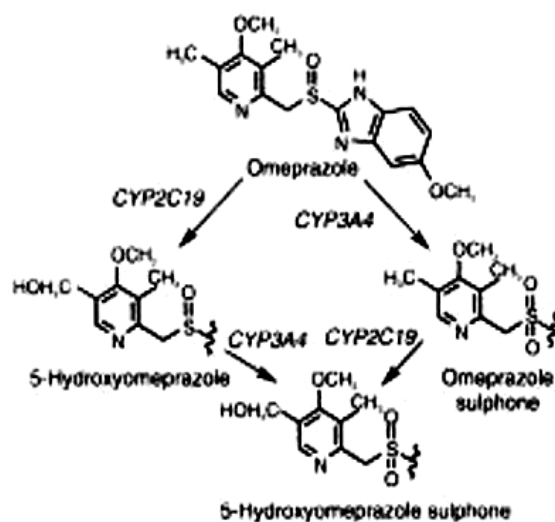
1.3.4.1. Metabolism of Omeprazole and Esomeprazole

Omeprazole is almost completely oxidized to its two major metabolites, 5-hydroxy omeprazole and omeprazole sulfone, and no unchanged drug appears in urine or faeces (Howden 1991). Omeprazole is mainly metabolized by CYP2C19 and CYP3A4 to 5-hydroxyomeprazole and omeprazole sulfone, respectively. Also, CYP2C19 contributes to the formation of omeprazole sulfone (Anderson et al 1993). Figure 1.8 shows the major metabolic pathways of omeprazole. For most PPIs, such as omeprazole, the activity of CYP2C19 determines the plasma concentration of omeprazole (Rost et al 1992). A recent study conducted by Klotz has shown that the AUC of omeprazole, lansoprazole, and rabeprazole are approximately 7.5, 4.5, and 4 times higher in PMs than in homozygous EMs (Klotz 2006). The eradication rate of *H. pylori* is higher in PMs (100%) compared with EMs (29%) and IMs (60%) (Furuta et al 1998).

The (*S*)-enantiomer of omeprazole is termed **esomeprazole**, which is metabolized slightly differently from racemic omeprazole and (*R*)-omeprazole (Miura et al 2005). Esomeprazole is also a substrate for CYP2C19 and CYP3A4, but its clearance is less affected by the CYP2C19 polymorphism than either the racemic form or (*R*)-enantiomer (Miura et al 2005). Thus, CYP2C19 is responsible for 70% of esomeprazole oxidation, while 90% of (*R*)-omeprazole is metabolized by CYP2C19 (Abelo et al 2000). Based on *in vitro* experiments, CYP2C19 and CYP3A4 account for approximately 70% and 30% of esomeprazole metabolites, respectively (Abelo et al 2000; Andersson et al 2001). Additional *in vitro* studies of have also shown that the inhibitory potential of esomeprazole is low

and restricted to effects on the oxidation of CYP2C19 substrates (Abelo et al 2000).

Figure 1.8. Major metabolic pathways of omeprazole (Anderson et al 1993)



1.3.4.2. Metabolism of Rabeprazole

Some studies have suggested that the major metabolic pathway of rabeprazole is non-enzymatic reduction to the thioether (Figure 1.7) (Ishizaki & Horai 1999). Thus, the polymorphism of CYP2C19 appears to have a lesser impact on rabeprazole metabolism than on omeprazole metabolism (Horn 2006; Ishizaki & Horai 1999; Lim et al 2004). In contrast with other PPIs, Ariizumi et al have reported that after 4 or 8 weeks treatment with 10 mg/day of rabeprazole, no significant differences in healing rate were seen among the different genotypes of CYP2C19 (Ariizumi et al 2006). However, it has been reported that the AUC of rabeprazole and its thioether

metabolite are significantly higher in PM than in EM subjects (Lin et al 2003). On day 1 and 4 of treatment with rabeprazole (20 mg twice/daily), the AUCs for rabeprazole and its thioether metabolite were higher in PM than EM subjects. The plasma gastrin concentration was not different between PMs and EMs on day 1, but it was higher in PMs by day 4 (Lin et al 2003). These conflicting findings may relate to difference in the dosage regimens that were used in rabeprazole therapy but require independent confirmation in the future.

The commercially available form of rabeprazole is the racemic mixture. Similar to other PPIs rabeprazole contains a chiral benzimidazole sulfoxide. The pharmacokinetic parameters of rabeprazole enantiomers in patients carrying three different CYP2C19 genotypes (homozygous EMs *1/*1, heterozygous EMs *1/*2 and *1/*3 and PMs *2/*2) have been determined. The results showed that (*R*)-rabeprazole produced higher plasma concentrations and higher AUC than (*S*)-rabeprazole in all genotypes. Furthermore, the elimination half-life of (*R*)-rabeprazole was significantly longer in PMs than in homozygous EMs. Thus, it was suggested that clearance of the (*R*)-enantiomer of rabeprazole is more dependent on CYP2C19 genotype than that of (*S*)-rabeprazole. However, the AUC ratios of R/S enantiomers in homozygous EMs, heterozygous EMs, and PMs (which were 1.8, 2.2, and 2.4) indicated that the overall metabolism of rabeprazole is less dependent on CYP2C19 compared to lansoprazole (Miura et al 2005).

1.3.4.3. Metabolism of Lansoprazole and Pantoprazole

Lansoprazole is metabolized by CYP2C19 and CYP3A4 to 5-hydroxylansoprazole and lansoprazole sulphone, respectively. According to *in vitro* studies in human hepatic microsomes, the affinity of lansoprazole for CYP2C19 is lower than that of omeprazole (Furuta et al 2005). Similar to omeprazole, lansoprazole induces the synthesis of CYP1A1 and CYP1A2 (Furuta et al 2005). Nevertheless, compared to omeprazole it does not appear to elicit clinically significant interactions with other drugs (Furuta et al 2005).

Pantoprazole, like the other PPIs, undergoes hepatic oxidation by CYP2C19 and CYP3A4. All studies to date have suggested that the potential of pantoprazole to inhibit the activity of CYPs appears to be lower than that of omeprazole and lansoprazole (Simon et al 1991).

Table 1.13 summarizes pharmacokinetic parameters of PPIs in subjects who are PM and EM for CYP2C19 (Ishizaki & Horai 1999).

Table 1.13. Pharmacokinetic parameters of PPIs in EMs and PMs of CYP2C19 (Ishizaki & Horai 1999).

PPIs	$t_{1/2}$		CL (ml/min/kg)		AUC _{EM} : AUC _{PM} (0-∞)	C _{max} : C _{max} PM
	EM	PM	EM	PM		
Omeprazole	0.6	2.1	18.39	1.24	1.0 : 6.3	1.0 : 3.1
	0.71	2.68	1.24	0.06(L/h/kg)	1.0* : 3.7§ : 20.0‡	1.0* : 2.6§ : 7.6‡
Lansoprazole	1.4	3.2	0.26(L/h/kg)	0.04(L/h/kg)	1.0 : 4.7	1.0 : 2.4
	1.9*	3.61	3.77	0.78	1.0* : 1.92§ : 4.11‡	1.0* : 1.32§ : 1.88‡
Pantoprazole	1.4	6.9	125.5 (mL/min)	20.19 (mL/min)	1.0 : 6.0	1.0 : 1.7
Rabeprazole	1	1.8	8.94	3.9	1.0 : 1.8	1.0 : 0.97

*Homozygous EMs, § Heterozygous EMs, ‡PMs

1.3.5. Interaction Profiles of PPIs

Since most of the gastric acid-related disorders are chronic, long term treatment seems necessary and consequently the likelihood of concomitant use of other drugs is increased (Blume et al 2006). Three types of interactions are common with PPI drugs:

- 1) Increasing gastric pH,
- 2) Interaction with the P-glycoprotein transporter protein and
- 3) interaction with CYPs.

1.3.5.1. Modulation of Gastric pH

Since PPIs increase gastric pH, they could modify the extent of ionization, solubility and the release of certain drugs (Blume et al 2006). Ketoconazole and itraconazole are two important drugs that are susceptible to this type of interaction (Chin et al 1995;

Jaruratanasirikul & Sriwiryajan 1998). Increased gastric pH decreases the solubility of the drugs in aqueous systems. After co-administration of omeprazole the AUC of ketoconazole and itraconazole is reduced by 80% and 64%, respectively (Chin et al 1995; Jaruratanasirikul & Sriwiryajan 1998). It seems that most of the significant interactions reported with rabeprazole are a result of increased gastric pH, e.g. with digoxin and ketoconazole. Gastric acid antisecretory effect of rabeprazole may increase intra-gastric pH and decrease the absorption of digoxin and ketoconazole (Blume et al 2006).

1.3.5.2. Interactions with the P-glycoprotein Transporters

P-glycoprotein transporters mediate the efflux of some drug substrates from enterocytes back into the gut lumen, or from the capillaries of the blood-brain barrier back into the blood stream (Potschka et al 2001).

P-glycoprotein inhibitors or inducers can affect the disposition of transporter substrates (Potschka et al 2001). It has been suggested that P-glycoprotein transporters might affect the access of some drugs to CYP3A4 present in the intestine (Blume et al 2006). PPIs, such as omeprazole, lansoprazole, and pantoprazole are substrates of this transporter system and have been reported to inhibit P-glycoprotein mediated efflux of digoxin (Paul-Magnus 2001).

1.3.5.3. Interactions with CYP Enzymes

Recent *in vitro* studies of the inhibitory effect of PPIs on four CYPs (CYP2C9, CYP2C19, CYP2D6, and CYP3A4) have shown that none of the drugs inhibited CYP2D6. However, CYP2C9, CYP3A4, and CYP2C19 are susceptible to inhibition by PPIs (Li et al 2004).

The inhibitory potency of PPIs toward CYP2C19 was determined in both human liver microsomes and with recombinant CYP (rCYP). In microsomes the inhibition constant (K_i) of omeprazole against CYP2C19 was between 2 and 9 μM and was between 17 and 21 μM for rabeprazole. Interestingly, the thioether metabolite of rabeprazole was more potent than the parent drug against CYP2C19 ($K_i = 2\text{-}8 \mu\text{M}$) (Li et al 2004). Moreover, omeprazole and rabeprazole were relatively non-potent inhibitors of CYP3A4, but the inhibitory potency of rabeprazole thioether was about 3-fold higher than that of rabeprazole ($K_i = 41.9 \mu\text{M}$ for omeprazole; $K_i = 50.7 \mu\text{M}$ for rabeprazole and 15 μM for rabeprazole thioether) (Li et al 2004).

1.3.5.3.1. Inhibition of CYP-dependent Drug Oxidation by Omeprazole

CYP2C19 mediates the formation of the major metabolite of diazepam, which is *N*-desmethyldiazepam (Anderson et al 1990). Although many factors *in vivo* could influence the inhibition of diazepam metabolism, it has been confirmed that the clearance of diazepam is reduced up to 25% to 50% during concomitant use of omeprazole, due to competitive inhibition of CYP2C19 (Caraco et al

1995). Another study indicated that omeprazole does not impair the clearance of diazepam in PMs for CYP2C19, but in EMs the clearance of diazepam is impaired (Ishizaki et al 1995). In agreement with this finding Andersson et al have reported that omeprazole only decreases the clearance of diazepam in CYP2C19 EM subjects (Andersson et al 1990).

Omeprazole was shown to reduce the clearance of both carbamazepine and phenytoin (Dixit et al 2001; Prichard et al 1987)

Phenytoin is principally metabolized by CYP2C9 to 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (*p*-HPPH) and also by CYP2C19 (Levy 1995). Omeprazole increases phenytoin plasma concentrations by about 25% due to the inhibition of CYP2C19 activity (Richard et al 1987).

CYP3A4 is the main enzyme involved in the metabolism of carbamazepine to carbamazepine-10, 11-epoxide (Levy 1995). Dixit et al demonstrated that multiple doses of omeprazole increased the C_{max}, AUC, and elimination half-life of a sustained-release preparation of carbamazepine (Dixit et al 2001).

Since omeprazole is a competitive inhibitor of CYP2C19 and this enzyme oxidizes warfarin to some extent, the metabolism of warfarin might be altered during omeprazole therapy. However, findings in this regards are conflicting (Blume et al 2006). Some studies have demonstrated that the hepatic metabolism of the *R*-enantiomer, but not the *S*-enantiomer, of warfarin is inhibited due to CYP2C19 inhibition. However, only one of these studies demonstrated a significant increase in the anticoagulation time of warfarin (Blume et al 2006). Thus, the clinical significance of the interaction is unclear.

CYP2C19 is an important enzyme in the bioactivation of the antimalarial prodrug proguanil, to cycloguanil. An *in vitro* study has shown that omeprazole (20 µmol/L) inhibits cycloguanil formation (Funck-Brentano et al 1997). Following an *in vivo* study in healthy subjects, the apparent oral clearance and metabolic clearance of proguanil to cycloguanil decreased significantly in the presence of omeprazole (Funck-Brentano et al 1997).

Omeprazole (40 mg) also inhibited the metabolism of moclobemide, an antidepressant with selective action at monoamine oxidase-A, that is principally a substrate for CYP2C19. Omeprazole inhibited moclobemide metabolism in CYP2C19 EM subjects but not in PM subjects (Yu et al 2001).

1.3.5.3.3. Induction of CYP Genes by Omeprazole

Studies in cultured cells have indicated that CYP1A1 and CYP1A2 mRNA expression is increased by omeprazole (Shin et al 1999; Quattrochi & Tukey 1993; Curi-Pedrosa et al 1993). Subsequent *in vivo* studies of caffeine clearance in breath or urine confirmed the induction of CYP1A2 by omeprazole. However, most of these studies have indicated that the induction of CYP1A2 is dose dependent. A study conducted on 12 EM, one intermediate metabolizer, and 5 PM for CYP2C19 (Rost et al 1992), using the ¹³C-caffeine breath test, showed that the AUC of omeprazole after 7 days treatment with 40 mg omeprazole was 4-fold higher in the PMs than in the EMs for CYP2C19. Also, the exhalation of ¹³CO₂ was significantly higher in PMs and

intermediate metabolizers than in the EMs. Thus, it appears that CYP1A2 induction at therapeutic dose of omeprazole (40 mg/daily) is dependent on CYP2C19 status. Another study by the same authors showed that CYP1A2 induction by omeprazole in EMs is dose-dependent (Rost et al 1994). Thus, CYP1A2 activity was increased only 8.5% by 40 mg omeprazole and 27.3% by a 120 mg dose. Furthermore, it has been suggested that omeprazole does not induce CYP1A2 activity at conventional therapeutic doses (20 mg/day) (Andersson et al 1998; Rizzo et al 1996; Sinues et al 2004). These findings indicate that the *in vivo* induction of CYP1A2 is dependent on the plasma concentration of omeprazole as well as CYP2C19 status (which influences the elimination of omeprazole). Moreover, it has been suggested that the induction of CYP1A2 by omeprazole may depend on the CYP1A2 genotype. An *in vivo* study with 12 subjects who were EMs with respect to CYP2C19 showed that the plasma ratio of caffeine/paraxanthine metabolite (used to reflect CYP1A2 activity) was significantly higher in subjects who were homozygous for *CYP1A2*IF* after 7 days treatment with omeprazole (120 mg daily) (Han et al 2002). As mentioned previously, the *CYP1A2*IF* genotype appears to be a more inducible allele (Sachse et al 1999).

1.3.5.3.3. Inhibition of CYP Enzymes by Rabeprazole

Recent study has shown that fluvoxamine, which is a potent CYP2C19 inhibitor, increases the AUC of rabeprazole and its thioether metabolite by 2.8-fold and 5.1-fold in homozygous EMs and 1.7-fold and 2.6-fold in heterozygous EMs, respectively (Uno et al 2005). Rabeprazole thioether may be demethylated by CYP2C19. Shirai et al have shown that the AUC of rabeprazole was not changed after repeated doses of rabeprazole in individuals with different CYP2C19 status. However, the AUC of rabeprazole thioether was significantly higher in CYP2C19 PMs (Shirai et al 2001). Although, rabeprazole itself may not influence CYP2C19 activity significantly, its thioether metabolite may be an inhibitor of the enzyme. Further studies will be useful to corroborate this possibility and then evaluate its effects on pharmacokinetics of co-administered drugs.

1.3.5.3.4. Induction of CYP Genes by Rabeprazole

Krusekopf et al have investigated the induction of omeprazole and rabeprazole on CYP1A1, 1A2 and 1B1 activity in HepG2 cells. After 4 hours treatment of HepG2 cells with omeprazole (100 μ M) and rabeprazole (50 μ M), both omeprazole and rabeprazole induced CYP1A1 and CYP1A2. Omeprazole was a more effective inducer (Krusekopf et al 2003). No other studies have yet evaluated the effect of rabeprazole on the activities of CYP1A2 or other CYPs. Further understanding of the effect of rabeprazole on CYP function and expression may assist dose prediction and adjustment of drug such as clozapine. Table 1.14 summarizes the clinically significant interactions of omeprazole and rabeprazole with antipsychotic agents

Table 1.14. Interactions of omeprazole and rabeprazole with antipsychotics and other drugs.

Drug	Major P450 involved	Omeprazole	Rabeprazole	References
Diazepam	CYP2C19	Clearance↓	No interaction	Caraco et al 1995
Phenytoin	CYP2C9/2C19	Clearance↓	Unknown	Richard et al 1987
Carbamazepine	CYP3A4/ P-glycoprotein	Clearance↓	Unknown	Dixit et al 2001; Prichard et al 1987
Clozapine	CYP1A2	Clearance↑	Unknown	Frick et al 2003; Mookhoek & Loonen 2004
Olanzapine	CYP1A2	Unknown	Unknown	www.nzhpa.org.nz
Triazolam	CYP3A4	Clearance↓	Unknown	Blume et al 2006
Warfarin	CYP2C19	Controversial results	Unknown	Blume et al 2006
Proguanil	CYP2C19	Clearance↓	Unknown	Funck-Brentano et al 1997

1.4. Aims of this study

The replacement of omeprazole with rabeprazole may offer therapeutic benefits in clozapine patients who require PPIs. However, to date there have been no in vivo studies that have evaluated the impact of rabeprazole on clozapine metabolism. The major aim of this study was to investigate and compare the effects of rabeprazole and omeprazole on plasma clozapine concentrations and therefore on apparent clozapine clearance in patients. Hence, the hypothesis of this research is that “Does rabeprazole has a similar effect as omeprazole on apparent clearance of clozapine?”

Therefore, the effect of the PPI-clozapine combinations on plasma clozapine concentration was assessed in sequential fashion in patients who were controlled by clozapine.

The results may assist the evaluation of the influence of rabeprazole on CYP1A2 activity, which is the most important enzyme in the metabolism of clozapine.

Because there are a number of potential covariates that could influence clozapine plasma concentrations, these variables were considered in the present analysis. Apart from CYP1A2 function these patient covariates included cigarette smoking, weight, Body Mass Index (BMI), age, and gender.

A model for clozapine pharmacokinetics was evaluated using the Abbottbase Bayesian Pharmacokinetic System (PKS) and internal and external data.

CHAPTER TWO: MATERIALS AND METHODS

2. Materials and Methods

2.1. Study Subjects

The study was approved by the Northern Sydney Health Human Research Ethics Committee and University Human Ethics Committee. Patients from Macquarie Hospital who were stabilized on clozapine were recruited for this study. These subjects were also receiving rabeprazole as part of their drug therapy for clozapine mediated gastrointestinal disturbances. They were not receiving other medication that could potentially interact with CYP1A2 and CYP2C19 (Appendix I). Enrolled patients did not have any coexisting medical conditions (such as renal impairment). Although cigarette smoking has been shown to induce CYP1A2 (Bondolfi et al 2005), smokers and non-smokers subjects were included in this study since the majority of schizophrenic patients are smokers (Ücok et al 2004).

The study was conducted over a period of 5 months. Relevant medical staff in the rehabilitation wards discussed the study with patients who were eligible for inclusion in this clinical trial. Patients who were willing to participate were provided verbal and written information (Appendix II) about the study, and written informed consent was obtained from each participant (Appendix III). Of the eligible patients all but one were happy to participate. Medical staff in the rehabilitation wards (Psychiatrists in charge, chief pharmacist and registered nurse) supervised enrolment and took blood.

To provide privacy and confidentiality for the enrolled patients all information was identified by patient codes and not by name.

Details required for population pharmacokinetic analysis such as demographics, dosing schedule, smoking and alcohol histories, and concurrent medication were obtained from the hospital files of the study patients at the Macquarie Hospital. Approximately 80% of all schizophrenic patients in hospital who received clozapine were smokers. Data on number of smoked cigarettes are recorded and controlled by nurses. Finally, 20 patients were recruited to this study. The student obtained the patient data and blood samples for analysis. The patients' data are presented in table 2.1.

2.2. Study design

A cross-over study design was used to compare the effects of co-administered rabeprazole and omeprazole on plasma clozapine concentrations in patients. Twenty patients who had been receiving clozapine and rabeprazole (with no other interacting medications) for at least two weeks and were at steady state were recruited into the first arm of the study (Phase 1). Clozapine dosage was determined by the attending clinician and the dose of rabeprazole was either 20 mg or 40 mg. Blood samples (10 ml each) were taken 30 minutes, 1 hour, 2 hours and 12 hours after a dose of clozapine.

In the second arm of the study (Phase 2), rabeprazole was switched to omeprazole (consistent with the rabeprazole dose in the first arm of the study i.e. 20 or 40 mg).

Samples were again collected at the intervals described above after the patients had received omeprazole and clozapine for at least one month. Table 2.1 summarizes these phases of the study.

Recruitment to the proposed control was not possible because patients were not available. Further, technical issues prevented the assay of the 5-hydroxy- metabolite of omeprazole in patient samples.

Table 2.1: Phase I and Phase II of the study

Phase I	Phase I	Phase II	Phase II
Clozapine + Rabeprazole (at least 2 weeks)	Time at which blood samples were taken relative to last dose	Clozapine + Omeprazole (1 month)	Time at which blood samples were taken relative to last dose
	30 min		30 min
	1hr		1hr
	2hr		2hr
	12hr		12hr

Blood samples from the patients were collected into heparinized tubes. Immediately after collection, blood samples were centrifuged (2000g x 10 minutes). The obtained plasma was stored in 1.5 mL Eppendorf tubes at -80°C until used in assays. The interval between sampling and assays was 5 to 6 months. Aravagiri & Marder 2001 have shown that clozapine and its metabolites (norclozapine and clozapine-N-oxide) stored in freezer at -70 °C are stable over a period of 6months (Aravagiri & Marder 2001).

Clozapine and norclozapine concentrations were estimated (described in section 2.3 of Materials and Methods). The clozapine concentrations were used to determine the pharmacokinetic parameters of clozapine, and the ratios of norclozapine/clozapine at trough levels were used to reflect CYP1A2 activity in the presence of rabeprazole in phase 1 and in the presence of omeprazole in phase 2. Statistical analysis was performed using GraphPad Prism (Version 4.00).

2.3. Analytical Method

The concentrations of clozapine and norclozapine were quantified by a high-performance liquid chromatography (HPLC) at the Austin and Repatriation Medical Centre in Heidelberg, Victoria. The frozen plasma samples were transported to Melbourne on dry ice by Australian Air Express.

A liquid-liquid extraction method was used to extract clozapine and norclozapine from plasma. To 0.5 mL of plasma, 10 μ L of internal standard (flunitrazepam, 5 mg/mL in methanol) was added. Clozapine and norclozapine were extracted by the addition of hexane (2.5 mL) and ethyl acetate (2.5 mL) and the sample was vortex mixed for one minute and then centrifuged (1000g x 5 min). The aqueous layer was frozen in a chilled ethanol bath at -30° C and the organic layer was transferred to a clean labeled tube. Samples then were dried under a stream of air before reconstitution in mobile phase (150 μ L) and injection (20 μ L) on to a Spherisorb CN 5 μ m 0.5 x 150 mm column (Alltech). Separation was achieved using a mobile phase of 60:40, acetonitrile: triethylamine (2.2 mmol/L, pH 5.5-6 adjusted with orthophosphoric acid) at a flow rate of 1.5 mL/min and detection at 254 nm.

Multipoint standard curves were constructed from clozapine (47, 94, 188, 375, 750 and 1500 ng/mL) and norclozapine (94, 188, 375 and 1500 ng/mL). The lower limit of quantitation for clozapine was 47 ng/mL and for norclozapine was 94 ng/mL. The upper limit of quantitation was 1500 ng/mL for both clozapine and norclozapine.

The inter-day accuracy and precision were assessed at low, medium and high concentrations across the standard curve and were <12.2% and <8.5% for clozapine and < 19.1% and <12.3% for norclozapine, respectively. The coefficients of determination (r^2) for standard curves were >0.995 in all cases.

2.4. Estimation of Clozapine Clearance

The Abbottbase Pharmacokinetic System (PKS) Software Version 1.10 (Abbott Laboratories, Abbott Diagnostics GMBH), was used to estimate the pharmacokinetic parameters of clozapine in the study participants by Bayesian forecasting.

Statistical analysis was performed using GraphPad Prism (Version 4.00).

2.4.1. Bayesian Model for Estimation of Pharmacokinetic Parameters of Clozapine Using Abbottbase Pharmacokinetic System (PKS)

PKS software allows the user to predict drug dose, calculate population pharmacokinetic parameters and estimate their variability.

The model was validated using concentration-time data from patients in another study (obtained by personal communication with researchers).

The values for development of a Bayesian model for clozapine were extracted from the literature (Doude van Troostwijk et al 2003b) and were as follows:

$$V_d \text{ (volume of distribution)} = 6 \pm 21 \text{ L/kg}$$

$$K_e \text{ (elimination rate constant of clozapine)} = 0.05 \pm 0.02 \text{ h}^{-1}$$

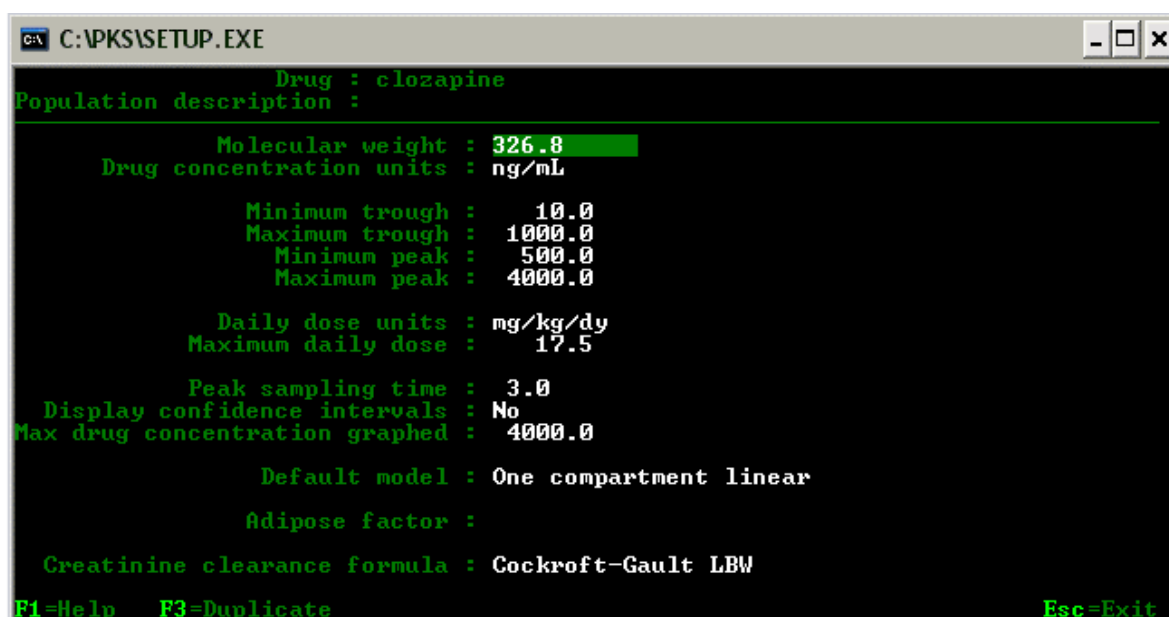
$$F \text{ (bioavailability)} = 0.34 \pm 0.12$$

$$K_a \text{ (absorption rate constant)} = 1 \pm 0.5 \text{ h}^{-1}$$

(Parameters used in the model of Doude van Troostwijk et al (2003b) were derived from three sources, Guitton et al 1998, Jann et al 1993, Miller et al 1994).

Doude van Troostwijk et al (2003b) used population PK software (MWPharm) and Bayesian analysis to estimate PK parameters of clozapine. The estimated PK-parameters in that study were obtained using single blood samples. In the present study at least four points were used to estimate PK-parameters of clozapine, which leads to more accurate estimations. Figure 2.1 shows the model set up panel of the PKs for clozapine.

Figure 2.1: Input data for validation of Abbottbase Pharmacokinetic System (PKS) for clozapine



The predicted concentrations, apparent clearance (CL/F) and apparent volume of distribution (V_d/F) of clozapine in both phases of the study were calculated by the PKS program after entering the actual concentrations of clozapine in individual patient samples taken at the indicated times after dosage (0.5, 1, 2 and 12 hr). The calculated clearance and volume of distribution data in this thesis are apparent clearance (CL/F) and apparent volume of distribution (V_d/F).

There are different methods for evaluation of the performance of Abbottbase Bayesian model:

1. Correlation between the observed and predicted plasma concentrations.

The bias (accuracy) is calculated by Mean Prediction Error (ME) and precision is calculated by Mean Square Error (MSE) or Root Mean Square Error (RMSE) (Sheiner & Beal 1981).

2. Correlation between the observed and predicted area under the curve (AUC).

For evaluation of the performance of the program the actual plasma concentrations of clozapine were plotted against the predicted plasma concentrations at all sampling time points in both phases of the study. In addition, the AUC_{0-12} of clozapine was calculated by the log trapezoidal method, in which the actual and predicted clozapine concentrations were entered. The ME and RMSE indicated bias and precision of this software.

The accuracy and precision of the developed model were also estimated using the external data on 38 subjects derived from the dataset taken obtained in another study (by personal communication with researchers). External model evaluation was performed by plotting the actual and predicted plasma concentration-time curve at trough level of clozapine. The ME and RMSE indicated bias and precision of this software.

2.5. Statistical analysis:

All the statistical analyses were performed using GraphPad Prism (Version 4.00).

The comparison of the clozapine clearance in the presence of rabeprazole and omeprazole was performed using the two-tailed paired student's t-test.

The influence of smoking on clozapine clearance was performed using one-way analysis of variance.

Pearson r test was performed to determine the correlation between both clozapine clearance and weight and BMI. The same statistical approach was used to determine the correlation between the ratio of plasma norclozapine/clozapine concentrations and weight and BMI.

Mann-Whitney test was applied to compare clozapine clearance in male and female and in two groups of patients with BMI>25 and BMI<25 in phases of the study.

CHAPTER THREE: RESULTS

3. Results

3.1. Subjects

Twenty patients were recruited in this study (Table 3.1). The demographic characteristics of the 20 evaluable patients are summarized in table 3.2. Fifteen males and five females were recruited in this study. Ninety percent of recruited patients were Caucasians. The ages ranged from 22 to 67 years (mean \pm SD: 43.74 ± 14.39), the heights were in the range of 159 to 188 cm (mean \pm SD: 173 ± 8.95), weights ranged from 60 to 128 kg (mean \pm SD: 89.37 ± 18.57) and the Body Mass Index (BMI) values were from 22 to 39.5 (mean \pm SD: 29.61 ± 5.60). Since 80% of admitted schizophrenic patients to hospital were smokers, just four nonsmokers could be recruited in this study and 16 patients were smokers. The daily dose of clozapine among the patients varied between 2.8 to 11.8 mg/kg/day (mean \pm SD: 6.1 ± 2.33). The PPI dosage was either 20 or 40 mg/day. The clozapine and norclozapine plasma concentrations were assayed in samples collected from the patients at 0.5, 1, 2 and 12 hours after clozapine intake during both phases of the study. The trough level for one patient was not determined because of sample losses during HPLC. The comparisons involving the trough levels, particularly the plasma ratio of norclozapine/clozapine concentrations of this patient was omitted from the analysis. In addition, a 2 hour sample for one of the patients was not available in the second phase of the study.

Table 3.1: patients' data

Patient	Sex	Weight kg	Height cm	Age Year	BMI	Cigarettes /day	Clozapine Dosage (mg)	PPI (mg)
MN-1	M	128	180	38	39.5	20	200m/500n	20
GR-2	M	69	177	55	22.0	14	100m/200n	20
MS-3	M	107	187	32	30.6	25	300m/400n	20
RE-4	M	68	173	52	22.7	Nil	400m/400n	20
DD-5	M	103	187	44	29.5	Nil	200 (13pm) / 350n	20
TE-6	M	68	175	67	22.2	Nil	350m/350n	40
PN-7	F	60	164	65	22.3	8	150m/250n	20
GH-8	M	84	166	51	30.5	20	50 (14pm) /400n	20
AI-9	M	101	188	31	28.6	30	150m/300n	20
FE-10	M	106	175	28	34.6	20	50m/400n	20
KN-11	M	86	185	26	25.1	15	100m/400n	40
DR-12	M	106	175	27	34.6	15-20	100m/500n	20
JL-13	F	88	159	36	34.8	Nil	100m/150n	20
DY-14	M	101	172	34	34.1	15	150m / 450 (17pm)	40
AA-15	F	80	163	60	30.1	12	200m/400n	20
AN-16	M	102	166	22	37	7	450 (12pm)	40
NN-17	M	74	175	47	24.2	20	250 (14pm)	40
IH-18	F	101	170	58	35	13	200m/400n	20
JL-20	F	66	162	58	25.1	6	400m/250n	20
TY-21	M	87	181	25	26.5	15	100m/300n	20

m: 8:00 am, n: 20:00pm

Table 3.2: Demographic characteristics of patients

Age	42.80 ± 14.62 year	
Height	174 ± 8.86 cm	
Weight	89.25 ± 18.09 kg	
BMI	29.45 ± 5.50	
Sex	5 female	
	15 male	
Smoking status	non-smoker	4
	1-8 Cigarette(s)/day	3
	9-15 Cigarettes/day	8
	>15 Cigarettes/day	5
Clozapine dosage	6.02 ± 2.30 mg/kg/day	
PPI dosage	20 or 40	

3.2. Relationship Between Norclozapine/Clozapine Ratio in Plasma and Clozapine Clearance

Clozapine clearance was calculated from the clozapine concentration data for each patient in conjunction with the PKS software. Details of the validation of the PKS model are given in section 3.4 below. The mean values of clozapine clearances were found to be similar at 0.443 ± 0.228 and 0.487 ± 0.264 L/hr/kg (mean±SD) in Phase 1 and Phase 2, respectively. Individual clozapine and norclozapine concentrations in plasma and the ratios at 12 hour after the first clozapine dose, and clozapine clearance in both phases of the study are presented in Table 3.3.

The ratio of norclozapine/clozapine concentrations at the trough level (12 h) was used as a marker of CYP1A2 activity and ranged from 0.22 to 0.915 (mean ± SD: 0.591 ± 0.198) in the presence of rabeprazole (phase 1) and from 0.279 to 1.267 (mean ± SD:

0.598 ± 0.235) in the presence of omeprazole (phase 2). Nineteen patients are included in these analyses because the trough level for one patient could not be determined in the second phase of the study. The concentrations of norclozapine in five patients in P1 and P2 and the concentrations of clozapine in one patient in P1 were below the LLOQ and calculated manually. However, since the concentrations were close to the LLOQ small decrease in precision is expected at these levels.

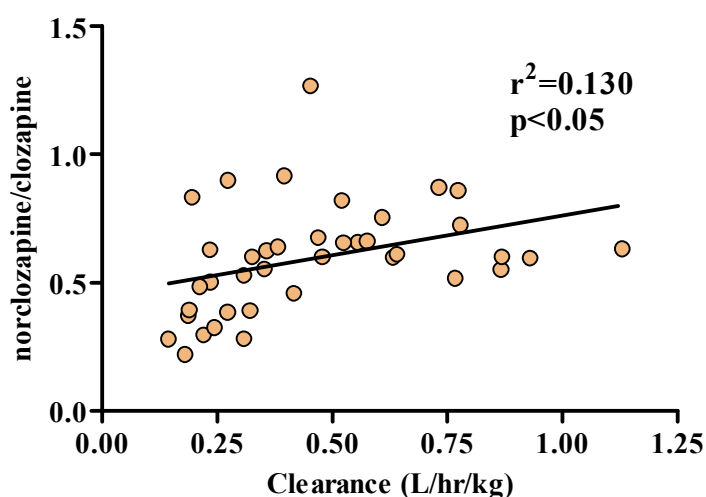
Table 3.3: Pharmacokinetic parameters of clozapine and norclozapine in both phases of the study

Patient	Clozapine (ng/mL) P1	nor-CLZ (ng/mL) P1	nor-CLZ/clozapine P1	Clearance (L/h/kg) P1	Clozapine (ng/mL) P2	nor-CLZ (ng/mL) P2	nor-CLZ/clozapine P2	Clearance (L/h/kg) P2
MN-1	241	158	0.656	0.525	350	137	0.391	0.322
GR-2	223	134	0.601	0.327	120	103	0.858	0.774
MS-3*	147	76	0.517	0.768	120	66	0.550	0.867
RE-4*	482	441	0.915	0.396	311	394	1.267	0.453
DD-5*	637	250	0.392	0.19	933	260	0.279	0.145
TE-6	208	124	0.596	0.93	125	79	0.632	1.13
PN-7	235	141	0.600	0.479	174	131	0.753	0.609
GH-8	545	177	0.325	0.245	451	238	0.528	0.309
AI-9	403	253	0.628	0.235	440	221	0.502	0.236
FE-10	100	87	0.870	0.732	116	84	0.724	0.779
KN-11	88	79	0.898	0.869	107	89	0.832	0.632
DR-12	451	271	0.601	0.274	632	378	0.598	0.196
JI-13*	382	84	0.220	0.181	318	94	0.296	0.221
DY-14	377	106	0.281	0.309	481	185	0.385	0.273
AA-15	213	141	0.662	0.576	185	125	0.676	0.47
AN-16	556	269	0.484	0.213	527	195	0.370	0.188
NN-17	152	84	0.553	0.353	108	66	0.611	0.641
IH-18	189	155	0.820	0.521	371	170	0.458	0.417
JL-20	466	291	0.624	0.358	321	211	0.657	0.556
TY-21	263	168		0.382				0.515
Mean	317.9	174.5	0.591	0.443	325.8	169.8	0.598	0.487
± SD	164.3	94.42	0.198	0.228	219.3	97.2	0.235	0.264
(CV%)	52	54	32	51	67	57	39	54

Nor-CLZ: norclozapine; P1: in presence of rabeprazole; P2: in presence of omeprazole; * non-smokers

The ratios of plasma norclozapine/clozapine concentrations were plotted as a function of the calculated clozapine clearance values obtained from both phases of the study (Figure 3.1). An analysis using Pearson's correlation coefficient indicated a statistically significant linear relationship between norclozapine/clozapine ratio and clearance of clozapine (Pearson $r = 0.361$, CI=95%, $p < 0.05$), which suggests that clozapine clearance is directly related to CYP1A2 activity. The equation of the line was determined by linear regression to be $y = 0.31x + 0.45$ ($r^2 = 0.130$).

Figure 3.1: Correlation between clozapine clearance and the norclozapine/clozapine ratio.

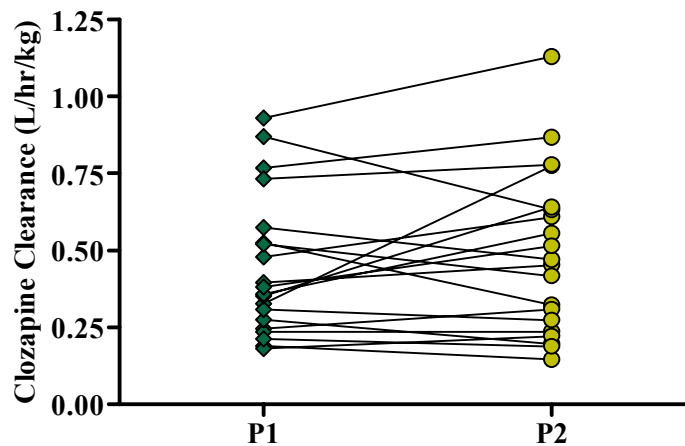


Number of XY Pairs	39
Pearson r	0.361
95% confidence interval	0.051 to 0.607
P value (two-tailed)	0.024
P value summary	*
Is the correlation significant? (alpha=0.05)	Yes
R squared	0.130

3.3. Comparison of the Effects of Omeprazole and Rabeprazole on Clozapine Clearance

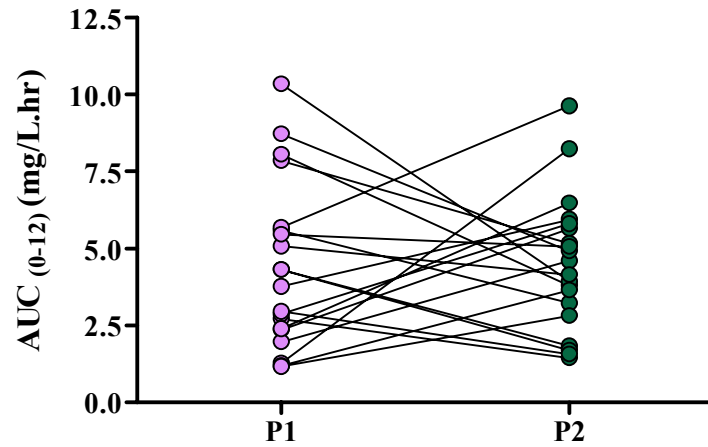
Clozapine clearance was quite variable within the patient group but intra-individual differences between the phases of the study were not significant. However, although there appeared to be a general trend toward an increase in clozapine clearance of approximately 10% in phase 2 of the study (Figure 3.2), the observed P value was 0.25. Further studies are required to clarify this point. Furthermore, no significant difference in the AUC₍₀₋₁₂₎ in the 2 phases of the study indicates that the total exposure to clozapine has not differed during the 2 phases of the study (Figure 3.3).

Figure 3.2: Comparison of the clozapine clearance in the presence of rabeprazole (P1) and in the presence of omeprazole (P2).



Paired t test	
P value	0.249
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.188 df=19
Number of pairs	20

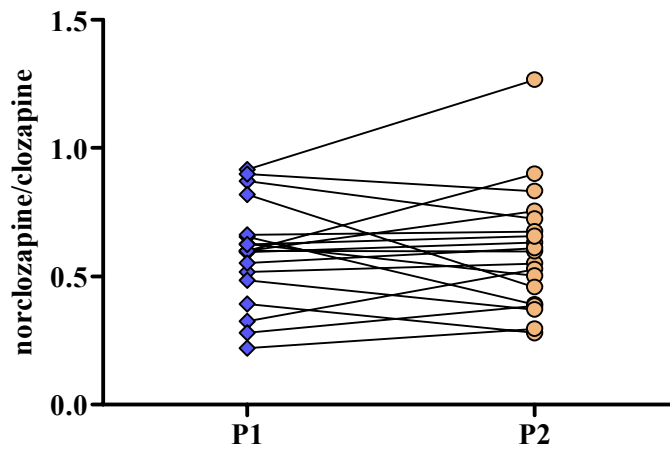
Figure 3.3: Comparison of the AUC₍₀₋₁₂₎ in the 2 phases of the study



Paired t test	
P value	0.922
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.099; df=19
Number of pairs	20

Supporting these observations the norclozapine/clozapine ratio at trough level was not different between the phases of the study. Figure 3.4 shows the distribution of the norclozapine/clozapine ratio in both phases of the study.

Figure 3.4: Comparison of the norclozapine/clozapine ratio in the presence of rabeprazole (P1) and in presence of omeprazole (P2).

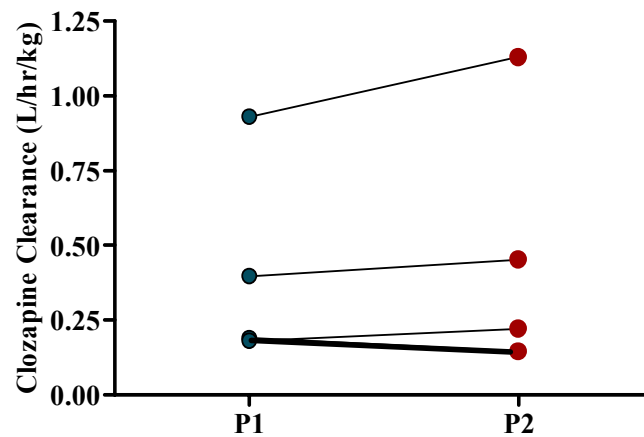


Paired t test	
P value	0.834
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.213 df=18
Number of pairs	19

To further evaluate the population differences between the study phases, the study group was divided into smokers and non-smokers. Again, however, neither the clozapine clearance nor the ratio of norclozapine/clozapine differed between the study phases in the non-smoker group (Figure 3.5 and 3.6) and smokers (Figures 3.7 and 3.8).

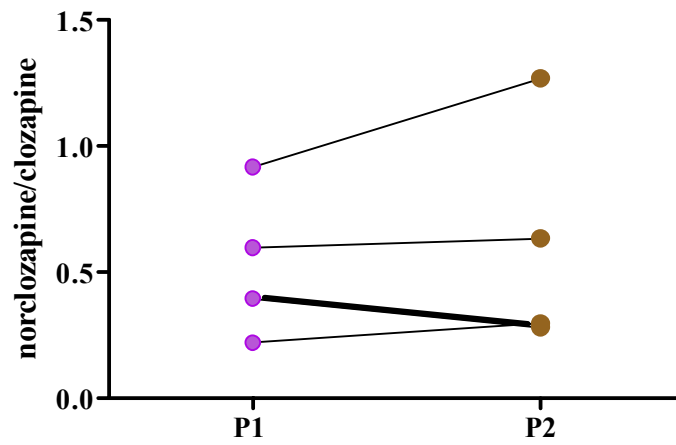
Closer examination of the clearance values and norclozapine/clozapine ratios in the non-smokers (as depicted in Figure 3.5 and 3.6) suggest that both clearance and norclozapine/clozapine ratios were higher in the omeprazole phase in 3 of the 4 non-smokers. However, investigation of the medical history of the only non-smokers who showed a decrease in both clozapine clearance and norclozapine/clozapine ratio in phase 2 of the study revealed no significant reason for this observation.

Figure 3.5: Comparison of the clozapine clearance the in the presence of rabeprazole (P1) and in the presence of omeprazole (P2) in 4 non-smoker patients. Bold line indicates the patient who showed a decrease in clozapine clearance in the presence of omeprazole.



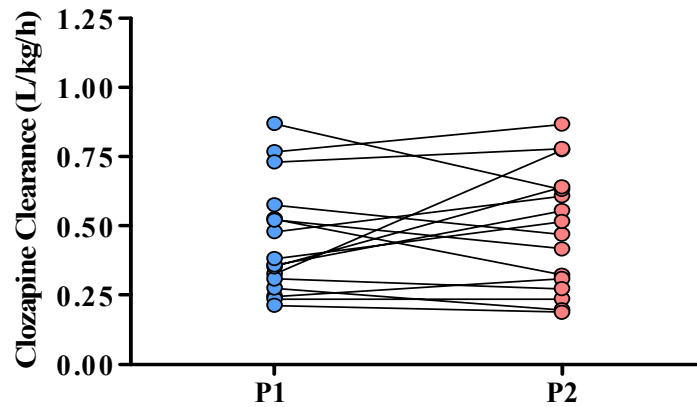
Paired t test	
P value	0.303
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=1.240 df=3
Number of pairs	4

Figure 3.6: Comparison of the norclozapine/clozapine ratio in the presence of rabeprazole (P1) and in the presence of omeprazole (P2), in non-smokers. Bold line indicates the patient who showed a decrease in the norclozapine/Clozapine ratio in the presence of omeprazole.



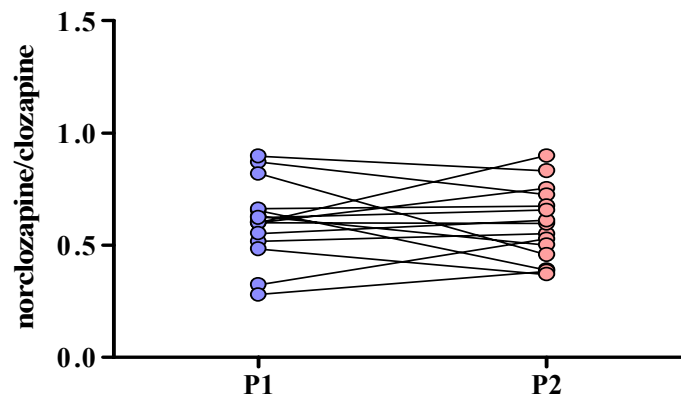
Paired t test	
P value	0.434
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.900 df=3
Number of pairs	4

Figure 3.7: Comparison of the clozapine clearance in the presence of rabeprazole (P1) and in the presence of omeprazole (P2) in smoker patients.



Paired t test	
P value	0.399
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.867; df=15
Number of pairs	16

Figure 3.8: Comparison of the norclozapine/clozapine ratio in the presence of rabeprazole (P1) and in the presence of omeprazole (P2), in smokers.

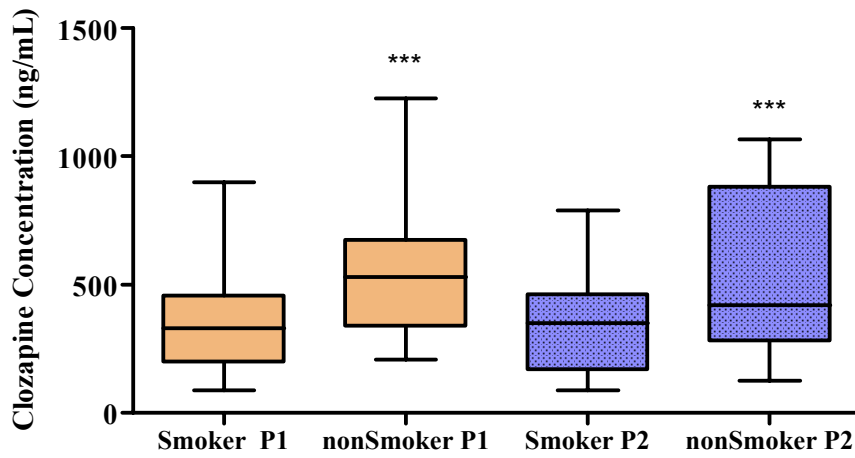


Paired t test	
P value	0.788
P value summary	Ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.274; df=14
Number of pairs	15

3.3.1. Influence of Smoking on Clozapine Concentrations

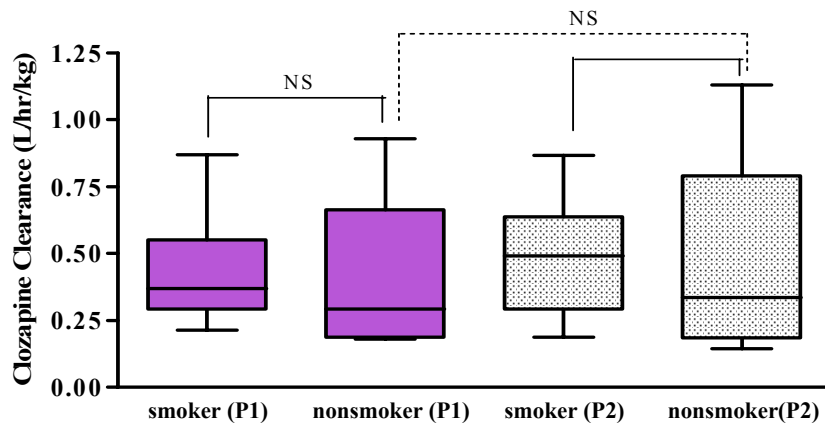
One-way analysis of variance was used to determine the impact of smoking on clozapine plasma concentrations. Dividing patients into smoker and non-smoker groups showed that clozapine trough levels are significantly higher in non-smokers than in smokers in both phases of the study (Figure 3.9). However, the mean clozapine clearance and the ratio of norclozapine/clozapine plasma concentrations in smokers **were not significantly different** to those in non-smokers in both phases of the study. Figure 3.10 and Figure 3.11 show the comparison of the influence of smoking on clozapine clearance and norclozapine/clozapine ratio in both phases of the study.

Figure 3.9: Influence of smoking on clozapine plasma concentrations in both phases of the study.



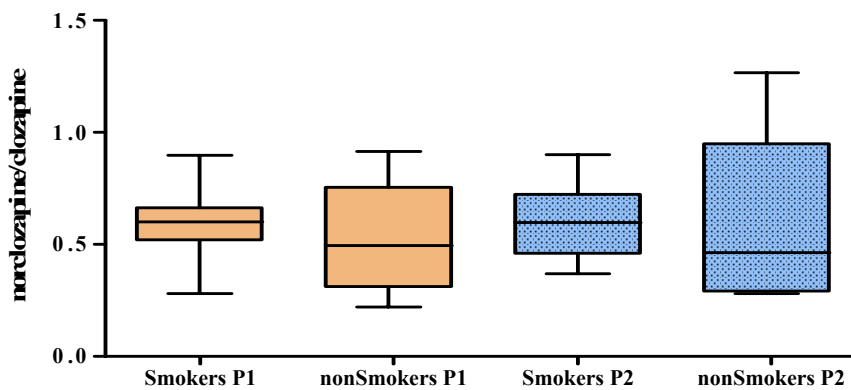
One-way analysis of variance	
P value	*** P<0.0001
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
F	8.845
R squared	0.146

Figure 3.10: Influence of smoking on clozapine clearance in both phases of the study.



One-way analysis of variance	
P value	0.969
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
F	0.083
R squared	0.007

Figure 3.11: Influence of smoking on norclozapine/clozapine ratio in both phases of the study.



One-way analysis of variance	
P value	0.935
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
F	0.141
R squared	0.012

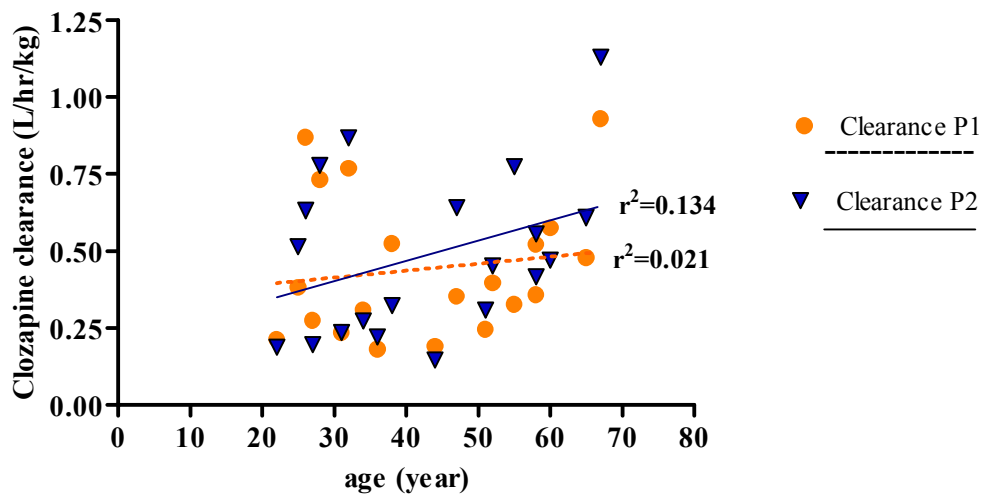
3.4. Impact of other covariates on clozapine clearance

3.4.1. Age

The relationship between age and clozapine clearance is shown in Figure 3.12.

Significant relationships were not apparent in either phase of the study.

Figure 3.12: The influence of age on clozapine clearance

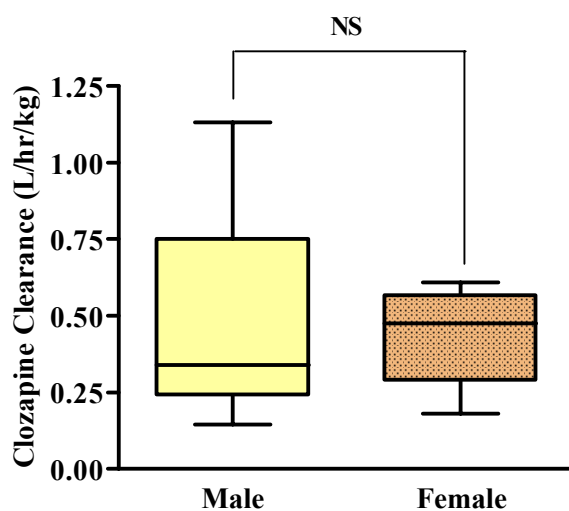


	P1	P2
Number of XY Pairs	20	20
Pearson r	0.145	0.366
95% confidence interval	-0.317 to 0.553	-0.092 to 0.696
P value (two-tailed)	0.540	0.113
P value summary	ns	ns
Is the correlation significant? (alpha=0.05)	No	No
R squared	0.021	0.134

3.4.2. Gender

The Mann-Whitney test was applied to compare clozapine clearance in male and female patients but the influence of gender was not significant. It is suggested from Figure 3.13 that the mean value of clozapine clearance may have been lower in males than in females. However, the small number of the subjects recruited in this study and the large inter-individual variation in clearance resulted in a non significant relationship. Figure 3.13 indicates the relationship between gender and clozapine clearance.

Figure 3.13: The effect of gender on clozapine clearance

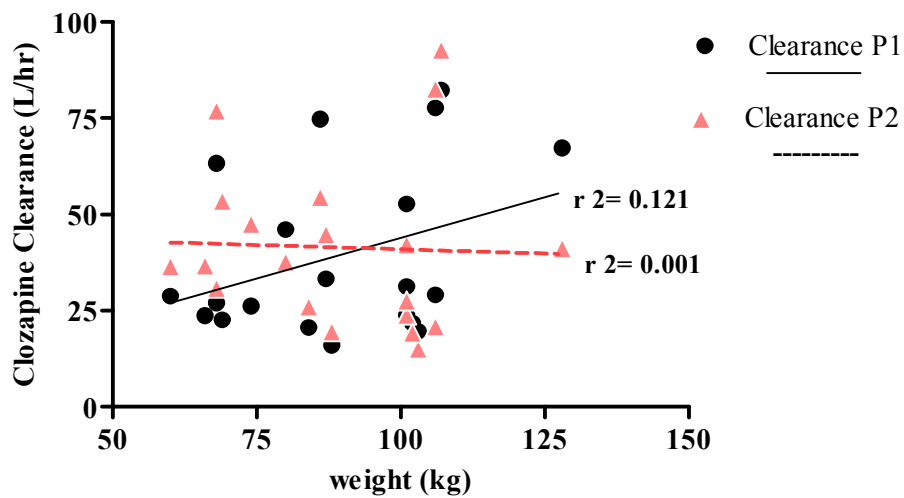


Mann Whitney test	
P value	0.960
Exact or approximate P value?	Gaussian Approximation
P value summary	ns
Are medians signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
Mann-Whitney U	138.0

3.4.3. Weight

No correlation was found between patients weight (kg) and clozapine clearance (L/hr) in either phase of the study. Figure 3.14 shows the relationship between weight and clozapine clearance.

Figure 3.14: The relationship between weight and clozapine clearance

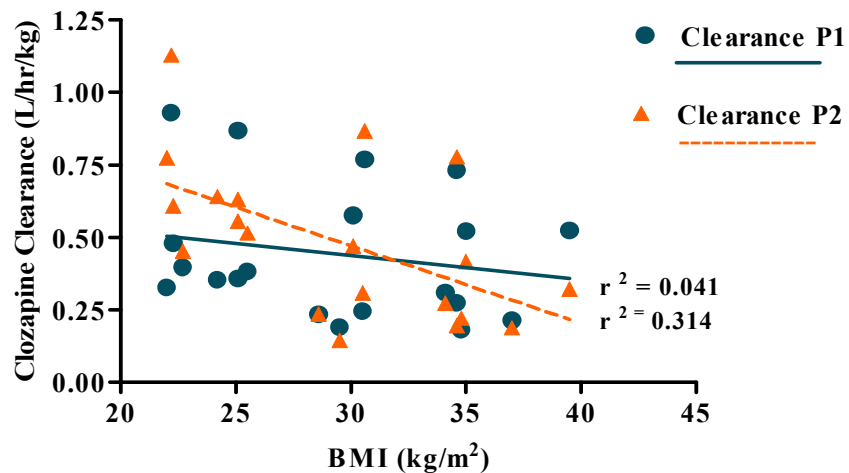


Number of XY Pairs	20	20
Pearson r	0.348	-0.037
95% confidence interval	-0.111 to 0.685	-0.472 to 0.412
P value (two-tailed)	0.132	0.877
P value summary	ns	ns
Is the correlation significant? (alpha=0.05)	No	No
R squared	0.121	0.001

3.4.4. Body Mass Index (BMI)

The relationship between BMI and clozapine clearance was investigated to test further whether there may be a relationship between patient weight and clozapine clearance. Significant negative correlation was observed between BMI and clozapine clearance in phase 2 (Pearson $r = -0.561$, $r^2 = 0.314$, $p < 0.05$), but not in phase 1. The results are shown in Figure 3.15.

Figure 3.15: Correlation between clozapine clearance and Body Mass Index (BMI).

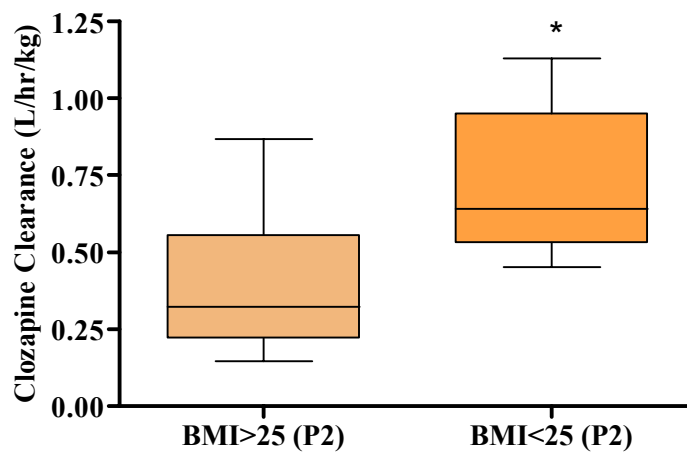


	P1	P2
Number of XY Pairs	20	20
Pearson r	-0.202	-0.561
95% confidence interval	-0.592 to 0.264	-0.804 to -0.157
P value (two-tailed)	0.393	0.010
P value summary	ns	*
Is the correlation significant? (alpha=0.05)	No	Yes
R squared	0.041	0.314

To further evaluate the relationship between clozapine clearance and BMI, the patients in phase 2 were separated into two groups based on BMI > 25 (n=15) and BMI < 25 (n=5), since 25 is considered borderline obesity. The mean value of

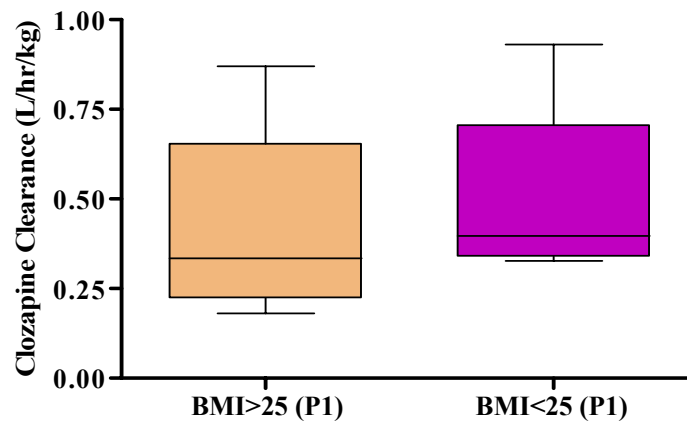
clozapine clearance was significantly greater in patients with BMI<25 than in patients with BMI>25 (Figure 3.16). When the same approach was taken for data in phase 1, the mean value of clozapine clearance was approximately 13.8% higher in patients with BMI<25. However, this did not attain statistical significance (Figure 3.17).

Figure 3.16: Comparison of clozapine clearance in two groups with BMI>25 and BMI<25 in Phase 2.



Mann Whitney test	
P value	0.036
Exact or approximate P value?	Gaussian Approximation
P value summary	*
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed

Figure 3.17: Comparison of clozapine clearance in two groups with BMI>25 and BMI<25 in Phase 1.



Mann Whitney test	
P value	0.431
Exact or approximate P value?	Gaussian Approximation
P value summary	ns
Are medians signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
Mann-Whitney U	26.00

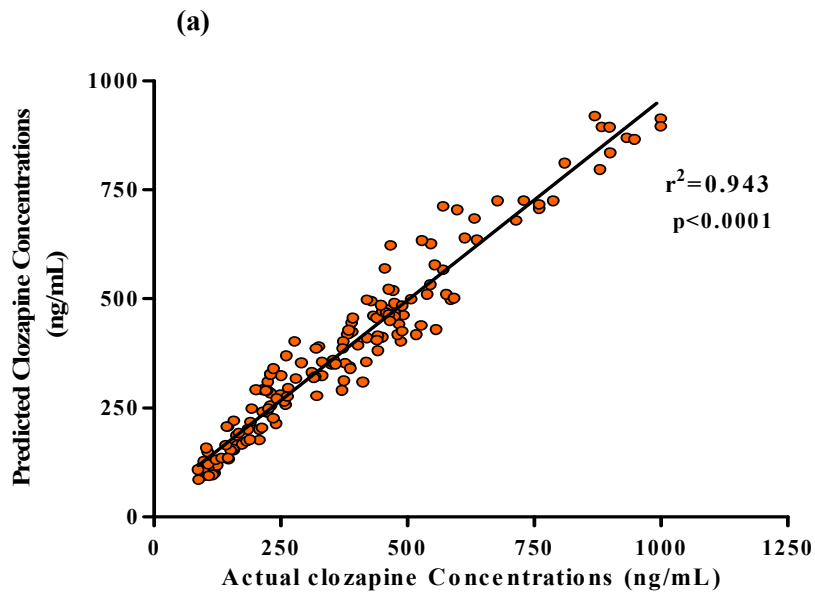
3.5. Evaluation of the Model Obtained with the Abbottbase Bayesian PKS Program

The accuracy and precision of the model that was developed using the Abbottbase Bayesian approach were evaluated using the internal and the external data.

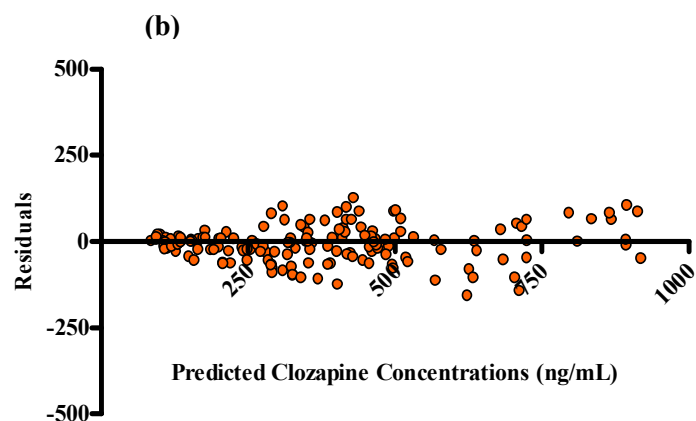
3.5.1. Internal Evaluation of PKS:

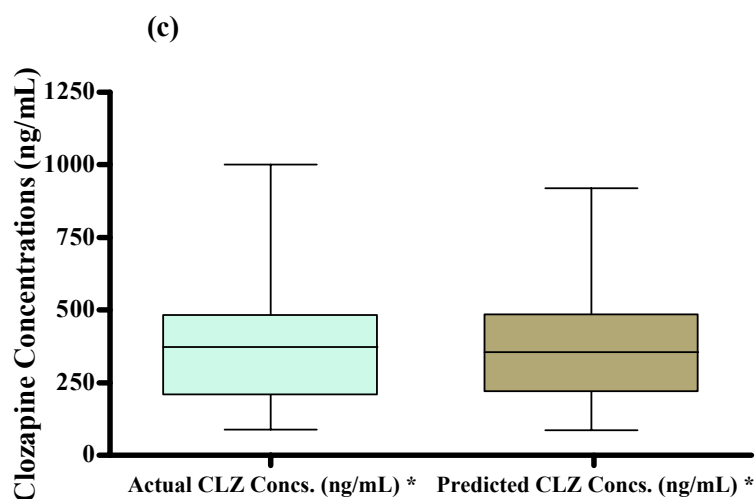
Details of the performance evaluation of Bayesian Abbottbase PKS modeling of the internal data are presented in Table 3.4. The results indicated strong correlation between the Bayesian predicted clozapine concentrations and actual clozapine concentrations (Pearson $r = 0.971$, 95% CI, $P < 0.0001$, $r^2 = 0.943$) for all sampling time points (Figure 3.18) and for each of the individual sampling time points at 0.5, 1, 2 and 12 h in both phases of the study (Table 3.4). Residual values indicated that the majority of the predicted clozapine concentrations are distributed around the identity line (Figure 3.18.b).

Figure 3.18: Graphical goodness-of-fit display for the Bayesian Abbottbase Pharmacokinetic System. **(a)** Correlation between the actual and Bayesian predicted clozapine concentrations (ng/mL) for all time points (0.5, 1, 2, 12 hr) in both phases of the study. **(b)** Plot of residuals of response versus predicted clozapine concentrations. **(c)** Difference between mean of the predicted and actual clozapine concentrations.



Pearson r	0.971
95% confidence interval	0.961 to 0.979
P value (two-tailed)	P<0.0001
P value summary	***
Is the correlation significant? (alpha=0.05)	Yes
R squared	0.943





Mean \pm SEM of column A	375.9 \pm 17.23
Mean \pm SEM of column B	382.9 \pm 16.30
Difference between means	-7.082 \pm 23.72

* CLZ Concs.: Clozapine Concentrations

Table 3.4: Comparison of observed and Bayesian predicted clozapine plasma concentrations at single sampling time point at 0.5, 1, 2, and 12 hr after the first dose of clozapine and all sampling time points in both phases of the study.

Sampling time (h)	r^2	RMSE (mg/mL)	RMSE %	ME (mg/mL)	ME %
0.5	0.921	0.054	13.5	-0.027	-6.8
1	0.929	0.053	13.2	-0.017	-4.25
2	0.927	0.061	15.2	-0.004	-1
12	0.969	0.038	9.5	-0.013	-3.27
All time points	0.943	1.65	0.1	-0.0071	-0.44

RMSE: Root Mean Square Error (precision).

ME: Mean Predicted Error (bias)

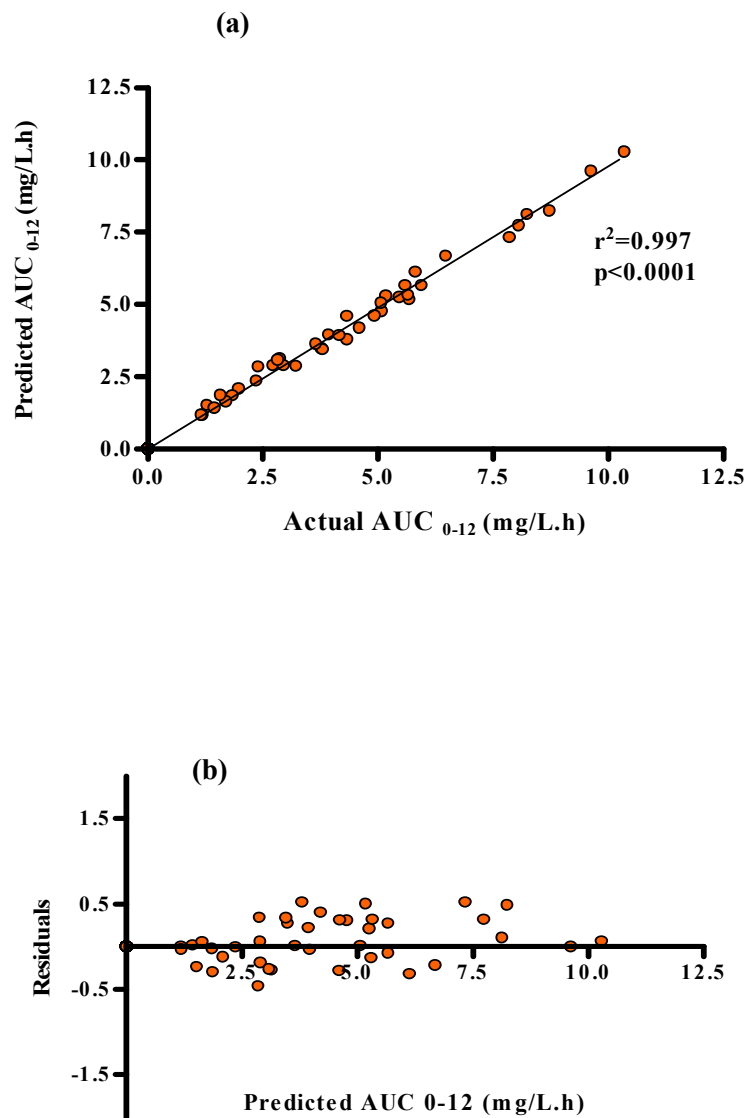
r^2 : All coefficient of determination (r^2) were statistically significant ($p < 0.0001$)

Note units are mg/mL instead of ng/mL to improve clarity.

The AUC_{0-12} of clozapine was calculated by the log trapezoidal method, using predicted and actual clozapine concentrations. The correlation between predicted and actual

AUC_{0-12} is shown in Figure 3.19. The results suggested a significant correlation between predicted and actual AUC_{0-12} . (95% CI, $\alpha=0.05$, $p<0.0001$, $r^2=0.997$).

Figure 3.19: (a) Comparison of predicted and observed AUC_{0-12} for clozapine at 0.5, 1, 2 and 12 h after clozapine in both phases of the study. (b) Plot of residuals versus predicted AUC_{0-12} .

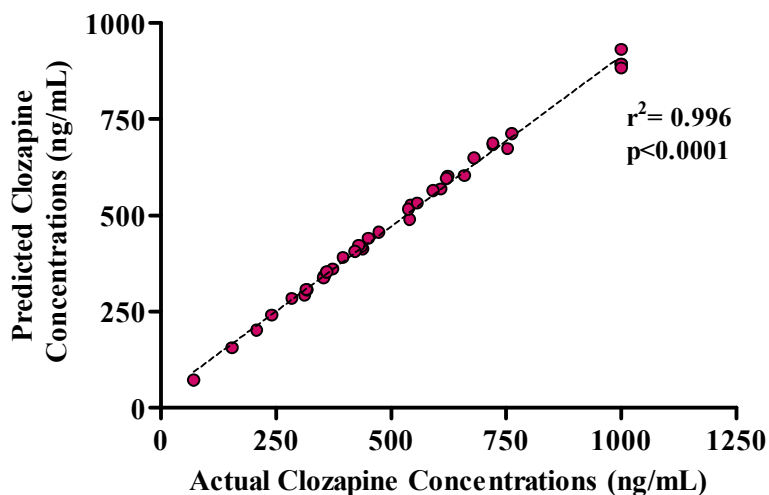


The overall results suggest that the Abbottbase prediction tended to overestimate the clozapine concentration with a precision (RMSE) ≤ 0.054 , 0.053, 0.061 and 0.038 mg/mL for samples collected at 0.5, 1, 2 and 12 h, respectively. The strong correlation between predicted and actual clozapine concentrations and AUC_{0-12} indicate that the prediction performance of Bayesian Abbottbase Pharmacokinetic System (PKS) for clozapine clearance is good.

3.5.2. External Evaluation of PKS

External model evaluation was determined by plotting the actual and predicted concentration-time curve of trough concentrations of plasma clozapine with 38 subjects derived from another study (obtained by personal communication with researchers). The results again showed a strong correlation between the predicted and actual concentration of clozapine concentrations (Pearson $r = 0.998$, 95% CI, $\alpha = 0.05$, $p < 0.0001$, $r^2 = 0.996$, Figure 3.20). The bias was low (about 0.08%) and the Abbottbase predictions again tended to overestimate the clozapine concentration with $\leq 3.5\%$ or 1.344 mg/mL at trough level (Table 3.5).

Figure 3.20: Comparison of predicted and observed clozapine concentrations at trough levels for a 38 patients external data set



Number of XY Pairs	38
Pearson r	0.998
95% confidence interval	0.996 to 0.999
P value (two-tailed)	P<0.0001
P value summary	***
Is the correlation significant? (alpha=0.05)	Yes
R squared	0.996

Table 3.5: Performance evaluation of the Bayesian Abbottbase Pharmacokinetic System (PKS) for the external data

Sampling time (h)	r^2	ME (mg/mL)	ME%	RMSE (mg/mL)	RMSE%
12	0.996	0.030	0.08	1.344	3.50%

RMSE: Root Mean Square Error (precision).

ME: Mean Predicted Error (bias)

r^2 : All coefficient of determination (r^2) were statistically significant ($p < 0.0001$)

Note units are mg/mL instead of ng/mL to improve clarity.

CHAPTER FOUR: DISCUSSION

4. Discussion

CYP1A2 contributes to the disposition of many drugs including the antipsychotics, clozapine and olanzapine, and methylxanthines such as caffeine and theophylline. It has been reported that, apart from cigarette smoke, certain diets and concurrent medications may also alter CYP1A2 activity. For instance, fluvoxamine, fluoroquinolones and oral contraceptives inhibit CYP1A2 activity whereas barbiturates increase enzyme activity (www.medicine.iupui.edu/flockhart/table.htm). A number of allelic variations of CYP1A2 have been described, which may contribute to inter-individual variation in CYP1A2 activity (Sachse et al 1999, Han et al 2001). It is important to note that, in contrast to genetic factors, the influence of environmental factors and other medications on enzyme activity are transient (Faber et al 2005). Although, there may be up to 60 fold variations in expression of the CYP1A2 gene, recent study has shown that no SNPs or haplotypes of the CYP gene have been significantly associated with CYP1A2 metabolic phenotype (Jiang et al 2006). Therefore, rather than genotyping, phenotyping tests might be useful for determination of CYP1A2 activity before and during drug therapy, particularly for those drugs with a narrow therapeutic index, such as clozapine. This information could assist medication efficacy assessment, possible interactions, avoid toxicity or side effects and nonresponse. Caffeine, a drug which is highly dependent on CYP1A2 for its metabolism, has been used as an *in vivo* probe for CYP1A2 phenotype. In this method CYP1A2 activity is assessed by measuring the paraxanthine/caffeine ratio in urine (Fuhr & Rost 1994).

It has been shown that clozapine clearance also correlates with CYP1A2 activity (Doude van Troostwijk et al 2003b). It is suggested that CYP1A2 activity is responsible for about 70% of the variation in oral clearance of clozapine (Bertilsson et al 1994). Indeed apart from the caffeine clearance test, some studies have shown that the ratio of norclozapine/clozapine correlates with CYP1A2 activity (Dailly et al 2002; Carrillo et al 1998; Bertilsson et al 1994).

The results show that there is a direct relation between clozapine clearance and the ratio of trough concentrations of norclozapine/clozapine in plasma (Pearson $r = 0.3607$; $p < 0.05$), which supports the assertion that clozapine clearance is directly related to CYP1A2 activity. Since some side effects of clozapine such as seizures, sedation and hypotension seem to appear at high plasma concentrations that exceed 1000 ng/mL (Chetty and Murray 2007) regular monitoring of clozapine plasma concentrations is recommended. Therefore, the ratio of norclozapine/clozapine could be a convenient substitute for the caffeine test, which can be performed in a context of therapeutic drug monitoring in patients who are taking clozapine. Furthermore, this test might be of clinical value in identification of an individual's metabolic capacity to avoid clozapine toxicity or adverse effects. Another advantage is that administration of caffeine would be avoided which could decrease the likelihood of impairment of clozapine administration.

Phenotyping tests may also assist the investigation the impact of drug exposure on the activity of particular CYPs, which could be used to detect drug-drug interactions caused by CYP induction or inhibition. Since omeprazole is amongst the CYP1A2 inducers, it might be expected that co-medication with clozapine and omeprazole

could influence clozapine elimination as a consequence of induction of CYP1A2 activity.

The concomitant use of clozapine and other agents for treatment of gastrointestinal disorders caused by clozapine therapy happens frequently. Omeprazole is a widely used PPI for the treatment of acid related disease. It has been reported that omeprazole therapy in two patients with schizophrenia decreased plasma clozapine concentrations, possibly as a consequence of CYP1A2 induction (Frick et al 2003). Replacement of omeprazole with pantoprazole, an alternative PPI agent with a weaker CYP1A2 induction capacity, resulted in an increase in plasma clozapine concentrations in patients (Mookhoek & Loonen 2004).

In vitro studies have indicated that the expression of CYP1A1 and 1A2 mRNAs increases in cells that have been treated with omeprazole (Shin et al 1999; Quattrochi & Tukey 1993; Curi-Pedrosa et al 1993). An *in vitro* comparison study in HepG2 cells showed that omeprazole and lansoprazole were more potent inducers of both CYP1A1 and 1A2 than rabeprazole. Moreover, this study showed that another PPI – pantoprazole – did not induce CYP1A2 (Krusekopf et al 2003).

We compared the CYP1A2 activity in the presence of both rabeprazole and omeprazole, using the ratio of norclozapine/clozapine as a probe for CYP1A2 activity, in patients with schizophrenia. There was no difference in the norclozapine/clozapine ratio after replacing concurrent rabeprazole with omeprazole. In support of this result, clozapine clearance was not different in patients who received either rabeprazole or omeprazole.

Some studies have indicated that the induction of CYP1A2 by omeprazole is dose dependent and that omeprazole is not a CYP1A2 inducer at conventional therapeutic doses (20 mg/day) (Andersson et al 1998; Rizzo et al 1996; Sinues et al 2004). Rost et al showed that at the higher therapeutic dose level of 40 mg/day, omeprazole induces CYP1A2 activity in subjects who are PMs for CYP2C19 but not EMs (Rost et al 1994). In contrast, another study on 14 healthy subjects compared the induction of omeprazole (20 mg), lansoprazole (30 mg) and pantoprazole (40 mg). Despite the increased AUC with three PPIs in PMs for CYP2C19, there was no evidence of CYP1A2 induction in patients of either genotype (Andersson et al 1998).

Another study reported that high dose omeprazole (40 and 60 mg/day, respectively) in two schizoaffective patients who were also smokers decreased plasma clozapine concentrations by 41.9% and 44.7%, respectively (Frick et al 2003). It was suggested that the induction of CYP1A2 by omeprazole may have led to the decrease in clozapine concentrations.

In our study, the daily dose of rabeprazole and omeprazole remained constant in both phases of the study. Sixteen of the patients were taking PPIs 20 mg/day, while the remainder received 40 mg daily.

The use of low therapeutic doses of the PPIs omeprazole and rabeprazole (20 and 40 mg/day) in the present study may explain the lack of induction of CYP1A2 activity. Clozapine clearance did not differ between either phase of the study.

Although, the present study did not evaluate CYP2C19 phenotype among the subjects, previous studies have shown that only 2 to 5% of Caucasians are PMs

for CYP2C19 (Rettie et al 2000); 90% of the recruited patients were Caucasian. Therefore, the non significant trend toward higher clozapine clearance in presence of omeprazole might have been affected by the likely low incidence of PMs for CYP2C19.

Mookhoek and Loonen reported that replacement of omeprazole with pantoprazole in 10 smokers led to a decrease in the mean serum clozapine concentration by 41 µg/L. However, in 3 non-smoking patients the clozapine concentrations increased by 134 µg/L. It was suggested that replacement of omeprazole, as established CYP1A2 inducer, by the much less potent pantoprazole may have caused the rise in clozapine concentrations in nonsmokers. In contrast, the remaining smoking subjects may have been maximally induced in terms of CYP1A2 activity (Mookhoek & Loonen 2003).

A comparison of smokers and non smokers in our study indicates that the smokers have lower mean plasma clozapine concentrations than non smokers (336.3 ng/mL vs 563.1 ng/mL, $P < 0.0001$, in the presence of rabeprazole and 336 vs 541.9, $P < 0.0001$, in the presence of omeprazole). The slightly higher clozapine clearance in smokers compared to non smokers did not reach statistical significance. Although the phenotyping marker (norclozapine/clozapine in plasma) suggested that CYP1A2 activity was greater in smokers, the difference was not also significant. The small number of non-smokers in this study may help to explain the non significant results in these analyses, although this is likely to remain a problem in studies of this type in patients with schizophrenia.

Despite the efficacy of clozapine in treatment of refractory schizophrenia, it has been reported that clozapine therapy is an additional risk factor for metabolic disorders (Lamberti et al 2006). Weight gain, elevation of liver enzymes, diabetes mellitus, fatty liver and lipid abnormalities such as increased total cholesterol levels and hypertriglyceridemia are among the side effects of clozapine (Rettenbacher et al 2006; Lamberti et al 2006; Henderson et al 2000). Moreover, weight gain may also be associated with elevation of liver enzymes in patients with schizophrenia treated with second generation antipsychotics (Rettenbacher et al 2006). For instance, Gaertner et al reported an elevation of at least one of the LFTs, usually aspartate transaminase (AST) or alanine transaminase (ALT), in 49% of 330 clozapine treated patients. This rate was 61% in patients who received clozapine monotherapy (Gaertner et al 1989).

Our results also suggested such an association between clozapine therapy and elevation of liver enzymes. Almost 60% of patients in our study showed an increase in at least one of the liver test functions, usually ALT or GGT (γ -glutamyl Transpeptidase).

The previous results do not support a differential induction effect of omeprazole or rabeprazole on CYP1A2 activity at the conventional dosages that were used in this study. Instead, a significant negative correlation between the BMI and clozapine clearance in phase 2 (in presence of omeprazole) may be informative. Inhibitory effects of lipids on clozapine metabolism are consistent with reports that clozapine clearance is significantly lower in obese patients with BMI>25.

Subsequent studies have suggested that BMI is a new source of CYP1A2 variability (Tantcheva-poor et al 1999; Hong et al 2004). Tantcheva-poor investigated the influence of covariates such as: age, gender, oral contraceptives, body height, body weight, BMI, number of cigarettes smoked, caffeine consumption and country of residence on CYP1A2 activity in 863 healthy Caucasians using caffeine clearance data derived from saliva. A significant relationship between BMI and caffeine clearance was reported. The estimated change relative to the defined basal caffeine clearance was 0.99 fold kg/m² in this study. The author pointed to the 1.16 fold higher caffeine clearance in an individual subject with a BMI 20 (kg/m²) compared with another subject with a BMI 35 (kg/m²) (Tantcheva-poor et al 1999).

Moreover, overall obesity which is indicated by BMI has been shown to be strongly related to increased levels of serum lipid and lipoprotein (Nakanishi et al 1999) and is also associated with the development of hepatic steatosis (Zhang et al 2007).

A recent study investigated the influence of fatty liver (steatosis) on CYP activity. It was shown that the expression and activity of several CYPs, including CYP1A2, 2A6, 2B6, 2C9, 2D6, 2E1 and 3A4 were reduced by 45% to 65% in hepatocytes that were treated with 1mM free fatty acids for 14h (Donato et al 2006).

Consistent with Donato et al, another recent *in vitro* study has shown a significant decrease in CYP1A2, 2C11, 2E1, and 3A activity in steatotic liver in rat (Zhang et al 2007). The results of this study also showed that the formation of norclozapine decreased significantly in steatotic liver, even though the formation of clozapine-*N*-oxide was unchanged. Interestingly, the fraction unbound of clozapine was

significantly lower in microsomes from steatotic liver compared with the control liver (Zhang et al 2007).

Thus, there are three potential explanations that may account for the negative correlation between clozapine clearance and BMI.

First, BMI could be a covariate that could reduce CYP1A2 activity, resulting in a lower clearance of clozapine in obese patients.

Secondly, fatty liver resulting from clozapine-induced metabolic syndrome could decrease CYP1A2 activity in obese patients.

Thirdly, the unbound fraction of clozapine in plasma of obese individuals could impair clearance. Since clozapine has a low hepatic extraction ratio (0.17 ± 0.11 ; Jann et al 1993), its clearance depends on protein binding. Therefore, the increased levels of serum lipoproteins may decrease the unbound fraction of clozapine in plasma and decrease clozapine clearance in obese patients according to the following expression:

$$CL_h = F_u CL_{int}$$

Where, CL_h is clozapine hepatic clearance, F_u is unbound fraction in plasma and CL_{int} is intrinsic enzyme activity.

Considered together, a decrease in clozapine metabolism may occur during weight gain in obese subjects. Lipid that accumulates in liver in patients with metabolic syndrome and steatosis may down regulate CYP1A2 expression and impair clozapine disposition by the mechanisms suggested above.

The influence of age and gender on clozapine metabolism is inconsistent. While some studies have shown the higher plasma clozapine concentrations in older patients (Haring et al 1989), Perry et al did not find a significant relationship between age and clozapine concentrations (Perry et al 1998).

The small number of patients recruited in many of these studies could explain the inconsistency of findings (Rostami-Hodjegan et al 2004). Rostami-Hodjegan et al reported that the higher plasma clozapine concentrations in females may be due to a higher body fat composition and therefore higher volume of distribution. Also, the lower CYP1A2 activity in females could explain the decrease in clozapine clearance that was observed (Rostami-Hodjegan et al 2004).

The current results did not find any statistically significant correlations between age and gender with clozapine clearance, which could be due in part to the small number of recruited patients in the study.

In conclusion, the present results are consistent with suggestions that the ratio of trough serum norclozapine/clozapine concentrations could be a convenient marker that reflects CYP1A2 activity in patients with schizophrenia who are receiving clozapine therapy.

The present study also suggests that the selection of either rabeprazole or omeprazole should not affect serum clozapine concentrations when used at common therapeutic dosage (20 to 40 mg/day). Because other studies have suggested that omeprazole is a CYP1A2 inducer at high dose, its effect now appears to be dose dependent.

Consistent with other studies, the clozapine concentrations were lower in smokers than in non smokers in both phases of the study.

For the first time we have found that a BMI higher than 25 may be associated with decreased clozapine clearance. It seems that there is a negative correlation between BMI and clozapine clearance which could be as a result of CYP1A2 down-regulation due to weight gain, lipid abnormalities and steatosis. The increased level of plasma lipoproteins in obese patients may decrease the unbound fraction of clozapine, reduce the availability of the drug for oxidation by CYP1A2 and decrease clozapine clearance. This issue is important for some drugs such as clozapine, which may affect clozapine efficacy and toxicity.

Appendix I

Patients' Co-mediations

Patients Code	Co-mediations
MN-1	Meloxicam, Quetiapine, Trihexiphenidyl
GR-2	Atorvastatin, Na valproate, Lactulose, Sertraline, Folic acid, Thiamine
MS-3	Quilonium (Lithium)
RE-4	Fybrogel
DD-5	Na valproate, Haloperidol, Atorvastatin
TE-6	Finastride, Amitriptyline, Coloxyl-Senna
PN-7	Na valproate, Sertraline, Thyroxin, Coloxyl-Senna, Lactulose, Metamucil
GH-8	Na valproate, Benztropine, Haloperidol, Duphalac, Coloxyl-Senna
AI-9	Sertraline, Lipitor, Coloxyl-Senna, Fybrogel
FE-10	Topiramate
KN-11	Na valproate
DR-12	Sertraline
JI-13	Sertraline, Na valproate, Atorvastatin, Microgynon, Insulin mixtard, Metformin
DY-14	Na valproate, Venlafaxine, Lactulose, Fybrogel
AA-15	Sertraline, Mixtard insulin, Lipitor, Coloxyl-Senna
AN-16	Sertraline, Lithium Retard, Nicotine gum
NN-17	Venlafaxine, Cephalexine, Lipitor, Thiamine
IH-18	Metformin, Lithium, Fybrogel
JL-20	Thyroxin, Na valproate, Folic acid, Coloxyl-Senna, Tamoxifen, Sertraline, Paracetamol
TY-21	None

Appendix II

Macquarie Hospital
Wicks Road, North Ryde 2113
PO Box 169, North Ryde NSW 1670
Telephone (02) 9888 1222 Facsimile (02) 9887 5684

NORTHERN SYDNEY
CENTRAL COAST
NSW HEALTH

Participant Information Sheet

Site: Macquarie Hospital

1. Study Title:

A comparison of the effect of omeprazole and rabeprazole on clozapine serum concentrations.

2. Your consent to participate:

We invite you to take part in this research project which will be conducted by Naghmeh Jabarizadekivi as part of a masters degree. Dr Glenys Dore and Dr. Mano Chetty will oversee the project.

This Participant Information Sheet contains detailed information about the research project. Its purpose is to explain to you as openly and clearly as possible all the procedures involved in this project before you decide whether or not to take part in it.

Please read this participant information sheet carefully. Feel free to ask questions about any information in the document. You may also wish to discuss the project with a relative or friend. Feel free to do this.

Once you understand what the project is about and if you agree to take part in it, you will be asked to sign the **Consent Form**. By signing the Consent Form, you indicate that you understand the information and that you give your consent to participate in the research project.

You will be given a copy of the Participant Information Sheet and Consent Form to keep as a record.

3. Purpose of the study:

You are already on the medications called rabeprazole and clozapine or clozapine alone. Rabeprazole treats the symptoms of heartburn, which can occur when you are on clozapine. Another similar drug, called omeprazole, is also used to treat symptoms of heartburn. It is reported that using both omeprazole and clozapine may reduce the clozapine level due to the increase the break down of clozapine by the enzymes in the liver. Rabeprazole is a new drug that belongs to the same family as omeprazole.

In this study we would like to see whether rabeprazole has similar affect on clozapine level.

In addition we would like to see how rabeprazole affects the liver enzymes that metabolise clozapine. All those changes may affect the dose of clozapine that has to be taken when these drugs are given together.

This study would help the clinicians in choosing the best dose for the patients.

4. Why has the participant been chosen?

You are taking clozapine or clozapine and rabeprazole as a part of your routine medication. You are invited to this study because we want to determine how omeprazole and rabeprazole affect the clozapine clearance. This may result in better dosing with clozapine. A total 30 patients will participate in this study.

5. Participation:

Participation in any research project is voluntary. If you do not wish to take part you are not obliged to. If you decide to take part or not to take part, or to take part and then withdraw, it will not affect your routine treatment, your relationship with those treating you or your relationship with the hospital.

The therapy for your illness is unaffected by your decision of whether or not to participate in this project. Treatment and clinical management of your condition will be the same regardless of your participation in the research project.

In the unlikely event that you suffer an injury as a result of participating in this research project, hospital care and treatment will be provided by the public health services at no extra cost to you.

6. Reimbursement for your participation:

You will be paid \$40 for your participation in this trial. If you are taking clozapine without rabeprazole or omeprazole, you will be given \$15 because you will be participating for a shorter period and a fewer number of blood samples will be taken.

7. Procedure:

A total of 30 patients will participate in this study.

You will already be taking clozapine and rabeprazole as a part of your routine medication. During the first part of this study, there is no change in your medication. The dosage of clozapine and rabeprazole you need will be decided by your doctor. The plasma clozapine will be measured after at least two weeks of treatment with these both of these medications. In addition to your usual blood sample that is taken about 12 hours after your medication, 3 further blood samples (10 ml each) will be taken at 30min, 1 hr and 2 hrs after you have taken clozapine. The blood sample will be taken by the clinical nurse specialist. Rabeprazole will then be switched to omeprazole and plasma concentration of clozapine will be measured after at least one month of taking medication, at the same sampling intervals as above.

On the first day after rabeprazole is changed to omeprazole, two blood samples will be taken. These blood samples will be used to analyse the activity of specific liver enzymes.

If you are taking clozapine without rabeprazole or omeprazole, in addition to your usual blood sample that is taken about 12 hours after your medication, 3 further blood samples (10 ml each) will be taken at 30min, 1 hr and 2 hrs after you have taken clozapine. This will be done once only.

8. The participant's responsibilities:

It is important to tell your doctor and the research staff about any treatments or medications you may be taking, including non-prescription medications, vitamins or herbal remedies and any changes to these during your participation in the study.

9. The side effects and possible disadvantages of taking part:

Omeprazole and rabeprazole work in the same way and they are well-tolerated drugs. Therefore the only one change from rabeprazole to omeprazole will not create serious side effects or interfere with your regular treatment.

Common side effects of omeprazole:

Headache, abdominal pain, nausea, vomiting, constipation, diarrhoea, flatulence.

Uncommon side effects of omeprazole:

Rash, dizziness, vertigo, increased liver enzymes, malaise.

Rare side effects of omeprazole:

Hypersensitivity reactions, e.g. fever, bronchospasm.

Some discomfort may be experienced when blood sample are taken.

10. Terminate the involvement:

If you wish to withdraw from the study at any time, you can do so by contacting Dr. Glenys Dore and signing the withdrawal form. Withdrawal from the study will not affect your treatment or care.

Before you make your decision, a member of the research team will be available so that you can ask any questions you have about the research project. You can ask for any information you want. Sign the consent form only after you have had a chance to ask your questions and have received satisfactory answers.

11. Privacy, confidentiality and disclosure of information:

Blood samples will be potentially identifiable with a code. The investigators will have access to the code that could identify individual samples. Both blood samples and information will be collected from the Macquarie Hospital and will be transferred to the Faculty of Pharmacy, Sydney University by the researcher (Naghmeh Jabarizadekivi). The original information will be held in a locked cupboard in the

Faculty of Pharmacy and electronic information will be held in password-protected files on the personal computer of the researcher (Naghmeh Jabarizadekivi) in the Faculty of Pharmacy, Sydney University. Data will be stored in files for 7 years after completion of the research. Thereafter all the data will be shredded or deleted. In any presentation of the findings of this study, identification of participants will not be possible.

We will not establish a human tissue bank using the blood samples obtained in this project. The samples will only be in the present research project and not in any other studies or for any commercial purpose.

This study does not have the capacity to provide information about an individual's future health or risk of having children with a genetic disorder, or information that may be relevant to the health of family members who are not the part of the study.

There is no potential for this research to detect or generate information of social significance (e.g. non-paternity or non-maternity) or information that may influence access to insurance/employment. There is no potential for this research to detect or generate information concerning genetic diseases.

Any information obtained in connection with this research project that can identify you will remain confidential and will only be used for the purpose of this research project and no other projects.

12. Results of project:

The investigators at Macquarie Hospital can provide you with the overall results of the study if you are interested.

13. Investigators, organizing and funding:

This project will be conducted mainly by investigators, Dr Mano Chetty and Naghmeh Jabarizadekivi from the Faculty of Pharmacy, University of Sydney and Dr Glenys Dore from Macquarie Hospital. Funding for this study was obtained from the

University of Sydney. Accordingly, we seek your consent for these blood samples to be measured in the Faculty of Pharmacy, Sydney university.

14. Further information or any other problems:

If you require further information or you have any problems concerning this project (for example, any side effects), you can contact Dr Glenys Dore at 98881222 or by dialling -9- in the hospital and asking to page her.

15. Other issues:

If you have any complaint about any aspect of the project, the way is being conducted or any question about your rights as a research participant, then you may contact Michael Herman

Position Held: Psychologist

Telephone: 98881222 or -9- in the hospital

You need to tell Michael Herman the name of one of the researchers given in section 12 above.

16. Ethical Guidelines:

This project will be carried out according to the *National Statement on Ethical Conduct in Research Involving Humans* (June 1999). This statement has been developed to protect the interests of people who agree to participate in human research studies.

The ethical aspects of this research project have been approved by the Northern Sydney Health Human Research Ethics Committee.

Appendix III

Macquarie Hospital
Wicks Road, North Ryde 2113
PO Box 169, North Ryde NSW 1670
Telephone (02) 9888 1222 Facsimile (02) 9887 5684

NORTHERN SYDNEY
CENTRAL COAST
NSW HEALTH

Consent form to Participate in a research Project

I, _____
(name of participant)

of _____
(street) (suburb/town) (state & postcode)

have been invited to participate in a research project entitled "**A comparison of the effect of the omeprazole and rabeprazole on clozapine serum concentrations**".

In relation to this project I have read the Participant Information Sheet and have been informed of the following points:

1. Approval has been given by the Human Research Ethics Committee (HREC) of Northern Sydney Central Coast Health.
2. The aim of the project is to investigate whether rabeprazole has a similar effect on clozapine clearance as omeprazole and to determine the activity of some specific liver enzymes in presence of rabeprazole and omeprazole.

Medications like omeprazole or rabeprazole are frequently used in the treatment of heartburn symptoms due to clozapine.

During this research project medication will be changed from rabeprazole to omeprazole.

3. The results obtained from the study may or may not be of direct benefit to my medical management.
4. The procedure will involve giving 3 additional blood samples (10ml at 30mins, 1hr, 2hrs) firstly after taking the rabeprazole-clozapine combination and secondly

after changing to the omeprazole-clozapine combination. In addition 2 blood samples will be taken on the first day after change over to omeprazole.

If I am taking clozapine without rabeprazole or omeprazole, only 3 additional samples (10ml at 30mins, 1hr, 2hrs) will be taken from me.

5. Omeprazole is well-tolerated drug and most adverse reactions are mild and transient. However, most of these side effects are similar to rabeprazole. Common side effects of omeprazole are headache, abdominal pain, nausea, vomiting, constipation, diarrhoea, flatulence. Uncommon side effects of omeprazole are rash, dizziness, vertigo, malaise, increased liver enzymes. Hypersensitivity is one of the rare reactions of omeprazole, such as fever and bronchospasm.

6. My involvement in this project may be terminated if any of the following circumstances develop:

- a. serious side effects
- b. poor response to the drug combination
- c. I decide to withdraw my consent
- d. at the discretion of the psychiatrist treating me

7. Should I develop a problem which I suspect may have resulted from my involvement in the project, I am aware that I may contact Dr Glenys Dore

On:
Macquarie Hospital
Telephone Number: 02- 98881222

Date:

Witness: _____
(Please print name)

Signature: _____
(of participant/volunteer)

Signature: _____
(of witness)

8. Should I have any problems or queries about the way in which the study was conducted, and I do not feel comfortable contacting the research staff, I am aware that I may contact the Consumer Consultant who is an independent person within the Hospital on 98881222.

9. I can refuse to take part in this project or withdraw from it at any time without affecting my medical care.

10. I understand that take out the participating in this research may or may not benefit my medical treatment directly however my participating may assist in the development of treatments and/or procedures for the future.

11. Participation in this project will not result in any extra medical and hospital costs to me. I will receive \$40 as reimbursement payment for my time and the blood

samples associated with my involvement in this study. If I am taking clozapine without rabeprazole or omeprazole, I will be given \$15 because I will be participating for a shorter period and a fewer number of blood samples will be taken.

12. I understand that my research records will be stored in a locked cupboard at the University of Sydney. Electronic information will be held on password-protected files on personal computer in the laboratory of the Faculty of Pharmacy, Sydney University. The research team, authorized personnel, and regulatory entities may have access to my study records to protect my safety and welfare.

13. I understand that my information will be identified by a numerical random code together with the first letter of my first name and the last letter of my last name. This information is potentially identifiable but all precautions will be taken by the clinical staff to ensure the information will be kept confidential.

14. If the results of my tests or information regarding my medical history are published, my identity will not be revealed.

15. While participating in this study, I should not take part in any other research project without approval from all of the investigators. This is to protect myself from possible injury arising from such things as extra blood drawing, extra x-ray, interaction of research drugs, or similar hazards.

16. During the course of this study, I will be informed of any significant new findings (either good or bad) such as changes in the risks or benefits resulting from participation in the research or new alternatives to participation that might cause me to change my mind about participating. If such new information is provided to me, my consent to participate will be re-obtained.

17. In giving my consent, I acknowledge that the Government Health Department Officials, and the personnel directly involved in the study, may examine my medical records only as they relate to this project.

18. I declare that I am over the age of 18 years.

After considering all these points, I accept the invitation to participate in this project.

I am aware that I will be given a copy of the Participant Information Sheet and Consent Form.

I also state that I have/have not participated in any other research project in the past 3 months.

If I have, the details are as :

follows: _____

Dr: _____ on: _____
(Phone and pager numbers)

Date: _____ Witness: _____
(Please print name)

Signature: _____ Signature: _____
(of participant/volunteer) (of witness)

Patient's consent:

Participant Name: _____

Date: _____

Signature: _____

Witness Name: _____

Date: _____

Signature: _____

Investigators' confirming statement:

I have given this research subject information on the study, which in my opinion is accurate and sufficient for the subject to understand fully the nature, risks and benefits of the study, and the rights of a research subject. There has been no coercion or undue influence. I have witnessed the signing of this document by the subject.

Date: _____

Investigator's Name: : _____

Investigator's signature: _____

Withdrawal form Participation

Protocol Title: A comparison of the effect of omeprazole and rabeprazole on clozapine serum concentration.

An option should I wish to withdraw my consent to participate in the research protocol entitled above is to contact the researcher and/or return this slip. I understand that if I withdraw the research protocol my medical care, my relationship with the hospital and medical attendants will not be affected.

Patient's Name:

Patient's Signature:

Date:

Please detach the withdrawal of Participant Section and send to Dr. Glenys Dore (Figtree Ward, Macquarie Hospital) and if I would like to speak to a member of the study investigation team I may contact Dr. Glenys Dore (02-98881222).

REFERENCES

- ABELO, A., ANDERSSON, T.B., ANTONSSON, M., NAUDOT, A. K. (2000). Stereoselective metabolism of omeprazole by human cytochrome P450 enzymes. *Drug Metab Dispos*, **28**, 966-72.
- ANDERSSON, T., HASSAN-ALIN, M., HASSELGREN, G., ROHSS, K. (2001). Drug interaction studies with Esomeprazole, the (s)-isomer of omeprazole. *Clin Pharmacokinet* **40**, 523-537.
- ANDERSSON, T., CEDERBERG, C., EDVARDSSON, G., HEGGELUND, A. & LUNDBORG, P. (1990). Effect of omeprazole treatment on diazepam plasma levels in slow versus normal rapid metabolizers of omeprazole. *Clin Pharmacol Ther*, **47**, 79-85.
- ANDERSSON, T., HOLMBERG, J., ROHSS, K. & WALAN, A. (1998). Pharmacokinetics and effect on caffeine metabolism of the proton pump inhibitors, omeprazole, lansoprazole, and pantoprazole. *Br J Clin Pharmacol*, **45**, 369-375.
- ANDERSSON, T.B., MINERS, J.Q. & VERONESE, M.E. (1993). Identification of human liver cytochrome P450 isoforms mediating omeprazole metabolism. *Br J Clin Pharmacol*, **36**, 521-530.
- ARAVAGIRI, M. & MARDER, S.A. (2001). Simultaneous determination of clozapine and its N-desmethyl and N-oxide metabolites in plasma by liquid chromatography/electrospray tandem mass spectrometry and its application to plasma level monitoring in schizophrenic patients. *J Pharmaceutical Biomed Analysis*, **26**, 301-311.
- ARIIZUMI, K., OHARA, S., KOIKE, T., INOMATA, Y., LIJIMA, K., SEKINE, H., NOGUCHI, M., SUGIYAMA, K., KAWAMURA, M. & SHIMOSEGAWA, T. (2006). Therapeutic effect

- of 10 mg/day rabeprazole administration on reflux esophagitis was not influenced by CYP2C19 polymorphism. *J Clin Gastroenterol Hepatol*, **21**, 1428-1434.
- BALDESSARINI, R.J. & FRANKENBURG, F.R. (1991). Clozapine: a novel antipsychotic agent. *N Engl J Med*, **324**, 746-754.
- BATTY, K.Y., DAVIS, T.M., ILETT, K.F., DUSCI, L.J. & LANGTON, S.R. (1995). The effect of ciprofloxacin on theophylline pharmacokinetics in healthy subjects. *Br J Clin Pharmacol*, **39**, 305-311.
- BELL, N.J.V., BURGET, D.W., HOWDEN, C.W., WILKINSON, G. & HUNT, R.H. (1992). Appropriate acid suppression for the management of the gastro-oesophageal reflux disease. *Digestion*, **51**, 56-67.
- BERTILSSON, L., CARRILLO, J.A., DAHL, M.L., LLERENA, A., ALM, C., BONDESSON, U., RODRIGUEZ DE LA RUBIA, I., RAMOS, S. & BENITEZ, J. (1994). Clozapine disposition varies with CYP1A2 activity determined by a caffeine test. *Br J Clin Pharmacol*, **38**, 471-473.
- BESANCON, M., SIMON, A., SACHS, G. & SHIN, J.M. (1997). Sites of reaction of the gastric H, K-ATPase with extracytoplasmic thio reagents. *J Biol chem*, **272**, 22438-22446.
- BLUME, H., DONATH, F., WARNKE, A. & SCHUNG, B.S. (2006). Pharmacokinetic drug interaction profiles of proton pump inhibitors. *Drug saf*, **29**, 769-784.
- BONDOLFI, G., MOREL, F., CRETOL, S., RACHID, F. & BAUMANN, P. (2005). Increased clozapine plasma concentrations and side effects induced by smoking cessation in 2 CYP1A2 genotyped patients. *Ther Drug Mon*, **27**, 539-43.
- BRADFORD, L.D. (2002). CYP2D6 allele frequency in European Caucasians, Asians, Africans, and their descendants. *Pharmacogenetics*, **3**, 229-243.
- BURGET, D.W., CHIVERTON, S.G. & HUNT, R.H. (1990). Is there an optional degree of acid suppression for healing duodenal ulcers? A model of the relationship between ulcer healing and acid suppression. *Gastroenterol*, **99**, 345-351.
- BYERLY, M.J. & DEVANE, C.L. (1996). Pharmacokinetics of clozapine and risperidone: a review of the literature. *J Clin Psychopharmacol*, **37**, 177-187.
- CALLAGHAN, J.T., BERGSTROM, R.F., PTAK, L.R. & BEASLEY, C.M. (1999). Olanzapine: Pharmacokinetic and pharmacodynamic profile. *Clin Pharmacokinet*, **37**, 177-193.
- CARACO, Y., TATEISHI, T. & WOOD, A.J.J. (1995). Interethnic difference in omeprazole's inhibition of diazepam metabolism. *Clin Pharmacol Ther*, **58**, 62-72.

- CARILLO, J.A. & BENITEZ, J. (2000). Clinically significant pharmacokinetic interactions between dietary caffeine and medications *Clin Pharmacokinet*, **39**, 127-153.
- CARRILLO, J.A., HARRAIZ, A.G., RAMOS, S.I. & BENITEZ, J. (1998). Effects of caffeine withdrawal from the diet on the metabolism of clozapine in schizophrenic patients. *J Clin Psychopharmacol*, **18**, 311-316.
- CARRILLO, J.A., HERRAIZ, A.G., RAMOS, S.I., GERVASINI, G., VIZCAINO, S. & BENITEZ, J. (2003). Role of the smoking-induced cytochrome P450 (CYP) 1A2 and polymorphic 2D6 in steady-state concentration of olanzapine. *J Clin Psychopharmacol*, **23**, 119-127.
- CASHMAN, J.R., YANG, Z., YANG, L. & WRINGTON, S.A. (1993). Stereo- and regioselective N- and S-oxidation of tertiary amines and sulfides in the presence of adult human liver microsomes. *Drug Metab Dispos*, **21**, 1379-1382.
- CATTEAU, A., BECHTEL, Y.C., POISSON, N., BECHTEL, P.R. & BONAITI-PELLIE, C. (1995). A population and family study of CYP1A2 using caffeine urinary metabolites. *Eur J Clin Pharmacol*, **47**, 423-430.
- CENTORRINO, F., BALDESSARINI, R.J., KANDO, J.C., FRANKENBURG, F.R., VOLPICELLI, S.A., PUOPOLO, P.R. & FLOOD, J.G. (1994). Serum concentrations of clozapine and its major metabolites: effects of cotreatment with fluvoxamine and valproate. *Am J Psychiatry*, **51**, 123-125.
- CHENG, Y.F., LUNDBERG, T., BONDESSON, U., LINDSTROM, L. & GABRIELSSON, J. (1998). Clinical pharmacokinetics of clozapine in chronic schizophrenic patients. *Eur J Clin Pharmacol*, **34**, 445-449.
- CHETTY, M. & MURRAY, M. (2007). CYP-mediated interactions: How predictable are they? *Curr Drug Metab*, **8**, 307-313.
- CHIN, B.E., BENDER, S., SIROT, E.J., CUCCHIA, G., JONZIER-PEREY, M., BAUMANN, P., ALLORGE, D. & BROLY, F. (2004). Nonresponse to clozapine and ultrarapid CYP1A2 activity: clinical data and analysis of CYP1A2 gene. *J Clin Psychopharmacol*, **24**, 214-219.
- CHIN, T.W., LOEB, M. & FONG, I.W. (1995). Effects of an acidic beverage (Coca-Cola) on absorption of ketoconazole. *Antimicrob Agents Chemother*, **39**, 1671-1675.
- CLOUSE, R.E. & LUSTMAN, P.J. (1983). Psychiatric illness and contraction abnormalities of the esophagus. *N Engl J Med*, **309**, 1337-1342.
- CRIBB, E.A. (2006). Role of polymorphic human cytochrome P450 enzymes in estrone oxidation. *Cancer Epidemiol Biomarkers Rev*, **15**, 551-558.
- CURI-PEDROSA, R., DAUJAT, M., PICHARD, L., OURLIN, J.C., CLAIR, P., GERVOT, L., LESCA, P., DOMERGUE, J., JOYEUX, H., FORTANIER, G. & MAUREL, P. (1993).

- Omeprazole and lansoprazole are mixed inducers of CYP1A and CYP3 in human hepatocytes in primary culture. *J Pharmacol Exp Ther*, **69**, 384-392.
- DAILLY, E., URIEN, S., CHANUT, E., CLAUDEL, B., GUERRA, N., FERNANDEZ, C., JOLLIET, P. & BOURIN, M. (2002). Evidence from a population pharmacokinetics analysis for a major effects of CYP1A2 activity on inter- and intraindividual variation of clozapine clearance. *Prog Neuropsychopharmacol Biol Psychiatry*, **26**, 699-703.
- DAIN, J.G., NICOLETTI, J. & BALLARD, F. (1997). Biotransformation of clozapine in humans. *Drug Metab Dispos*, **25**, 603-609.
- DALY, A.K. & KING, B.P. (2003). Pharmacogenetics of oral coagulants. *Pharmacogenetics*, **13**, 247-252.
- DAYER, P., DESMEULES, J. & STRIBERNI, R. (1992). In vitro forecasting of drugs that may interfere with codeine bioactivation. *Eur J Drug Metab Pharmacokinet*, **17**, 115-120.
- DE LEON, J. (2004). Atypical antipsychotic dosing: the effect of smoking and caffeine. *Psychopharmacol*, **55**, 491-493.
- DE LEON, J., ARMSTRONG, S.C. & COZZA, K.L. (2006). Clinical guidelines for psychiatrists for the use of pharmacogenetic testing for CYP450 2D6 and CYP450 2C19. *Psychosomatics*, **47**, 75-85.
- DE LEON, J., SUSCE, M.T., PAN, R.M., FAIRCHILD, M., KOCH, W.H. & WEDLUND, P.J. (2005). The CYP2D6 poor metabolizer phenotype may be associated with risperidone adverse drug reactions and discontinuation. *J Clin Psychiatry*, **66**, 15-27.
- DESTA, Z., ZHAO, X., SHIN, J.G. & FLOCKHART, D.A. (2002). Clinical significance of the cytochrome P450 2C19 genetic polymorphism. *Clin Pharmacokinet*, **41**, 913-958.
- DEV, V. & RANIWALLA, J. (2000). Quetiapine: a review of its safety in the management of schizophrenia. *Drug saf*, **23**, 296-307.
- DEVANE, C.L. & NEMEROFF, C.B. (2001). Clinical pharmacokinetics of quetiapine *Clin Pharmacokinet*, **40**, 509-522.
- DIXIT, R.K., CHAWLA, A.B., KUMAR, N. & GRAG, S.K. (2001). Effect of omeprazole on the pharmacokinetics of sustained-release carbamazepine in healthy male volunteers. *Methods Find Exp Clin Pharmacol*, **23**, 37-39.
- DONATO, M.T., LAHOZ, A., JIMENEZ, N., PEREZ, G., SERRALTA, A., MIR, J., CASTELL, J.V. & GOMEZ-LECHON, J. (2006). Potential impact of steatosis on cytochrome P450 enzymes of human hepatocytes isolated from fatty liver grafts. *Drug Metab Dispos*, **34**, 1556-1562.

- DOUDE VAN TROOSTWIJK, L.J.A.E., KOOPMANS, R.P. & GUCHELAAR, H.J. (2003a). Two novel methods for the determination of CYP1A2 activity using the paraxanthine/caffeine ratio. *Fundam Clin Pharmacol* **7**, 355-362.
- DOUDE VAN TROOSTWIJK, L.J.A.E., KOOPMANS, R.P., VERMEULEN, H.D.B. & GUCHELAAR, H.J. (2003b). CYP1A2 activity is an important determinant of clozapine dosage in schizophrenic patients. *Eur J Pharm Sci*, **20**, 451-457.
- DUMORTIER, G., LOCHU, A., COLEN DE MELO, P., GHRIBI, O., ROCHE-RABREAU, D., DEGRASSAT, K. & DESCE, J.M. (1996). Elevated clozapine plasma concentrations after fluvoxamine initiation *Am J Psychiatry*, **153**, 738-739.
- EIERMANN, B., ENGEL, G., JOHANSSON, I., ZANGER, U.M. & BERTILSSON, L. (1997). The involvement of CYP1A2 and CYP3A4 in the metabolism of clozapine. *Br J Clin Pharmacol*, **44**, 439-446.
- FABER, M.S., JETTER, A. & FUHR, U. (2005). Assessment of CYP1A2 activity in clinical practice: Why, How, and When? *Pharmacol Toxicol*, **97**, 125-134.
- FACCIOLA, G., AVENOSO, A., SCORDO, M.G., MADIA, A., VENTIMIGLIA, A., PERUCCA, E. & SPINA, E. (1999). Small effect of valproic acid on plasma concentrations of clozapine and its major metabolites in patients with schizophrenia or affective disorder. *Ther Drug Monit*, **21**, 341-345.
- FANG, J., BAKER, G.B., SILVERSTONE, P.H. & COUTTS, R.T. (1997). Involvement of CYP3A4 and CYP2D6 in the metabolism of haloperidol. *Cell Mol Neurobiol*, **17**, 227-233.
- FANG, J., BOURIN, M. & BAKER, G.B. (1999). Metabolism of risperidone to 9-hydroxyrisperidone by human cytochrome P450 2D6 and P4503A4. *Naunyn Schmiederbergs Arch Pharmacol*, **359**, 147-151.
- FINLEY, P. & WARNER, D. (1994). Potential impact of valproic acid therapy on clozapine disposition. *Biol Psychiatry*, **36**, 487-488.
- FLEMING, J. & CHETTY, M. (2005). Psychotropic drug interactions with valproate. *Clin Neuropharmacol*, **28**, 96-101.
- FREEMAN, D.J. & OYEWUMI, L.K. (1997). Will routine therapeutic monitoring have a place in clozapine therapy? *Clin Pharmacokinet*, **32**, 93-100.
- FRICK, A., KOPITZ, J. & BERGEMANN, N. (2003). Omeprazole reduces clozapine plasma concentrations. A case report. *Pharmacopsychiatry*, **36**, 121-123.
- FUHR, U. & ROST, K.L. (1994). Simple and reliable CYP1A2 phenotyping by the paraxanthine/caffeine ratio in plasma and in saliva. *Pharmacogenetics*, **4**, 109-116.

- FUJIYAMA, K., FUJIOKA, T., KODAMA, R. & NASU, M. (1994). Effect of E3810, a novel proton pump inhibitor, against *Helicobacter pylori*. *Am J Gastroenterol*, **89**, 1371 (Abstract).
- FUNCK-BRENTANO, C., BECQUEMONT, L., LENEVEU, A., ROUX, A., JAILLON, P. & BEAUNE, P. (1997). Inhibition by omeprazole of proguanil metabolism: mechanism of the interaction in Vitro and prediction of in vivo results from the in vitro experiments. *Pharmacol Exp Ther*, **280**, 730-738.
- FURUTA, T., SHIRANI, N., SUGIMOTO, M., NAKAMURA, A., HISHIDA, A., ISHIZAKI, T. (2005). Influence of CYP2C19 pharmacogenetic polymorphism on proton pump inhibitor-based therapies. *Drug Metab Pharmacokinet*, **20**, 153-67.
- FURUTA, T., OHASHI, K., KAMATA, T. & ET, A. (1998). Effect of genetic differences in omeprazole metabolism on cure rates for *Helicobacter pylori* infection and peptic ulcer. *Ann Intern Med*, **129**, 1027-30.
- GAERTNER, H.J., FISCHER, E. & HOSS, J. (1989). Side effects of clozapine. *Psychopharmacol*, **99**, S97-S100.
- GONZALEZ, F.J. (1998). The molecular biology of cytochrome P450s. *Pharmacol Rev*, **40**, 243-288.
- GONZALEZ, F.J. & TUKEY, R.H. (2004). Drug Metabolism. In *Goodman & Gilman's: The Pharmacological Basis of Therapeutics* ed. Brunton, L., Lazo, J. & Parker, K. pp. 71-91: McGraw-Hill
- GUITTON, C., KINOWSKI, J.M., GOMENI, R. & BRESSOLLE, F. (1998). A kinetic model for stimulation fit of clozapine and norclozapine concentrations in chronic schizophrenic patients during long-term treatment. *Clin Drug Invest*, **16**, 35-43.
- GUZEY, C., AAMO, T. & SPIGSET, O. (2000). Risperidone metabolism and the impact of being ultrarapid metabolizer. *J Clin Psychiatry*, **61**, 600-601.
- HAGG, S., SPIGSET, O., MJORNDAL, T. & DAHLQVIST, R. (2000). Effect of caffeine on clozapine pharmacokinetics in healthy volunteers. *Br J Clin Pharmacol*, **49**, 59-63.
- HAN, X.M., OUYANG, D.S., CHEN, P.X., SHU, Y., JIANG, C.H., TAN, Z.R. & ZHOU, H.H. (2002). Inducibility of CYP1A2 by omeprazole *in vivo* related to genetic polymorphism of *CYP1A2*. *Br J Clin Pharmacol*, **54**, 540-543.
- HAN, X.M., OUYANG, D.S., LU, P.X., JIANG, C.H., SHU, Y., CHEN, X.P., TAN, Z.R. & ZHOU, H.H. (2001). Plasma caffeine metabolite ratio (17X/137X) *in vivo* associated with G- 2964A and C734A polymorphism of human CYP1A2. *Pharmacogenetics*, **11**, 429-435.

- HARING, C., MEISE, U., HUMPEL, C., SARIA, A., FLEISCHHACKER, W.W. & HINTERHUBER, H. (1989). Dose-related plasma levels of clozapine: influence of smoking behavior, sex and age. *Psychopharmacol*, **99**, S38-S40.
- HASLEMO, T., EIKESETH, P.H., TANUM, L., MOLDEN, E. & REFSUM, H. (2006). The effect of variable cigarette consumption on the interaction with clozapine and olanzapine *Eur J Clin Pharmacol*, **62**, 1049-1053.
- HEIM, M.H. & MEYER, U.A. (1992). Evolution of highly polymorphic human cytochrome P450 gene cluster: CYP2D6. *Genomics*, **14**, 49-58.
- HEMERYCK, A. & BELPAIRE, M. (2003). Selective Serotonin Reuptake Inhibitors and Cytochrome P-450 mediated drug-drug interactions: An update. *Curr Drug Metab*, **3**, 13-37.
- HENDERSON, D.C., CAGLIERO, E., NASRALLAH, R.A., HAYDEN, D.L., SCHOENFELD, D.A. & GOFF, D.C. (2000). Clozapine, diabetes mellitus, weight gain, and lipid abnormalities: A five year naturalistic study. *Am J Psychiatry*, **157**, 975-981.
- HERRAN, A., DE SANTIAGO, A., SANDOYA, M., FERNANDEZ, M.J., DIEZ-MANRIQUE, J.F. & VASQUEZ-BARQUERO, J.L. (2000). Determinants of smoking behavior in outpatients with schizophrenia. *Schizophr Res*, **41**, 373-381.
- HIEMKE, C., WEIGMANN, H., HARTTER, S., DAHMEN, N., WETZEL, H. & MULLER, H. (1994). Elevated levels of clozapine in serum after addition of fluvoxamine. *J Clin Psychopharmacol*, **14**, 279-281.
- HONG, C.C., TANG, B.Q., HAMMOND, G.L., TRICHLER, D., YAFFE, M. & BOYD, N.F. (2004). Cytochrome P450 1A2 (CYP1A2) activity and risk factors for breast cancer; a cross sectional study. *Breast Cancer Res*, **6**, R352-R365.
- HORN, J. (2006). Understanding the pharmacodynamic and pharmacokinetic differences between proton pump inhibitors- focus on pKa and metabolism. *Aliment Pharmacol Ther symp ser* **2**, 340-350.
- HOWDEN, C.W. (1991). Clinical pharmacology of omeprazole. *Clin Pharmacokinet*, **20**, 38-49.
- HU, Y.-M., XU, J.-M., MEI, Q., XU, X.-H. & XU, S.-Y. (2005). Pharmacodynamic effects and kinetic disposition of rabeprazole in relation to CYP2C19 genotype in healthy Chinese subjects. *Acta Pharmacologica Sinica*, **26**, 384-388.
- HUANG, J.-Q. & HUNT, R.H. (2001). pharmacological and pharmacodynamic essentials of H2-receptor antagonists and proton pump inhibitors for the practicing physician. *Best Pract Res Clin Gastroenterol*, **15**, 355-370.
- ISHIZAKI, T., CHIBA, K., MANABE, K., KOYAMA, E., HAYASHI, M., YASUDA, S., HORAI, Y., TOMONO, Y., YAMATO, C. & YOYOKI, T. (1995). Comparison of the interaction potential of a new proton pump inhibitor, E3810, versus omeprazole with

- diazepam in extensive and poor metabolizers of S-mephenytoin 4'-hydroxylation. *Clin Pharmacol Ther*, **58**, 155-164.
- ISHIZAKI, T. & HORAI, Y. (1999). Cytochrome P450 and metabolism of proton pump inhibitors- emphasis on rabeprazole. *Aliment Pharmacol Ther*, **13**, 27-36.
- JANN, M.W., GRIMSLEY, S.R., GRAY, E.C. & CHANG, W.H. (1993). Pharmacokinetics and pharmacodynamics of clozapine. *Clin Pharmacokinet*, **24**, 161-176.
- JARURATANASIRIKUL, S. & SRIWIRIYAJAN, S. (1998). Effects of omeprazole on the pharmacokinetics of itraconazole. *Eur J Clin Pharmacol*, **54**, 159-161.
- JERLING, M., MERLE, Y., MENTRE, F. & MALLETT, A. (1997). Population pharmacokinetics of clozapine evaluated with nonparametric maximum likelihood method. *Br J Clin Pharmacol*, **44**, 447-453.
- JIANG, Z., DRAGIN, N., JORGE-NEBERT, L.F., MARTIN, M.V., GUENGERICH, F.P., AKLILLU, E., INGELMAN-SUNDBERG, M., HAMMONS, G.J., LYN-COOK, B.D., KADLUBAR, F.F., SALDANA, S.N., SORTER, M., VINKS, A.A., NASSR, N., VON RICHTER, O., LIN, L. & NEBERT, D.W. (2006). Search for association between the human CYP1A2 genotype and CYP1A2 metabolic phenotype. *Pharmacogenetics Genomics*, **16**, 359-367.
- JOOS, A.A., FRANK, U.G. & KASCHKA, W.P. (1998). Pharmacokinetic interaction of clozapine and rifampicin in a forensic patient with an atypical mycobacterial infection. *J Clin Psychopharmacol*, **18**, 83-85.
- JUNGHAN, U., ALBERS, M. & WOGGON, B. (1993). Increased risk of hematological side-effects in psychiatric patients treated with clozapine and carbamazepine? *Pharmacopsychiatry*, **26**, 262.
- KANDO, J.C., TOHEN, M., CASTILLO, J. & CENTORRINO, F. (1994). Concurrent use of clozapine and valproate in affective and psychotic disorders. *J Clin Psychiatry*, **55**, 255-257.
- KAROW, A. & LAMBERT, M. (2003). Polypharmacy in treatment with psychotropic drugs: underestimated phenomenon. *Curr Opin Psychiatry*, **16**, 713.
- KAROW, A. & NABER, D. (2002). Subjective well-being and quality of life under atypical antipsychotic treatment. *Psychopharmacol*, **162**, 3-10.
- KATZUNG, B.G. & TREVOR, A.G. (2005). Drug used in gastrointestinal disorders. In *Katzung and Trevor's Pharmacology: Examination and Board Review*. ed. Katzung, B.G., Trevor, A.G. & Masters, S.B.: McGraw-Hill Education: Singapore, 507-512.
- KESSLER, R.C., MCGONAGLE, K.A., ZHAO, S., NELSON, C.B., HUGHES, M., ESHLEMAN, S., WITTCHEN, H.U. & KENDLER, K.S. (1994). Lifetime and 12-month prevalence

- of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry*, **51**, 8-19.
- KLOTZ, U. (2006). Clinical impact of CYP2C19 polymorphism on the action of proton pump inhibitors: a review of a special problem. *Int J Clin Pharmacol Ther*, **44**, 297-302.
- KROMER, W., KRUGER, U., HUBER, R., HARTMANN, M. & STEINJANS, V.W. (1998). Differences in pH-dependent activation rates of substituted benzimidazoles and biological in vitro correlation. *Pharmacol*, **56**, 57-70.
- KRUSEKOPF, S., ROOTS, I., HILDEBRANDT, A.G. & KLEEBOEG, U. (2003). Time-dependent transcriptional induction of CYP1A1, CYP1A2 and CYP1B1 mRNAs by H⁺/K⁺ -ATPase inhibitors and other xenobiotics. *Xenobiotica*, **33**, 107-118.
- KUO, F.J., LANE, H.Y. & CHANG, W.H. (1998). Extrapyramidal symptoms after addition of fluvoxamine to clozapine *J Clin Psychopharmacol*, **18**, 483-484.
- LAKER, M.K. & COOKSON, J.C. (1997). Reflux oesophagitis and clozapine. *Int Clin Psychopharmacol*, **12**, 37-39.
- LAMBERTI, J.S., OLSON, D., CRILLY, J.F., OLIVARES, T., WILLIAMS, G.C., TU, X., TANG, W., WIENER, K., DVORIN, S. & DIETZ, M.B. (2006). Prevalence of the metabolic syndrome among the patients receiving clozapine. *Am J Psychiatry*, **163**, 1273-1276.
- LAMMERS, C.H., DEUSCHLE, M., WEIGMANN, H., HARTER, S., HIEMKE, C., HEESE, C. & HEUSER, I. (1999). Coadministration of clozapine and fluvoxamine in psychotic patients-clinical experience. *Pharmacopsychiatry*, **32**, 76-77.
- LANE, H.Y., JANN, M.W., CHANG, Y.C. & AL, E. (2001). Repeated ingestion of grapefruit juice does not alter clozapine's steady state plasma levels, effectiveness, and tolerability. *J Clin Psychiatry*, **62**, 812-817.
- LANE, H.Y., SU, K.P., CHANG, W.H. & JANN, M.W. (1998). Elevated plasma clozapine concentrations after Phenobarbital discontinuation. *J Clin Psychiatry*, **59**, 131-133.
- LEVY, R.H. (1995). Cytochrome P450 isoenzymes and antiepileptic drug interaction. *Epilepsia*, **36**, S8-13.
- LI, X.-Q., ANDERSSON, T.B., AHLSTRÖM, M. & WEIDOLF, L. (2004). Comparison of inhibitory effects of the proton pump inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on human cytochrome P450 activities. *Drug Metab Dispos*, **32**, 821-827.
- LIEBERMAN, J.A. (1998). Maximizing clozapine therapy: managing side effect. *J Clin Psychiatry*, **59**, 38-43.

- LIM, P.W.Y. & GOH, K.L. (2004). Efficacy and safety of rabeprazole in treating gastroesophageal reflux disease. *J Clin Gastroenterol Hepatol*, **19**, S61-S68.
- LIN, C.-J., YANG, J.-C., UANG, Y.-S., CHERN, H.-D. & WANG, T.-H. (2003). Time-dependent amplified pharmacokinetic responses of rabeprazole in cytochrome P450C19 poor metabolizers. *Pharmacother*, **23**, 711-719.
- LINNET, K. & OLESEN, O.V. (1997). Metabolism of clozapine by cDNA-expressed human cytochrome P450 enzymes. *Drug Metab Dispos*, **25**, 1379-1382.
- LINNOILA, M., VIUKARI, M., VAISANEN, A. & AUVINEN, J. (1980). Effect of anticonvulsants on plasma haloperidol and thioridazine levels. *Am J Psychiatry*, **137**, 819-821.
- LONGO, L.P. & SALZMAN, C. (1995). Valproic acid effects on serum concentrations of clozapine and nortriptyline. *Am J Psychiatry*, **154**, 650.
- LOZANO, J.J., LOPEZ-DE-BRINAS, E., CENTENO, N.B., GUIGO, R. & SANZ, F. (1997). Three-dimensional modeling of human cytochrome P450 1A2 and interaction with caffeine and MeIQ. *J Computer-Aided mol des*, **11**, 395-408.
- MADABUSHI, R., FRANK, B., DREWELow, B., DERENDORF, H. & BUTTERWECK, V. (2006). Hyperforin in St. John's wort drug interactions. *Eur J Clin Pharmacol*, **63**, 225-33.
- MADDALENA, A.S., FOX, M., HOFMANN, M. & HOCK, C. (2004). Esophageal dysfunction on psychotropic medication. A case report and literature review. *Pharmacopsychiatry*, **37**, 134-138.
- MARINKOVIC, M., TIMOTIJEVIC, I., BABINSKI, T., TOTIC, S. & PAUNOVIC, V.R. (1994). The side-effects of clozapine: a four year follow-up study. *Prog Neuropsychopharmacol Biol Psychiatry*, **18**, 537-544.
- MASELLIS, M., BASILE, V.S., V., O., MELTZER, H.Y., MACCIARDI, F.M. & KENNEDY, J.L. (2000). Pharmacogenetics of antipsychotic treatment: lessons learned from clozapine. *Biol Psychiatry*, **47**, 252-266.
- MCCARTHY, R.H. & TERKELSEN, K.G. (1994). Esophageal dysfunction in two patients after clozapine treatment. *J Clin Psychopharmacol*, **14**, 281-283.
- MICELI, J.J., SMITH, M., ROBARGE, L., MORSE, T. & LAURENT, A. (2000). The effects of ketoconazole on ziprasidone pharmacokinetics: a placebo-controlled crossover study in healthy volunteers. *Br J Clin Pharmacol*, **49**, 71S-76S.
- MILLER, D.D. (1991). Effect of phenytoin on plasma clozapine concentrations in two patients. *J Clin Psychiatry*, **52**, 23-25.
- MILLER, D.D., FLEMING, F., HOLMAN, T.L. & PERRY, P.J. (1994). Plasma clozapine concentrations as a predictor of clinical response: a follow up study. *J Clin Psychiatry*, **55(supp B)**, 117-121.

- MILLER, F.A. & RAMPLING, D. (1982). Adverse effects of combined propranolol and chlorpromazine therapy. *Am J Psychiatry*, **139**, 1198-1199.
- MIURA, M., KAGAYA, H., TADA, H., UNO, T., YASUI-FURUKORI, N., TATEISHI, T. & SUZUKI, T. (2005). Enantioselective disposition of rabeprazole in relation to CYP2C19 genotype. *Br J Clin Pharmacol*, **61**, 315-320.
- MOOKHOEK, E.J. & LOONEN, J.M. (2004). Retrospective evaluation of the effect of omeprazole on clozapine metabolism. *Pharm World Sci*, **26**, 180-182.
- MURRAY, M. (2006). Role of CYP pharmacokinetics and drug-drug interactions in the efficacy and safety of atypical and other antipsychotic agents. *J Pharm Pharmacol*, **58**, 871-885.
- NAKANISHI, N., NAKAMURA, K., ICHIKAWA, S., SUZUKI, K. & TATARA, K. (1999). Relationship between lifestyle and serum lipid and lipoprotein levels in middle-aged Japanese men. *Eur J Epidemiol*, **15**, 341-348.
- NEBERT, D.W. & DALTON, T.P. (2006). The role of cytochrome P450 enzymes in endogenous signaling pathways and environmental carcinogenesis. *Nat Rev Cancer*, **6**, 947-960.
- NEBERT, D.W. & RUSSELL, D.W. (2002). Clinical importance of the cytochromes P450. *Lancet*, **360**, 1155-1162.
- NELSON, D.R., KOYMANS, L., KAMATAKI, T., STEGEMAN, J.J., FEYEREISEN, R., WAXMAN, D.J., WATERMAN, M.R., GOTOH, O., COON, M.J., ESTRABOOK, R.W., GUNSALUS, I.C. & NEBERT, D.W. (1996). P450 superfamily: update on new sequence, gene mapping, accession numbers and nomenclature. *Pharmacogenetics*, **6**, 1-42.
- NEWCOMER, J.W. (2005). Second-generation (atypical) antipsychotics and metabolic effects: a comprehensive literature review. *CNS Drugs*, **19**, 1-93.
- OLESEN, O.V. & LINNET, K. (2001). Contributions of five human cytochrome P450 isoforms to the N-demethylation of clozapine in vitro at low and high concentrations. *J Clin Pharmacol*, **41**, 823-832.
- OZDEMIR, V., KALOW, W., POSNER, P., COLLINS, E.J., KENNEDY, J.L., TANG, B.K., ALBERS, L.J., REIST, C., ROY, R., WALKES, W. & AFRA, P. (2001). CYP1A2 activity as measured by caffeine test predicts clozapine and active metabolite norclozapine steady-state concentration in patients with schizophrenia. *J Clin Psychopharmacol*, **21**, 398-407.
- PAULI-MAGNUS, C., REKERSBRINK, S., KLOTZ, U. & FROMM, M.F. (2001). Interaction of omeprazole, lansoprazole, and pantoprazole with P-glycoprotein. *Naunyn-Schneidebergs Arch Pharmacol*, **364**, 551-557.

- PERRY, P.J., BEVER, K.A., ARNDT, S. & COMBS, M.D. (1998). Relation between patients variables and plasma clozapine concentrations: a dosing nomogram. *Biol Psychiatry*, **44**, 733-738.
- PIRMOHAMED, M. & PARK, B.K. (2003). Cytochrome P450 enzymes polymorphism and adverse drug reactions. *Toxicol*, **192**, 23-32.
- PIRMOHAMED, M., WILLIAMS, D., MADDEN, S., TEMPLETON, E. & PARK, B.K. (1995). Metabolism and bioactivation of clozapine by human liver in vitro. *J Pharmacol Exp Ther*, **272**, 984-990.
- POTSCHKA, H., FEDROWITZ, M. & LOSCHER, W. (2001). P-glycoprotein and multidrug resistance-associated protein are involved in the regulation of extracellular levels of the major antiepileptic drug carbamazepine in the brain. *Neuroreport*, **12**, 3557-3560.
- POTTER, W.Z. & HOLLISTER, L.E. (2004). Antipsychotic agents and lithium. In *Basic and Clinical Pharmacology*. ed. Katzung, B.G. New York: McGraw-Hill. 464-482.
- PRAKASH, C., KAMEL, A., CUI, D., WHALEN, R.D., MICELI, J.J. & TWEEDIE, D. (2000). Identification of the major human liver cytochrome P450 isoform(s) responsible for the formation of the primary metabolites of ziprasidone and prediction of possible drug interactions. *Br J Clin Pharmacol*, **49**, 35S-42S.
- PRICHARD, P.J., WALT, R.P., KITCHINGMAN, G.K., SOMERVILLE, K.W., LANGMAN, J.S., WILLIAMS, J. & RICHENS, A. (1987). Oral phenytoin pharmacokinetics during omeprazole therapy. *Br J Clin Pharmacol Ther*, **244**, 543-545.
- PRIOR, T.I. & BAKER, G.B. (2003). Interactions between the cytochrome P450 system and second- generation antipsychotics. *J Psychiatry Neurosci*, **28**, 99-112.
- QUATTROCHI, L.C. & TUKEY, R.H. (1993). Nuclear uptake of the Ah (Dioxin) receptor in response to omeprazole: Transcriptional activation of the human CYP1A1 gene. *Mol Pharmacol*, **43**, 504-508.
- RAASKA, K. & NEUVONEN, P.J. (2000). Ciprofloxacin increases serum clozapine and N-desmethylozapine: a study in patients with schizophrenia. *Eur J Clin Pharmacol*, **56**, 585-589.
- RAITASUO, V., LEHTOVAARA, R. & HUTTUNEN, M.H. (1993). Carbamazepine and plasma levels of clozapine. *Am J Psychiatry*, **150**, 169.
- RASMUSSEN, B.B., MAENPAA, J., PELKONEN, O., LOFT, S., POULSEN, H.E., LYKKESFELDT, J. & BROSEN, K. (1995). Selective serotonin reuptake inhibitors and theophylline metabolism in human liver microsomes: potent inhibited by fluvoxamine. *Br J Clin Pharmacol*, **39**, 151-159.

- RAUNIO, H., PASANEN, M., MAENPAA, J., HAKKOLA, J. & PELKONEN, O. (1995). Expression of extrahepatic cytochrome P450 in human. In: Pacifici GM, Fracchia GN, eds. *Advances in drug metabolism in man. Luxemburg: European Commission*, 234-287.
- RETTENBACHER, M.A., BAUMGARTNER, S., EDER-ISCHIA, U., EDLINGER, M., GRAZIADEI, I., HOFER, A., HUBER, R., HUMMER, M., KEMMLER, G., WEISS, E. & FLEISCHHACKER, W.W. (2006). Association between antipsychotic-induced elevation of liver enzymes and weight gain. *J Clin Psychopharmacol*, **26**, 500-503.
- RETTIE, A.E., KOOP, D.R. & HAINING, R.L. (2000). Metabolism drug interactions. *Lippincott William & Wilkins*, 75-86.
- REYNOLDS, J.C. (1990). The clinical importance of drug interactions with antiulcer therapy. *J Clin Gastroenterol*, **12**, 54S-63S.
- RITTMANNBERGER, H., MEISE, U., SCHAUFLINGER, K., HORVATH, E., DONAT, H. & HINTERHUBER, H. (1999). Polypharmacy in psychiatric treatment. Patterns of psychotropic drug use in Austrian psychiatric clinics. *Eur Psychiatry*, **14**, 33-40.
- RIZZO, N., PADOIN, C., PALOMBO, S., SCHERRMANN, J.M. & GIRRE, G. (1996). Omeprazole and lansoprazole are not inducers of cytochrome P4501A2 under conventional therapeutic conditions. *Eur J Clin Pharmacol*, **49**, 49-495.
- ROBINSON, M. (2001). New-generation proton pump inhibitors: overcoming the limitation of early-generation agents. *Eur J Gastroenterol Hepatol*, **13**, S43-S47.
- ROBINSON, M. (2004). The pharmacodynamics and pharmacokinetics of proton pump inhibitors- overview and clinical implication. *Aliment Pharmacol Ther*, **20**, 1-10.
- ROOTS, I., GERLOFF, T., MEISEL, C., KIRCHHEINER, J., GOLDAMMER, M., KAISER, R., LASCHINSKI, G., BROCKMOLLER, J., CASCORBI, I., KLEEBOEG, U. & HILDEBRANDT, A.G. (2004). Pharmacogenetics-based new therapeutic concepts. *Drug Metab Rev* **36**, 617-638.
- ROST, K.L., BROSCICKE, H., BROCKMOLLER, J., SCHEFFLER, M., HELGE, H. & ROOTS, I. (1992). Increase of cytochrome P450 1A2 activity by omeprazole: evidence by the ¹³C-[N-3-methyl]-caffeine breath test in poor and extensive metabolizers of S-mephenytoin. *Clin Pharm Ther*, **52**, 170-180.
- ROST, K.L., BROSCICKE, H., HEINEMEYER, G. & ROOTES, I. (1994). Specific and dose-dependent enzyme induction by omeprazole in human being. *Hepatol*, **20**, 1204-1212.
- ROSTAMI-HODJEGAN, A., AMIN, A.M., SPENCER, E.P., LENNARD, M.S., TUCKER, G.T. & FLANAGAN, R.J. (2004). Influence of dose, cigarette smoking, age, sex, and

- metabolic activity on plasma clozapine concentrations: A predictive model and nomograms to aid clozapine dose adjustment and assess compliance in individual patients. *J Clin Psychopharmacol*, **24**, 70-78.
- SACHSE, C., BROCKMOLLER, J., BAUER, S. & ROOTS, I. (1999). Functional significance of a C-->A polymorphism in intron 1 of the cytochrome P450CYP1A2 gene tested with caffeine. *Br J Clin Pharmacol*, **47**, 445-449.
- SCHABER, G., STENENS, I., GAERTNER, H.J., DIETZ, K. & BREYER-PFAFF, U. (1998). Pharmacokinetics of clozapine and its metabolites in psychiatric patients: plasma protein binding and renal clearance. *Br J Clin Pharmacol*, **46**, 453-459.
- SCHRENK, D. (1998). Impact of dioxin-type induction of drug-metabolizing enzymes on the metabolism of endo- and xenobiotics. *Biochem Pharmacol*, **55**, 1155-1162.
- SCLAR, D.A., TARTAGLION, T.A. & FINE, M.J. (1994). Overview of issues related to medical compliance with implications for the outpatient management of infectious disease. *Infect Agents Dis*, **3**, 266-273.
- SEPPALA, N.H., LEINONEN, E.V., LEHTONEN, M.L. & KIVISTO, K.T. (1999). Clozapine serum concentrations are lower in smoking than in non-smoking schizophrenic patients. *Pharmacol Toxicol*, **85**, 244-246.
- SHEINER, L.B. & BEAL, S.L. (1981). Some suggestions for measuring predictive performance. *J Pharmacokinet Biopharm*, **9**, 503-512.
- SHIN, H., PICKWELL, G.V., GUENETTE, D.K., BILIR, B. & QUATTROCHI, L.C. (1999). Species differences in hepatocyte induction of CYP1A1 and CYP1A2 by omeprazole. *Hum Exp Toxicol*, **18**, 95-105.
- SHIRAI, H., FUTURA, T., MORIYAMA, Y., OKOCHI, H., KOBAYASHI, K., TAKASHIMA, M., XIAO, F., KOSUGE, K., NAKAGAVA, K., HANAI, H., CHIBA, K., OHASHI, K. & ISHIZAKI, T. (2001). Effects of CYP2C19 genotype differences in the metabolism of omeprazole and rabeprazole on intragastric pH. *Aliment Pharmacol Ther*, **15**, 1929-1937.
- SILVER, H., KUSHNIR, M. & KAPLAN, A. (1996). Fluvoxamine augmentation in clozapine-resistant schizophrenia: An open pilot study. *Biol Psychiatry*, **40**, 671-674.
- SIMON, W.A., BUDINGEN, C., FAHR, S., KINDER, B., KOSKE, M. (1991). The H⁺, K⁺ ATPase inhibitor pantoprazole (BY1023/SK&F96022) interacts less with cytochrome P450 than omeprazole and lansoprazole. *Biochem Pharmacol* **42**, 347-55.

- SINUES, B., FANLO, A., BERNAL, M.L., VAL, M. & MAYAYO, E. (2004). Omeprazole treatment: genotoxicity biomarkers, and potential to induce CYP1A2 activity in human. *Hum Exp Toxicol*, **23**, 107-113.
- SPINA, E., AVENOSO, A., FACCIOLA, G., FABRAZZO, M., MONTELEONE, P., MAJ, M., MADIA, A., PERUCCA, E. & CAPUTI, A.P. (1998). Effect of fluoxetine on plasma concentrations of clozapine and its major metabolites in patients with schizophrenia. *Int Clin Psychopharmacol*, **13**, 141-145.
- SPINA, E., AVENOSO, A., FACCIOLA, G., SCORDO, M.G., ANCIONE, M. & MADIA, A. (2001). Plasma concentrations of risperidone and 9-hydroxyrisperidone during combined treatment with paroxetine. *Ther Drug Monit*, **23**, 223-227.
- SPINA, E., AVENOSO, A., SCORDO, M.G., ANCIONE, M., MADIA, A., GATTI, G. & PERUCCA, E. (2002). Inhibition of risperidone metabolism by fluoxetine in patients with schizophrenia: a clinically relevant pharmacokinetic drug interaction. *J Clin Psychopharmacol*, **22**, 419-423.
- SPINA, E. & DE LEON, J. (2007). metabolic drug interaction with newer antipsychotics: A comparative review. *Basic Clin Pharmacol Toxicol*, **100**, 4-22.
- STEDMAN, C.A.M. & BARCLAY, M.L. (2000). Comparison of the pharmacokinetics, acid suppression and efficacy of proton pump inhibitors. *Aliment Pharmacol Ther*, **14**, 963-978.
- STRAIN, J.J., CHIU, N.M., SULTANA, K., KARIM, A., CALIENDO, G., MUSTAFA, S. & STRAIN, J.J. (2004). Psychotropic drug versus psychotropic drug: update *Gen Hosp Psychiatry*, **26**, 87-105.
- SUZUKI, Y., SOMEYA, T., SHIMODA, K., HIROKANE, G., MORITA, S., YOKONO, A., INOUE, Y. & TAKAHASHI, S. (2001). Importance of the CYP2D6 genotype for drug metabolic interaction between chlorpromazine and haloperidol. *Ther Drug Monit.*, **23**, 363-368.
- SYVAHLAHTI, E.K.G., LINDBERG, R., KALLIO, J. & DE VOCHT, M. (1986). Inhibitory effects of neuroleptics on debrisoquine oxidation in man. *Br J Clin Pharmacol*, **22**, 89-92.
- TANAKA, E. (1998). Clinically important pharmacokinetics drug-drug interactions: role of cytochrome P450 enzymes *J Clin Pharm Ther*, **23**, 403-416.
- TANTCHEVA-POOR, H., ZAILGLER, M., RIETBROCK, S. & FUHR, U. (1999). Estimation of cytochrome P-450 CYP1A2 activity in 863 healthy Caucasians using a saliva-based caffeine test. *Pharmacogenetics*, **9**, 131-144.
- TAYLOR, D. (1997). Pharmacokinetic interactions involving clozapine. *Br J Psychiatry*, **171**, 109-112.

- TESTA, B. & KRAMER, S.D. (2007). The biochemistry of drug metabolism - An introduction Part2. Redox Reactions and their enzymes. *Chem Biodivers*, **4**, 257-405.
- THUERAUF, N. & LUNKENHEIMER, J. (2006). The impact of CYP2D6-polymorphism on dose recommendation for current antidepressants. *Eur Arch Psychiatry Clin Neurosci*, **256**, 287-293.
- TOTH, P. & FRANKENBURG, F.R. (1994). Clozapine and seizures: a review. *Can J Psychiatry*, **39**, 236-238.
- TUGNAIT, M., HAWES, E.M., MCKAY, G., EICHELBAUM, M. & MIDHA, K.K. (1999). Characterization of the human hepatic cytochromes P450 involved in the in vitro oxidation of clozapine. *Chem Biol Interact*, **118**, 171-189.
- ÜCOK, A., POLAT, A., BOZKURT, O. & METERIS, H. (2004). Cigarette smoking among patients with schizophrenia and bipolar disorders. *Psychiatry Clin Neurosci* **58**, 434-437.
- UNO, T., SHIMIZU, M., YASU-FURUKORI, N., SUGAWARA, K. & TATEISHI, T. (2005). Different effects of fluvoxamine on rabeprazole pharmacokinetics in relation to CYP2C19 genotype status. *Br J Clin Pharmacol*, **61**, 309-314.
- VAINER, J.L. & CHOUINARD, G. (1994). Interaction between caffeine and clozapine. *J Clin Psychopharmacol*, **14**, 284-285.
- VAN DER WEIDE, J., STEIJNS, L.S. & VAN WEELDEM, M.J. (2003). The effect of smoking and cytochrome P450 CYP1A2 genetic polymorphism on clozapine clearance and dose requirement. *Pharmacogenetics*, **13**, 169-172.
- VANDEL, P., TALON, J.M., HAFFEN, E. & SECHTER, D. (2007). Pharmacogenetic and drug therapy in psychiatry - the role of the CYP2D6 polymorphism. *Curr Pharm Des*, **13**, 241-250.
- WARRINGTON, S., LEE, D., BAISLEY, K., DELEMONS, B., LOMAX, K. & MOROCUTTI, A. (2002). Effect of single doses of rabeprazole 20 mg and pantoprazole 40 mg on a 24-hour intragastric acidity in gastroesophageal reflux disease patients with nocturnal heartburn. *Aliment Pharmacol Ther*, **16**, 1301-1307.
- WEDLUND, P.J. (2000). The CYP2C19 enzyme polymorphism. *Pharmacol*, **61**, 174-183.
- WEIGMANN, H., GEREK, S., ZEISIG, A., MULLER, M., HARTTER, S. & HEIMKE, C. (2001). Fluvoxamine but not sertraline inhibits the metabolism of olanzapine: evidence from a therapeutic drug monitoring. *Ther Drug Monit*, **23**, 410-413.
- WETZEL, H., ANGHELESCU, I., SZEGEDI, A., WIESNER, J., WEIGMANN, H., HARTER, S. & HIEMKE, C. (1998). Pharmacokinetic interactions of clozapine with selective

- serotonin reuptake inhibitors: different effects of fluvoxamine and paroxetine in a prospective study. *J Clin Psychopharmacol*, **18**, 2-9.
- WIETHOLTZ, H., ZYSSET, T., MARSCHALL, H.U., GENERET, K. & MATERN, S. (1995). The influence of rifampin treatment on caffeine clearance in healthy man. *J Hepatol*, **22**, 78-81.
- WILKINSON, G.R. (2005). Drug metabolism and variability among patients in drug response. *N Engl J med*, **352**, 2211-21.
- WILLIAMS, M.P. & POUNDER, R.E. (1999). The pharmacology of rabeprazole. *Aliment Pharmacol Ther*, **13**, 3-10.
- WILLIAMS, M.P., SERCOMBE, J., HAMILTON, M.I. & POUNDER, R.E. (1998). A placebo control trial to assess the effects of 8 days of dosing with rabeprazole versus omeprazole on 24-h intragastric acidity and plasma gastrin concentrations in young healthy male subjects. *Aliment Pharmacol Ther*, **12**, 1079-1089.
- WILNER, K.D., DEMATTOS, S.B., ANZIANO, R.J., APSELOFF, G. & GERBER, N. (2000). Ziprasidone and the activity of cytochrome P450 2D6 in healthy extensive metabolizers. *Br J Clin Pharmacol*, **49**, 43S-47S.
- WONG, Y.W.J., YEH, C. & THYRUM, P.T. (2001). The effects of concomitant phenytoin administration on the steady-state pharmacokinetics of quetiapine *J Clin Psychopharmacol*, **21**, 89-93.
- YASUDA, S., OHNISHI, A., OGAWA, T., TOMONO, Y., HASEGAWA, J., NAKAI, H., SHIMAMURA, Y. & MORISHITA, N. (1994). pharmacokinetic properties of E3810, a new proton pump inhibitor, in healthy male volunteers. *Int J Clin Pharmacol Ther*, **32**, 466-473.
- YU, K.S., YIM, D.S., CHO, J.Y., PARK, S.S., PARK, J.Y., LEE, K.H. & SHIN, S.G. (2001). Effect of omeprazole on the pharmacokinetics of moclobemide according to the genetic polymorphism of CYP2c19. *Clin Pharm Ther*, **69**, 266-273.
- ZHANG, W.V., RAMZAN, I. & MURRAY, M. (2007). Impaired microsomal oxidation of the atypical antipsychotic agent clozapine in hepatic steatosis. *J Pharmacol Exp Ther*, **322**, 770-777.

