View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by NORA - Norwegian Open Research Archives

Pharmacokinetic Variability of Olanzapine -A Study Based on Therapeutic Drug Monitoring Data

Dissertation for the Degree Philosophiae Doctor (Ph.D.)

Tore Haslemo

Oslo 2012

Department of Pharmaceutical Biosciences
School of Pharmacy
Faculty of Mathematics and Natural Sciences
University of Oslo

Center for Psychopharmacology,
Diakonhjemmet Hospital
Oslo

Diakonhjemmet Hospital

UNIVERSITAS OSLOENSIS MDCCCXI

© Tore Haslemo, 2012

Series of dissertations submitted to the
Faculty of Mathematics and Natural Sciences, University of Oslo
No. 1227

ISSN 1501-7710

All rights reserved. No part of this publication may be
reproduced or transmitted, in any form or by any means, without permission.

Cover: Inger Sandved Anfinsen.
Printed in Norway: AIT Oslo AS.

Produced in co-operation with Akademika publishing.
The thesis is produced by Unipub merely in connection with the
thesis defence. Kindly direct all inquiries regarding the thesis to the copyright
holder or the unit which grants the doctorate.

Contents

ACKNOWLEDGEMENTS

I would like to thank my supervisor Professor Espen Molden for crucial support and motivation during the work with this thesis. I kept you busy many nights reading manuscripts, and you always provided prompt and ingenious feedback for the projects. I also thank supervisor Professor Helge Refsum for sharing his experience, and for facilitating all the scientific work at Center for Psychopharmacology during these years.

I would like to thank MD Per Håkon Eikeseth for the introduction to working with psychiatric patients. The experience gave me a new perspective on the field of psychopharmacology. Further, I am honoured to work with such an excellent group of researchers as Professor Erik Eliasson and the rest of my co-authors at Karolinska Institutet, Stockholm. I would also like to thank MSc Kristine Olsen for all your contribution to paper IV.

I am grateful to BSc Hilde Lunde and MSc Niclas Lunder helping me out during the method development and analyses of the serum samples. All my colleagues at Center for Psychopharmacology have contributed to this work, thank you for all your help and for making Diakonhjemmet such a nice place to work.

The project was funded by a PhD grant from the South-Eastern Norway Regional Health Authority and made possible by Center for Psychopharmacology, Diakonhjemmet Hospital.

I thank my family, friends and fellow students for providing relevant, and even more importantly, irrelevant support for this thesis.

Unni - Thank you for enduring the good and bad days of the PhD-period, including the breakthroughs, setbacks, frustration and fun. You have supported, persisted, pushed and pulled in all the right places.

Tore Haslemo
Oslo, 2012

LIST OF PAPERS

Paper I
The effect of variable cigarette consumption on the interaction with clozapine and olanzapine.
Haslemo T, Eikeseth PH, Tanum L, Molden E, Refsum H.
Eur J Clin Pharmacol. 2006 Dec;62(12):1049-53.

Paper II
The effect of ethinylestradiol-containing contraceptives on the serum concentration of olanzapine and *N*-desmethyl olanzapine.
Haslemo T, Refsum H, Molden E.
Br J Clin Pharmacol. 2011 Apr;71(4):611-5.

Paper III
*UGT1A4*3* Encodes Significantly Increased Glucuronidation of Olanzapine in Patients on Maintenance Treatment and in Recombinant Systems
T Haslemo, I Loryan, N Ueda, B Mannheimer, L Bertilsson, M Ingelman-Sundberg, E Molden and E Eliasson
Clin Pharmacol Ther advance online publication, June 20, 2012; doi:10.1038/clpt.2012.46

Paper IV
Valproic acid significantly lowers serum concentration of olanzapine – an interaction effect comparable to smoking.
Haslemo T, Olsen K, Lunde H, Molden E.
Submitted Therapeutic Drug Monitoring, 04 May 2012

**Update due Aug 2012:

Paper III published Clin Pharmacol Ther. 2012 Aug;92(2):221-7.

Paper IV accepted for publication Ther Drug Monitoring, 11 Jul 2012.

ABBREVIATIONS

AED	antiepileptic drug
AGNP	arbeitsgemeinschaft fur neuropsychopharmakologie und pharmakopsychiatrie
AhR	aromatic hydrocarbon receptor
BMI	body mass index
CATIE	Clinical Antipsychotic Trials of Intervention Effectiveness
C/D ratio	concentration/dose ratio, i.e. dose-adjusted serum concentration
CNS	central nervous system
CYP	cytochrome P450
D2	dopamine 2
DDD	defined daily dose
DDI	drug-drug interaction
ECC	ethinyl estradiol-containing contraceptive
FDA	Food and Drug Administration (USA)
FMO	flavin-containing monooxygenase
H2	histamine 2
LC	liquid chromatography
MDR	multi drug resistance
MS	mass spectrometry
NSAID	non-steroidal anti-inflammatory drug
PAH	poly-aromatic hydrocarbons
PBC	progestogen based contraceptive
SNP	single nucleotide polymorphism
TDM	therapeutic drug monitoring
UDP	uridine diphosphate
UGT	UDP glycosyltransferase

ABSTRACT

Olanzapine is one of the most commonly used antipsychotic drugs in treatment of schizophrenia and bipolar disorder. The interindividual variability in pharmacokinetics of olanzapine is extensive, with a 10-20-fold difference in serum concentration despite equivalent dosing. The aim of this thesis was to identify and evaluate factors that influence the pharmacokinetics of olanzapine, and thereby provide knowledge that can be applied in order to individualize treatment with olanzapine.

Therapeutic drug monitoring samples from patients treated with olanzapine were used as data material in all four studies of the thesis. The serum concentration of olanzapine and metabolites were quantified with liquid chromatography-mass spectrometry (LC-MS) analyses. Overall, cigarette smoking, age and gender were shown to be significant determinants of olanzapine variability. Non-smokers generally obtained a two-fold higher dose-adjusted serum concentration (C/D ratio) compared to smokers. Age and gender were also shown to be significant determinants of olanzapine C/D ratio, but the numerical effects of these factors were less than those mentioned above. Furthermore, it was shown that comedication with the antiepileptic drugs valproic acid and carbamazepine substantially affected C/D ratio of olanzapine. The estimated reductions were approximately 30% and 50%, respectively. Concurrent use of ethinyl-estradiol containing contraceptives and a mutation in the gene encoding uridine diphosphate glycosyl transferase 1A4 (UGT1A4) did not affect serum concentration of olanzapine significantly, but both were shown to have significant impact on metabolic pathways of olanzapine.

In conclusion, the present thesis reveals that cigarette smoking, age, gender and comedication with valproic acid or carbamazepine are significant factors which contribute to the variability in pharmacokinetics of olanzapine. Summarized, a female, non-smoking patient ≥60 years receiving olanzapine monotherapy, would on average obtain a more than 3-fold higher C/D ratio compared to a male, smoking patient <60 years comedicated with valproic acid or carbamazepine. To improve the therapeutic effect and reduce the risk of side effects, these factors should be considered as a basis for individualized dosing of olanzapine in clinical practice.

1 INTRODUCTION

1.1 Schizophrenia and bipolar disorder

Schizophrenia is considered the costliest mental illness in terms of both human suffering and social expenditure. The lifetime prevalence is estimated to be 0.3-0.7%.[1] Schizophrenia is a developmental syndrome deriving from multiple genetic and environmental factors.[2] Although schizophrenia is often viewed as a single disease, it is now recognized that the diagnostic category probably comprises a group of disorders with heterogeneous etiology.[3] Patients with schizophrenia experience positive symptoms, including hallucinations and delusions, thought disorders, and negative symptoms like social withdrawal.[4]

Numerous long term prognostic studies in schizophrenic patients have shown that the clinical outcome is good for 20-50% of the patients, with different definitions like total remission, no readmission and symptomatic recovery. A similar proportion of the patients are characterized by a poor outcome (15-45%), with definitions like severe chronic social- or intellectual deficit, moderate to severe symptoms at follow up or chronic psychotic symptoms.[4] This heterogeneity of outcome is mainly unexplained.[5] Some clinical predictors of poor outcome have been identified, including early onset, long period of untreated psychosis, prominent negative symptoms and poor premorbid adjustment.[6] Early response to treatment may also be a predictor of good outcome.[7;8] Though some biochemical predictors for metabolic side effects of antipsychotic medication have been identified, no clinically relevant biochemical markers for the outcome of schizophrenia are known.[8]

Bipolar disorder has been described as a cyclical illness, with manic and/or depressive episodes interspaced with normal euthymic periods. Further evidence suggest that patients with bipolar disorder experience a more subtle chronic course than initially thought, characterized by residual symptoms, emotional dysregulation, sleep disturbances, cognitive impairment, and increased risk of psychiatric and medical comorbidity.[9] Bipolar disorder is grouped into several categories based on the severity and cycling of the symptoms (e.g. bipolar I, bipolar II, rapid cycling and mixed states). Severe episodes of mania might involve psychosis and are often accompanied by periods of depression. Bipolar disorder shares some of the symptoms and biology with schizophrenia suggesting some shared etiological mechanisms.[10]

Antipsychotic drugs are effective in treatment of schizophrenia and bipolar disorder, and have been available for sixty years. They have been demonstrated to be important in prevention of relapse in schizophrenic patients.[11] Since the discovery of chlorpromazine in 1952, more than 30 antipsychotic drugs have been marketed. Traditionally the antipsychotic drugs have been divided into the first generation (typical) and second generation (atypical) agents. The first generation agents are characterized by good clinical effect against the positive symptoms, but poor response against negative symptoms. Moreover, their use has been limited by severe extrapyramidal side effects such as spasms, motor restlessness and involuntary movements. In comparison, the second generation antipsychotics better address negative symptoms of schizophrenia, and cause little extrapyramidal side effects. However, these latter are accompanied by metabolic side effects (weight gain, lipid- and glucose disturbances), which are seldom for first generation agents.

Common for antipsychotics is the ability to block dopamine 2 (D2) receptors, which explains the effect towards positive symptoms via reduction of mesolimbic hyperactivity in dopaminergic transmission.[12] However, as the negative symptoms of schizophrenia are related to *decreased* dopamine activity in the mesocortical dopamine pathway, this represents a major challenge for pharmacologic treatment of schizophrenia. Ideally, agents used in treatment of schizophrenia should exert opposite effects on the same receptor system in different parts of the brain. The antipsychotic drug aripiprazole was developed to address this challenge by acting as a partial dopamine agonist, instead of being D2-antagonists like the other antipsychotics. Besides the effect on D2-receptors, second generation antipsychotics antagonize serotonin 5HT2A-receptors.

1.2 Olanzapine

Olanzapine is a second generation antipsychotic approved for use in treatment of schizophrenia and bipolar disorder. It was first approved for clinical use in the European Union in 1996, and has become one of the most commonly used antipsychotic drugs worldwide. Olanzapine was the most prescribed antipsychotic drug in Norway in 2010, both in terms of defined daily doses (DDD) and number of users.[13] WHO defines DDD of olanzapine to 10 mg.[14] According to data from the Norwegian Prescription Database, it was dispensed a total of 4 336 324 DDDs to 15799 of unique olanzapine users in Norway in 2010. These numbers relates to olanzapine dispensed directly to patients through Norwegian pharmacies, and do not

include sale to hospitals and other institutions.[13] In Norway, olanzapine is now marketed in four different formulations, i.e. ordinary tablets, orally disintegrating tablets, solution for injection and depot injection.[15] Indications include acute psychosis in patients with schizophrenia or manic episode, long-term treatment of schizophrenia and prevention of manic episodes in bipolar patients. In the US market olanzapine is also approved in set combinations with the antidepressant fluoxetine, for use in treatment-resistant depression and bipolar I depression.[16]

Olanzapine improves negative and positive symptoms of schizophrenia.[17] It is considered one of the most effective antipsychotic drugs, with lower rates of discontinuation and greater reduction in symptoms compared to both first- and other second-generation antipsychotic agents.[18;19] The favourable effects, however, are accompanied by more frequent side effects than certain other antipsychotics.[11] Weight gain, dyslipidemia, type II diabetes and heart disease are all serious adverse effects associated with olanzapine, which have limited its use in some patient groups, e.g. the elderly.

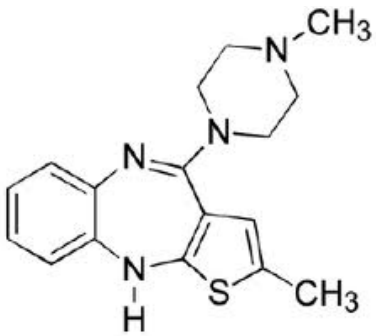

Figure 1 Chemical structure of olanzapine.
(2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]benzodiazepine)

The molecule is a thienobenzodiazepine (**Figure 1**), with structural and functional similarities to clozapine. Olanzapine is well absorbed after administration of ordinary tablets, with maximum concentration reached after 5-8 hours and unaffected by food intake. Olanzapine is highly bound to plasma proteins (approx. 93%), primary to albumin and to a lesser extent alfa1-acid glycoprotein.[20] On average, the elimination half life and clearance of olanzapine are reported to be 33 h and 26.1 L/h respectively. Bioavailability is 0.8 and the hepatic extraction ratio is 0.4.[21;22] Metabolism of olanzapine is covered in section 1.4.4.

1.3 Drug metabolism

The human body has a number of detoxification and transport systems to aid excretion of foreign substances (xenobiotics), including drugs. Psychoactive drugs, including olanzapine, are lipophilic compounds. This is a necessity in order to pass the blood brain barrier and exert pharmacodynamic effect in the central nervous system (CNS). This also means that most psychoactive drugs need to be metabolized to more water-soluble molecules prior to excretion via urine or bile.

Drug metabolism is traditionally divided into “phase I” and “phase II” reactions. Phase I reactions involve processes which modify the primary molecular structure by reactions such as reduction, oxidation or hydrolysis. Some phase I metabolites are sufficiently polar to be directly excreted in urine or bile, but phase I reactions may also facilitate further metabolism (phase II) of the molecules. The cytochrome P450 (CYP) superfamily is regarded as the most important enzyme system in phase I metabolism of drugs, but other families such as the flavin-containing monooxygenase (FMO) system are also of relevance.

Phase II metabolism involves conjugation of the primary molecules to so called co-substrates, which are polar, small-molecular compounds like glucuronic acid, amino acids, sulphate or glutathione. Phase II enzymes often conjugate these latter compounds to functional groups on the molecule resulting from phase I metabolism, or like in the case of olanzapine - via direct glucuronidation of a functional group on the parent molecule. While phase I metabolites may display relevant pharmacological activity, phase II reactions often result in inactive molecules. The superfamily of uridine diphosphate glycosyltranferase (UGT) enzymes is one of the most important for phase II metabolism of many drugs.

1.3.1 Cytochrome P450

The CYP enzymes are involved in oxidative metabolism of many clinically used drugs. More than 50 different human CYP enzymes are identified,[23] but CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A are regarded the most important ones in the metabolism of xenobiotics.[24] Some CYP enzymes also metabolize endogenous compounds, e.g. fatty acids, hormones and cholesterol, but those mentioned above are usually of minor relevance in endogenous metabolism.[25]

CYP enzymes are located in the endoplasmatic reticulum of cells that provide external tissue barriers, e.g. enterocytes, hepatocytes, dermatocytes and lung epithelial cells, in order to limit systemic exposure of foreign substances. In addition, CYP enzymes are expressed to some extent in the brain, where they may locally contribute to metabolism of endogenous substrates and drugs.[26]

The individual variability in CYP enzyme phenotype is extensive due to genetic and environmental factors.[27;28] Most of the CYP enzymes are prone to induction and inhibition, making CYP-related drug-drug interactions (DDI) a relevant issue regarding clinical safety and effect of many drugs. Details and examples are listed in section 1.5.

1.3.2 Uridine diphosphate glycosyltranferase

The superfamiliy of uridine diphosphate (UDP) glycosyltranferase (UGT) enzymes is important in conjugative (phase II) metabolism of drugs, but also play a crucial role in metabolism of many endogenous substrates.[29] UGT metabolism leads to increased water solubility of the substrate and therefore aids excretion through the kidneys. Glucuronidation will usually inactivate substrates, by increasing size and polarity of the molecule, and hamper binding to drug receptors. The UGT1A and UGT2B subfamilies, the most important ones in drug metabolism, acts by adding UDP glucuronic acid to an electron-rich (e.g. nitrogen, oxygen, carboxyl or sulphur) part of a substrate.

There are identified 15 human enzymes within the UGT1A and UGT2B subfamilies,[30] they are expressed mainly in liver, but also found in the gastrointestinal tract and kidney.[31] Enzymes within these families catalyze the conjugation of glucuronic acid (**Figure 2**) to the

respective substrates. This is in contrast to UGT3 and UGT8, which catalyze the attachment derivates from other sugar residues, e.g. galactose and N-acetylglucosamine.[32] Despite conjugation of different co-substrates, most authors still refer to UGT as UDP *glucuronosyltransferases*.[29]

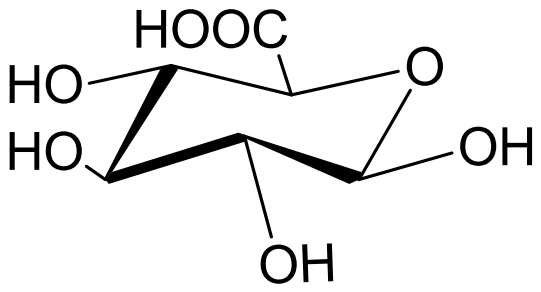

Figure 2 Structure of glucuronic acid

Individual differences in metabolism via UGTs could be of importance both with respect to disease risk and drug response. Regarding the former, inherent deficiency in the function of UGT1A is associated with conditions like the Gilbert and Crigler-Najjar syndromes, which causes toxic hyperbilirubinemia.[33;34] Newborns and infants lack UGT capacity,[35] and might develop lethal accumulation of UGT substrates. This is exemplified by the "gray baby syndrome" that might develop in children after administration of UGT substrate chloramphenicol.

In contrast to the CYP system, substrate specificity is lower in the UGT system. Several UGTs catalyze conjugation of the same substrates, especially in *O*-glucuronidation. N-glucuronidation is an exception to this, with UGT1A4 and the less studied UGT2B10,[36] being specialized in performing *N*-glucuronidation. Some of these metabolites are rather specific to humans (e.g. *N*-glucuronides from amines, amides and aromatic *N*-heterocycles).[37] This complicates *in vivo* extrapolation of *in vitro* results from animal models.[38] UGT1A4 are considered the most important subfamily in relation to psychotropic drug, catalyzing N-glucuronidation of antidepressants, antipsychotics and antiepileptics.[38]

1.3.3 Flavin-containing monooxygenase

The flavin-containing monooxygenases (FMOs) are mainly known to metabolize exogenous substances like toxins, pesticides and drugs.[39] There are identified five functional isoforms of FMO (FMO1-5).[40] Expression of FMO1, FMO2 and FMO4 is limited in human beings, and FMO3 is believed to be the most prominent in drug metabolism.[40] FMO enzymes are expressed in kidney (FMO1 and 4), lung (FMO2 and 4) liver (FMO 3, 4 and 5) and intestine (FMO4).[41] In an average human liver, the FMO3 level is approximately 65% compared to the widely expressed CYP3A4.[42] FMO3 therefore has the potential to be an important enzyme in the metabolism of drugs. Still, there has been limited focus on FMO3 and other FMO enzymes compared to other drug-metabolizing enzymes, such as CYP and UGT enzymes. However, some *in vivo* studies have demonstrated that substances like some H2-receptor antagonists (ranitidine and cimetidine), NSAIDS (sulindac and benzydamine) and nicotine are substrates of FMO3.[43] Little is known about the potential induction or inhibition of FMO3, but a spontaneous 'back-reduction' of FMO3-mediated metabolites to the parent drug has been described.[44] This phenomenon might have underestimated the impact of FMO3 as an enzyme of relevance in the context of drug metabolism and potential drug interactions.

1.3.4 Metabolism of olanzapine

The most important eliminating mechanism for olanzapine is metabolism via multiple pathways. Summarized, the most relevant enzymes for metabolism of olanzapine are uridinediphosphate (UDP) glycosyltransferase 1A4 (UGT1A4), cytochrome P450 1A2 (CYP1A2) and flavin-containing monooxygenase 3 (FMO3).[21] These mediate formation 10-*N*-glucuronide olanzapine, *N*-desmethyl olanzapine and *N*-oxide olanzapine, respectively (**Figure 3**).[20;45;46] Other enzymes are also involved in the metabolism of olanzapine (e.g. CYP2D6 and CYP3A), but appear to play minor roles in the overall clearance.[45]

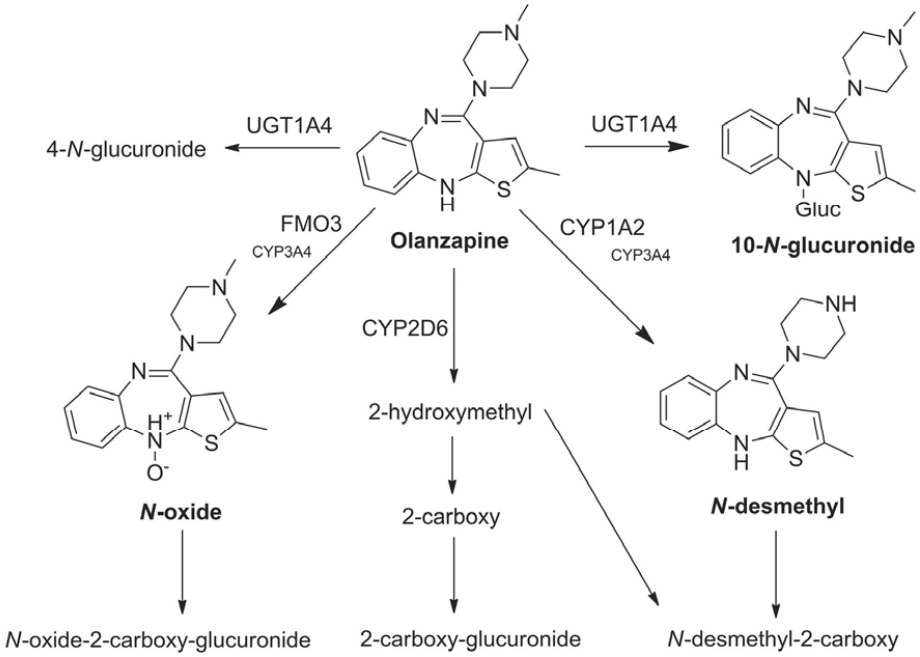

Figure 3 Pathways of olanzapine metabolism in humans (based on [20;45;47])

1.4 Pharmacokinetic variability

1.4.1 Genetic polymorphism

Genetically determined differences in function of drug-metabolizing enzymes are generally important factors for interindividual variability in pharmacokinetics. The phenotypical consequences of genetic variants include defective, reduced or increased expression or activity of the protein of relevance.

Within the field of pharmacogenetics, most attention has been paid to genetic polymorphisms of drug-metabolizing enzymes, in particular CYP enzymes, where the clinical relevance of genetic variability is best documented for CYP2C9, CYP2C19 and CYP2D6.[48-51] Examples include 10-fold higher AUC and more adverse drug reactions of atomoxetine in CYP2D6 poor metabolizers compared to extensive metabolizers.[52] The antiplatelet drug clopidogrel is a prodrug activated by CYP2C19, and carriers of reduced function CYP2C19 alleles treated with this drug are shown to have more major adverse cardiovascular events compared to

extensive metabolizers.[53] Genetic polymorphisms also exist for other CYP enzymes, for example CYP1A2,[54] but pharmacogenetic variability has so far appeared to be of clinical relevance mostly within the CYP2 family. Genetic testing of *CYP2D6*, *CYP2C19* and *CYP2C9* is a valuable tool for predicting dosing of many drugs that are substrates of these enzymes. Algorithms have been published to individualize drug doses based on the respective genotypes,[43;55-57] but it seems to be a way to go before the potential benefit of genotyping is exploited in clinical practice.[58]

Regarding UGT enzymes, genetic polymorphism with relevant functional consequences has been described for UGT1A4. An example is the *UGT1A4 142T>G* variant allele (*UGT1A4*3*), encoding an amino acid substitution (L48V), which has been reported to be of relevance for the pharmacokinetics of clozapine and lamotrigine.[59;60] A pilot study indicated that *UGT1A4*3* impacts olanzapine metabolism in humans as well,[61] but a limitation of the studies performed so far is the absence of homozygous **3* carriers.[60-63] A recent study also proves that differences in other UGT enzymes can be crucial for the disposition of drugs. A substance under development for respiratory diseases, MK-7246, displayed 25 fold higher AUC in homozygous carriers of *UGT2B17*2* compared to carriers of the wild type.[64] New discoveries are being made, but genetic variation of UGTs are complicated by processes beyond genetic polymorphism, i.e. variation due to epigenetic factors and splicing.[65] This calls for more information regarding genetic and environmental variation of UGTs.

Although poorly investigated, genetic variability might also be an important determinant for interindividual differences in FMO3-mediated metabolism. The expression of FMO3 is shown to vary more than 10-fold in an adult liver, and this variability might be associated with genetic factors.[66] *FMO3* mutations have been linked to clinical response of olanzapine in one study,[67] but studies published on the pharmacokinetics of olanzapine in relation *FMO3* genetics are so far lacking.

1.4.2 Non-genetic factors

Drug-drug interactions

In a clinical pharmacological context, drug-drug interactions (DDIs) represent one of the most important non-genetic factors behind individual pharmacokinetic variability. There are several pharmacokinetic DDI mechanisms, but inhibition or induction of drug-metabolizing enzymes

is generally considered as most relevant. Some drugs act as competitive inhibitors, while others act more 'mechanistically' by enzyme inactivation (reversible or irreversible). In general, all kind of enzyme inhibition has rapid onset, but the duration of inhibition depends on the mechanism. Inhibition of drug metabolism usually leads to increased serum levels of an active parent compound, however, the opposite might be the case for prodrugs. The pharmacokinetic sensitivity of enzyme inhibition will largely depend on the complexity of pathways involved in elimination of the substrate.

Up-regulation of enzyme levels is the mechanism behind enzyme induction. Induction could be viewed as a response to increase the 'xenobiotic defence' following prolonged exposure of foreign substances. An inducer acts by stimulating binding of nuclear receptors and thereby increases transcription of genes encoding the respective enzymes. Induction is usually achieved during a time period of days to weeks. Many inducers act rather unspecifically (e.g. carbamazepine) by simultaneously up-regulating a number of enzymes involved in drug metabolism.[68;69] The activity of CYP1A2, involved in olanzapine metabolism, could be induced or inhibited by a number of substances (see **Table 1** for examples).[70]

Table 1 Examples of substrates, inducers and inhibitors of CYP1A2 (based on Zhou et al.[70])

SUBSTRATES	INDUCERS	INHIBITORS
Caffeine	Carbamazepine	Ciprofloxacin
Clozapine	Cruciferous vegetables	Contraceptives (estrogens)
Duloxetine	Cigarette smoke	Fluvoxamine
Melatonin	Grilled/smoked meat/food	Rofecoxib
Olanzapine	Rifampicin	
Theophylline		
Tizanidine		

There are described several sources to variability in UGT1A4, including genetic polymorphism and interactions with endogenous and exogenous substances. Reports have been published on genetic variants in UGT1A4 and the effect of these, but with conflicting evidence and possibly also substrate specific effects.[60;63;71;72] Some inducers are reported for UGT-enzymes, i.e. smoking, carbamazepine, alcohol, but the effects are mostly small and

inconsistent.[73] Good probe substances and specific inducers/inhibitors of the various UGT isoforms are essential, but are complicated by the fact that several of the UGTs overlap in regard to metabolism of substrates. **Table 2** provides examples of UGT1A4 substrates, inducers and inhibitors.

Table 2 Examples of substrates, inducers and inhibitors of UGT1A4
Based on Kaivosaari et al., Kiang et al. and Chen et al.[38;74;75]

SUBSTRATES	INDUCERS	INHIBITORS
Amitriptyline	Carbamazepine	Bilirubin
Asenapine	Smoking	Valproic acid
Chlorpromazine	Estradiol	
Clozapine		
Imipramine		
Irinotecan		
Lamotrigine		
Olanzapine		
Progestins		
Promethazine		

Lifestyle and dietary factors

Not only drugs are able to inhibit or regulate the transcription of enzymes – other xenobiotics, endogenous substances (hormones), pollutants (smoke) and dietary factors can also mediate these processes. Poly-aromatic hydrocarbons (PAH) are examples of substances that trigger up-regulation/induction of metabolism. PAHs belong to a class of substances that are often carcinogenic, teratogenic and mutagenic. Humans are exposed to PAHs through different sources, e.g. cigarette smoke, burned- or grilled food. PAHs are lipophilic ring structures that act in human cells by binding to the aromatic hydrocarbon receptor (AhR), which will cause up-regulation of enzymes (e.g. CYP1A). Activation of other nuclear receptors like constitutive androstane receptor (CAR) and pregnane X receptor (PXR) are also causes of induction.[76] A classic example of dietary factors causing enzyme inhibition is grapefruit juice, but other food and herbal products are also reported to affect metabolizing enzyme e.g. the inducer hypericum/St John's wort.[77;78] Furthermore, infection, inflammation and cancer are

examples of disease states that also affect enzymes.[79] Increased knowledge of all these factors is important, but controlling or estimating the effects of all these factors in the clinic is impossible. Although systemic concentrations do not necessarily correlate well with the concentration and pharmacodynamic effects of a certain drug, measuring the concentrations of drugs in blood, provides valuable information about pharmacokinetic variability of drugs.

1.5 Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) has been available for several decades as a tool for quality assurance and individualization of drug treatment. TDM is today more or less implemented in treatment monitoring of immunosuppressants, anti-infective agents (HIV and antimicrobials), anti-cancer agents, psychoactive agents, anticoagulants and antiepileptics.[80-82] With regard to psychoactive drugs, TDM is important because few other objective biological effect markers exist.

Older AEDs, tricyclic antidepressants and lithium have a well-documented concentration-effect relationship, a so-called therapeutic window.[80;83] The evidence for a concentration-effect relationship is not that extensive for newer antidepressants and antipsychotics, although many of these also have curvilinear concentration relationships of clinical- and side effects.[84] In addition to being a tool for dose individualization, TDM may also aid the detection of adherence problems, pharmacokinetic DDIs and other risk factors related to drug use. The concept of TDM "Nouveau", a term introduced by Bengtsson in 2004,[85] state that the patients should be their own controls in sequence serum concentration measurements. The suggested strategy is to measure drug concentration when the response is good, obtaining a 'therapeutic' target concentration for the individual patient, applied as a reference for future monitoring.

Pharmacogenetic testing has become an important part of TDM for detection of vulnerable patients, with the advantage of getting this information prior to initiating drug treatment. Genotype-based dose adjustments are relevant for many drugs[55;86], and might reduce the use of "trial and error" in dosing of potent psychoactive drugs. Applying this into clinic could reduce the negative experience with drugs, aiding long term adherence and trust in patients. The US Food and Drug Administration (FDA) has in recent years increasingly been recommending pharmacogenetic tests prior to treatment with drugs like warfarin and clopidogrel.[87]

1.5.1 Therapeutic drug monitoring of olanzapine

The AGNP-TDM consensus guidelines strongly recommend that serum concentration is monitored in patients using olanzapine, and suggests a therapeutic range of 20-80 ng/ml (64-255 nmol/L).[84] Serum concentrations have been related to clinical effect and side effects of olanzapine in several studies.[88-92] For instance, a follow up analysis of serum concentration-versus-clinical response data from the CATIE trial revealed a significantly higher discontinuation rate in patients with low serum concentrations of olanzapine, while patients with high serum concentrations had a significantly higher discontinuation rate due to side effects.[19;93] There is an extensive interindividual variability in pharmacokinetics of olanzapine, and TDM studies have shown 25-fold differences in C/D ratio between patients.[94-96] A recent study in adolescents have also shown large intraindividual variability of olanzapine and metabolite levels, recommending repeated measurements to obtain more precise estimates of the pharmacokinetics in individual patients.[97]

Discontinuation of treatment is an important reason of relapse in schizophrenic patient.[98] The CATIE trial reported that 65-80% of the included patients discontinued antipsychotic treatment before 18 months of treatment. The median time to discontinuation was between 5-8 weeks for the atypical antipsychotics.[19] This represents one of the main problems of treatment with antipsychotics, and emphasizes the importance of TDM as a tool to avoid discontinuation of treatment.

TDM is also of value for preventing serious side effects caused by changes in treatment or environment of patients. Studies have reported increased serum concentrations, serious side effects and intoxications of antipsychotics olanzapine and clozapine following smoking cessation.[91;99] One might argue that these episodes could be prevented by changing the dosing based on experience and the clinic only. The typical concept of increasing doses to reach clinical effect, and stop when side effects occur, is still commonly used. This strategy does not take into account the “silent”, but potentially fatal, side effects of atypical antipsychotics. A study by Ray et al. showed increased risk of serious ventricular arrhythmias and sudden cardiac death in users of antipsychotics. The risk was shown to be dose-dependant,[100] which necessarily means that serum concentration is of importance as well.

2 AIM OF THE THESIS

The objective of this thesis was to identify and evaluate factors that contribute to the pharmacokinetic variability of olanzapine. Cigarette smoking (paper I), drug-drug interactions with oral contraceptives (paper II) and antiepileptic drugs (paper IV), and *UGT1A4*3* polymorphism (paper III) were investigated during this work. The studies also allowed exploration of the effect of age and gender on serum concentrations of olanzapine and metabolites. All studies were based on TDM data from Norwegian psychiatric patients.

3 SUMMARY OF RESULTS

Paper I: The effect of variable cigarette consumption on the interaction with clozapine and olanzapine.

This paper describes the effect of variable cigarette smoking (i.e. cigarette 'dose') on serum concentrations of olanzapine and clozapine, in a group of 73 psychiatric patients (n=40 olanzapine, n=33 clozapine). Dose-adjusted serum concentrations (C/D ratios) were on average twofold higher in non-smokers compared to cigarette smokers for olanzapine and clozapine. A total of 80% of the patients included were smokers, and these were divided into subgroups based on daily cigarettes (i.e. 1-6 [n=0], 7–12 [n=10], 13–19 [n=8], ≥20 [n=13]). There were no significant differences in C/D ratios between subgroups of smokers ($p>0.7$). None of the patients smoked 1-6 cigarettes daily, so we were not able to identify the lower threshold needed for the enzyme induction to occur. A daily consumption of 7-12 cigarettes did, however, seem to be sufficient to obtain maximum induction of the metabolism of olanzapine and clozapine. Studying the absolute serum concentrations, it appeared that non-smokers were dosed too high, rather than cigarette smokers being dosed too low. This could possibly be explained by the fact that cigarette smokers have been overrepresented in this patient group and therefore predicted the therapeutic dose interval of olanzapine during drug development. Based on these findings, it seems reasonable that non-smokers should receive approximately half the dose of cigarette smokers when starting treatment with olanzapine or clozapine. Moreover, in a clinical setting, it seems adequate to classify patients into smokers and non-smokers when individualizing the dose based on smoking habits.

Paper II: The effect of ethinylestradiol-containing contraceptives on the serum concentration of olanzapine and N-desmethyl olanzapine.

Ethinylestradiol-containing contraceptives (ECC) are known to inhibit CYP1A2 metabolism, but the potential interaction with the CYP1A2 substrate olanzapine had not previously been investigated. This study was carried out to explore the effect of ECC and progestogen-based contraceptives (PBC) on serum concentrations of olanzapine and the metabolite *N*-desmethyl olanzapine, which is formed via CYP1A2. Olanzapine-treated female patients aged 18-40 years who used oral contraceptives were recruited from the TDM service at Diakonhjemmet Hospital along with a control group that did not use oral contraceptives. During a time period of 18 months, a questionnaire was by routine sent to the prescribing physician to collect information regarding contraceptive use and smoking habits among all female patients aged 18-40. A total of 149 questionnaires valid for inclusion were returned, 10 out of these were from patients that used ECC and 10 from patients that used PBC. Although users of ECC had 35-40% lower serum levels of *N*-desmethyl olanzapine compared to users of PBC ($p=0.02$) and the control group ($p=0.01$), the olanzapine levels were not significantly different between the three subgroups ($p>0.9$). The results of the study show that ECC inhibits CYP1A2-mediated metabolism of olanzapine. However, this inhibition does not produce a clinically relevant interaction between ECC and olanzapine.

Paper III: UGT1A4*3 encodes significantly increased glucuronidation of olanzapine in patients on maintenance treatment and in recombinant systems

This study investigated the effect of *UGT1A4*3* on the metabolism of olanzapine *in vitro* and *in vivo*. A total of 558 psychiatric patients treated with olanzapine were genotyped. In patients that met the inclusion criteria (n=407), dose-adjusted serum concentration (C/D ratio) of olanzapine were compared between subgroups with different *UGT1A4*3* genotype. Moreover, serum samples from 129 of these were available for reanalysis of 10-*N*-glucuronide olanzapine, which is formed via UGT1A4. Frequencies of heterozygous and homozygous carriers of *UGT1A4*3* were 16% and 1.8% respectively. Olanzapine C/D ratios were not significantly different between the *UGT1A4*3* subgroups ($p>0.4$), but heterozygous and homozygous carriers of *UGT1A4*3* had significantly higher levels of the 10-*N*-glucuronide metabolite compared to the control group without the **3* mutation. The numerical differences were 1.4-fold ($p=0.01$) and 2.5 fold ($p<0.001$) higher levels of 10-*N*-glucuronide in heterozygous and homozygous carriers of *UGT1A4*3*, respectively, compared to patients homozygous for the wild-type. In line with these in vivo findings, microsomes expressing UGT1A4.3 exhibited significantly higher glucuronidation activity of olanzapine compared to microsomes expressing UGT1A4.1. Overall, the study shows that *UGT1A4*3* encodes significantly higher glucuronidation activity of olanzapine, but this is not of clinical relevance for olanzapine treatment.

Paper 4: Valproic acid significantly lowers serum concentration of olanzapine – an interaction effect comparable to smoking.

Olanzapine is frequently combined with mood stabilizing antiepileptic drugs (AEDs) in patients with bipolar disorder and schizophrenia. This study aimed to investigate the possible effect of AEDs on serum concentrations of olanzapine and three major metabolites. Patients were divided into subgroups based on coadministration of different AEDs, and a control group of patients that used olanzapine without concurrent AEDs. A total of 598 serum samples, from 430 patients, were included. Linear mixed models were used to investigate the effect of different AEDs, age, sex and cigarette smoking on dose-adjusted serum concentrations (C/D ratios) of olanzapine and metabolites. Significant effects on olanzapine C/D ratio were found in subgroups comedicated with valproic acid (-32%, n=166 samples, $p<0.001$), lamotrigine and valproic acid (-31%, n=12, $p=0.003$) and carbamazepine (-50%, n=8, $p<0.001$) compared to the control group. Age ≥60 (+35%, $p<0.001$), female gender (+11%, $p=0.008$) and cigarette smoking (-32%, $p<0.001$) were also highly significant predictors of olanzapine concentrations compared to patients younger than 60 years, males and non-smokers respectively. The interaction of valproic acid on olanzapine C/D ratio in the present study was comparable to the effect of cigarette smoking and should be considered clinically relevant. However, data on three major olanzapine metabolites did not explain the observed changes in olanzapine levels in valproic acid users, so the mechanism behind this interaction needs to be further investigated.

4 DISCUSSION

This thesis has established that smoking habits, age, gender and comedication with certain mood-stabilizing antiepileptic drugs are important factors for interindividual variability in the pharmacokinetics of olanzapine. Together these factors explain approximately one third of the interindividual variability in dose-adjusted serum concentrations (C/D ratios) of olanzapine in TDM samples. By correcting for the factors shown to be significant determinants of olanzapine pharmacokinetics, it could be possible to predict a 3-4 fold individual difference in C/D ratio.

The recommended therapeutic serum concentration range for olanzapine is 4-fold, i.e. 20-80 ng/ml (64-255 nmol/L).[84] By taking into account the factors evaluated as important determinants in the present thesis, it would be possible to individualize dosing of olanzapine to obtain safer and more effective treatment of many patients with schizophrenia or bipolar disorder.

The *UGT1A4*3* variant allele and comedication with ethinylestradiol-containing contraceptives were also possible factors of importance for interindividual variability in olanzapine pharmacokinetics. Although these factors significantly affected metabolic pathways of olanzapine, they were both shown *not* to be of importance for the variability of olanzapine C/D ratio in the present thesis.

4.1 Impact of age, gender and weight on olanzapine serum concentration

4.1.1 Age

In study III and IV, patients older than 60 years had significantly higher C/D ratios of olanzapine compared to younger patients, i.e. about 20% and 35%, respectively. Age has earlier been reported to positively correlate with olanzapine levels, e.g. Weiss and colleagues found a 9.4% increase in olanzapine C/D ratio per decade of life, while Gex Fabry et al. reported a 27% higher olanzapine serum concentrations in patients ≥60 years.[101;102] On the other hand, Skogh et al. did not find a relationship between age and olanzapine levels in a study based on TDM data.[94;101;102] Thus, there has been some uncertainty regarding the impact of age on serum concentration of olanzapine. However, the consistent findings in the present

studies (paper III and IV), including a large number of patients, strongly indicate that advanced age is associated with increased risk of olanzapine serum concentrations above the suggested therapeutic range. Although the use of antipsychotics in certain groups of elderly patients have been questioned, i.e. FDA has warned about increased mortality in elderly patients with dementia,[103] approximately 20% of the subjects included in study III and IV were older than 60 years.

The increased C/D ratio of olanzapine in patients older than 60 years is probably due to an age-related decline in physiological parameters, such as cardiac output (blood flow), liver and kidney function, rather than reduced activities of metabolic enzymes. This is supported by parallel increases in parent drug and metabolites in study III and IV. Increased levels of both parent compound and the measured metabolites in the elderly are also in line with a recent TDM study of several antidepressants.[104] This study concluded that patients above 65 years had 1.5-2-fold increased levels of antidepressants and metabolites.

4.1.2 Gender

The about 10-30% higher estimated C/D ratios of olanzapine in females than in males in study III and IV, is in line with earlier studies reporting 10-40% higher olanzapine concentrations in women compared to men.[90;94;96;102;105] A gender difference in olanzapine pharmacokinetics may potentially reflect differences in drug-metabolizing phenotypes between males and females, a hypothesis which is actualized by the apparent inhibition of CYP1A2 metabolism/activity by estrogens.[106] However, study IV also reported higher levels of all metabolites in women, significant for 10-*N*-glucuronide olanzapine and *N*-oxide olanzapine. This might indicate that further elimination of the metabolites is also impaired.

The effect of female gender on olanzapine serum concentration is in line with the earlier mentioned study of antidepressants, reporting 1.1-1.5-fold increased serum levels in women compared to men.[104] Thus, one might speculate if the higher C/D ratio in women compared to men share some of the same mechanisms as for increased age, e.g. lower blood flow to drug-eliminating organs. Moreover, gender differences in body composition may lead to higher volumes of distribution of lipophilic drugs in females than in males, which theoretically could result in higher steady-state trough levels of most psychotropic agents in female patients. Finally, gender differences in weight might also be of importance, but there is conflicting evidence regarding the impact of body weight on olanzapine levels.

4.1.3 Weight

In Study I, information about the patients' weight and height was also collected. We found no significant effects of these variables on olanzapine C/D ratio in this limited data material (n=40). In the subsequent studies (II-IV), height and weight were not included mainly because this kind of information is not available from the TDM requisition forms, which these latter studies were based on.

Regarding other studies investigating the impact of body weight on olanzapine pharmacokinetics, Patel et al. found a 3-4% higher absolute concentrations of olanzapine for every 10 kg patient weight above 80 kg.[105] On the contrary, in a study by Skogh et al., the body mass index (BMI) of the patients did not correlate with olanzapine C/D ratios. The latter observation is supported by data from the CATIE project where weight was not significantly associated with olanzapine clearance in a population pharmacokinetic model.[96]

4.2 Impact of lifestyle and dietary factors on olanzapine serum concentration

4.2.1 Cigarette smoking

Cigarette smokers have been reported to display about 40-65% lower serum concentrations of olanzapine compared to non-smokers.[91;105;107-109] The impact of cigarette smoking on serum concentration of olanzapine was therefore evaluated in all four studies of the present thesis. Study I and III found significantly lower olanzapine levels in cigarette smokers, i.e 52% and 55% lower C/D ratio compared to non-smokers, respectively. The differences in mean C/D ratios in smokers vs. non-smokers were somewhat lower in study II (-28%, data not presented in paper II) and study IV (-32%) probably because different patient populations were studied in II/IV and I/III.

In study I, no significant difference in C/D ratios of olanzapine and clozapine was found between light (7-12 cigarettes per day) and heavy smokers (>20 cigarettes per day). However, in paper IV, we speculated that less smoking among bipolar than schizophrenic patients might have been the reason to the lower observed effect of smoking on olanzapine C/D ratio in study II and IV compared to study I and III.

A weakness of study I was the limited number of patients in each subgroup, as well as the statistical analyses, which were based on univariate tests without adjustment for covariates (age, gender etc.). Results from other studies investigating the impact of various number of cigarettes on the in vivo metabolism of CYP1A2 substrates (caffeine, clozapine or olanzapine) are inconsistent to some degree. In a study with male Chinese patients, olanzapine AUC was on average 45% lower in light smokers (1-5 cigarettes) and 65% lower in heavy smokers (more than 5 cigarettes) compared to non-smokers. Light smokers had significantly higher AUC compared to heavy smokers ($p<0.05$).[107] Another study found a typical dose-response relationship between the number of cigarettes smoked per day and caffeine clearance.[110] In this study, including Caucasian subjects, subgroups of smokers had on average 1.22-fold (1-5 cigarettes daily; n=85), 1.47-fold (6-10 cigarettes; n=90), 1.66-fold (11-20 cigarettes; n=140) and 1.72-fold (>20 cigarettes, n=70) higher caffeine clearance compared to non-smokers (n=401).[110] These data suggest that there is a dose effect of number of daily cigarettes, which support the lower observed effect of smoking on olanzapine C/D ratio in study II and IV than in studies I and III.

4.2.2 Dietary factors

Different types of food and drinks have been reported to influence CYP1A2 activity, e.g. roasted, burned or grilled organic foods,[111;112] which may affect clearance of olanzapine. However, dietary factors are challenging to control for and information about dietary habits was not included in study I-IV. Thus, these are potential confounders in the present thesis, as smokers and non-smokers, or females and males, theoretically might have different dietary habits. Another aspect regarding dietary habits is that they may differ between various geographical populations. Along with genetic factors, potential differences in dietary habits need to be kept in mind when comparing studies in populations living in different parts of the world. This was illustrated by a study comparing CYP1A2 activity in Northern- and Southern European populations, i.e. Swedes and Serbs.[113] This showed a significantly lower CYP1A2 activity in the Serbs than in Swedes.[113] The roasting process of coffee is believed to produce PAHs and thereby induce CYP1A2 in heavy coffee drinkers.[114] Thus, a potential explanation for the differences in CYP1A2 activities observed between Swedes and Serbs, might be geographical variability in coffee consumption.

4.3 Effect of interacting drugs on olanzapine serum concentration

Patients treated with olanzapine are commonly comedicated with psychotropic drugs, e.g. mood-stabilizing AEDs, antidepressants and other antipsychotics. The potential for drug-drug interactions (DDIs) are therefore relevant for olanzapine users.

Recent findings suggest that at least 30-50% of bipolar and schizophrenic patients are treated with mood stabilizers.[115-117] Lithium is considered first line treatment for bipolar patients, but use of lamotrigine and valproic acid has increased markedly at the expense of lithium and carbamazepine during the last 15 years.[115] Mood stabilizers, in particular valproic acid, are also used in treatment of hostility and aggression in psychiatric patients.[118] The increased clinical use of valproic acid and lamotrigine in psychiatry is probably due to a limited risk of side effects and potential for DDIs. However, findings in study IV show that use of valproic acid in psychiatric patients implies an interaction potential with olanzapine. In quantitative terms, the change in serum concentration of olanzapine during concurrent use of valproic acid is comparable to the interaction effect reported in combination with CYP1A2 inducers or inhibitors.[119-122]

4.3.1 Valproic acid

Valproic acid is metabolised by several UGT enzymes, including UGT2B7 and UGT1A4.[123;124] Olanzapine and lamotrigine are also extensively metabolised by UGT1A4, but adding valproic acid to each of these drugs seems to have opposite effects on their respective serum concentrations. Comedication with valproic acid leads to a more than two-fold increase in lamotrigine levels,[125;126] probably due to inhibition of UGT-mediated metabolism of the latter. On the other hand, decreased serum concentration of olanzapine has been observed in combination with valproic acid,[127;128] which is in accordance with the significantly lower C/D ratio of olanzapine (-32%, $p<0.001$) reported in paper IV. However, the lowering of olanzapine was accompanied with a parallel and significant reduction in serum concentration of 10-*N*-glucuronide olanzapine (-26%, $p<0.001$). Thus, the latter actually suggests that valproic acid inhibits UGT1A4-mediated olanzapine metabolism, but that this mechanism is overruled by another interacting mechanism resulting in reduced serum concentration of olanzapine.

Regarding alternative and hitherto unknown interacting mechanism of valproic acid, it has been shown that valproic acid induces gene expression of MDR1 (*ABCB1*, P-glycoprotein) and CYP3A4.[129] CYP3A4 has been shown to be able to metabolize olanzapine, but the relative impact of this enzyme is believed to minor for its overall clearance.[45] Interestingly, however, a follow-up study of the CATIE trial significantly linked a SNP in *CYP3A43* gene (rs472660) to the olanzapine clearance by explaining 5-10% of the interindividual variability (p=5.9e-7).[93] Among the 25 tested SNPs, including several mutations in genes encoding enzymes involved in the metabolism of olanzapine, i.e. *CYP1A2*, *CYP2D6, CYP3A* and *FMO3*, the mentioned *CYP3A43* mutation was the only one that significantly predicted olanzapine clearance. This might suggest that CYP3A43, and potentially other isoforms within the CYP3A subfamily, is more important for olanzapine metabolism than earlier suggested.[45] Olanzapine is also shown to be a substrate for MDR1,[130] but interactions with MDR1 is believed only to cause minor changes in olanzapine serum levels.[131] The potential induction of valproic acid on MDR1 activity is therefore unlikely to explain the reduction in olanzapine concentration during concurrent use.

4.3.2 Oral contraceptives

Oral contraceptives containing estrogen

Oral contraceptives containing ethinyl estradiol (ECC) have been shown to increase serum concentration of the CYP1A2 probe tizanidine almost fourfold.[132] Study II investigated the effect of ECC on olanzapine and *N*-desmethyl olanzapine concentrations. A significantly reduced formation of the CYP1A2-mediated metabolite *N*-desmethyl olanzapine was found, but no effect on parent olanzapine concentration was observed. While ethinyl estradiol is shown to inhibit CYP1A2,[106] use of ethinyl estradiol also appears to induce glucuronidation activity, as reflected by reduced serum levels of the UGT substrate lamotrigine reported in multiple studies.[75;133-135] This 'dual mechanism' could have been the reason why the decrease in *N*-desmethyl olanzapine concentration was not accompanied by increased concentration of olanzapine in females using ECC (paper II). However, the lack of effect of ECC on serum concentration of olanzapine could also reflect that CYP1A2 plays a limited role in its overall clearance.

Determination of the UGT-mediated metabolite 10-*N*-glucuronide olanzapine could have ruled out this hypothetical dual interaction mechanism of ECC on olanzapine. Unfortunately, reference substance of this metabolite was unavailable at the time when study II was conducted. In any case, study II revealed that combined use of ECC does not produce a clinically relevant interaction with olanzapine, but confirmed the CYP1A2-inhibitory activity of ethinyl estradiol.

4.4 Effect of genetics on serum concentration of olanzapine

UGT1A4 142T>G

Several polymorphisms in the UGT1A4 gene have been described,[136] but most attention so far has been paid to the 142T>G (L48V) polymorphism representing the *UGT1A4*3* variant allele. There is conflicting evidence about the influence of *UGT1A4*3* on the serum concentration of olanzapine. Studies by Ghotbi et al. and Mao et al. conclude that the serum concentration of olanzapine is significantly reduced in patients being heterozygous carriers of *UGT1A4*3*.[61;62] However, this was not replicated in study III, including both heterozygous and homozygous *UGT1A4*3* carriers. Nozawa et al. have also studied the impact of *UGT1A4*3* on levels of olanzapine in Japanese patients.[108] This study only included seven heterozygous carriers of *UGT1A4*3*, but in accordance with findings of paper III, olanzapine concentration was not significantly different in patients with *UGT1A4*3* compared to patients without this variant allele.[108]

Among the studies which have investigated the influence of *UGT1A4*3* on serum concentration of olanzapine, the one presented in this thesis (paper III) is clearly the largest one (n=407). In study III, we managed to include 10 homozygous carriers of *UGT1A4*3*, whereas the other studies only included heterozygous carriers. The fact that no significant (or close to significant) reduction in olanzapine level was observed in patients homozygous for *UGT1A4*3*, strongly suggest that this variant allele is of no clinical relevance for the interindividual variability of olanzapine serum concentration.

Despite that *UGT1A4*3* appears to have no or limited impact on the serum concentration of olanzapine, it is little doubt that this mutation encodes increased glucuronidation of olanzapine. The substantially increased concentration of 10-*N*-glucuronide olanzapine observed in homozygous *UGT1A4*3* carriers, was supported by a significantly higher

intrinsic clearance of olanzapine by microsomes expressing UGT1A4.3 (paper III). The in vitro part of study III, showed that the increased glucuronidation activity was due to a lower affinity constant (Km value) of olanzapine to UGT1A4.3 compared to UGT1A4.1. Overall, it is therefore possible that *UGT1A4*3* could be an important factor for the interindividual pharmacokinetic variability of other UGT1A4 substrates than olanzapine. In addition to lamotrigine,[60] a recent publication reported a significant impact of *UGT1A4*3* on interindividual variability in serum concentrations of clozapine and *N*-desmethyl clozapine.[59]

Other polymorphisms

Several studies have investigated the effect of polymorphisms in various candidate genes on olanzapine concentrations, e.g. *CYP1A2*, *CYP2D6*, *MDR1 (ABCB1)* and *FMO3*.[56;61;93;109;137;138] Laika et al. recently found the *CYP1A2*1F* variant haplotype to be of importance for olanzapine serum concentrations, with a 22% lower observed dose-adjusted serum concentrations in homozygous *CYP1A2*1F* carriers compared to non-carriers of this haplotype.[92] However, the actual impact of *CYP1A2*1F* on CYP1A2 phenotype has been questioned,[56;61;114;139] and was therefore not included in the present thesis. With respect to *CYP2D6* genotype, previous studies by Carrillo et al. and Hägg et al. have concluded that variability in CYP2D6 metabolism is of no importance for olanzapine C/D ratio.[109;137] Still, we decided to include *CYP2D6* genotype as a covariate in study III, with the hypothesis that the relevance of CYP2D6 phenotype might depend on *UGT1A4* genotype. However, *CYP2D6* genotype turned out to be of no significance for olanzapine C/D ratio regardless of *UGT1A4* genotype.

Overall, most studies have failed to show an association between olanzapine pharmacokinetic and pharmacogenetic factors. An exception is *CYP3A43*,[93] and further studies should concentrate on the role of *CYP3A* variant alleles as potential determinants of olanzapine serum concentration.

4.5 Methodological considerations

There are methodological limitations in using TDM data as material for studying interindividual pharmacokinetic variability. A naturalistic setting involves less control with factors like adherence, comedication and environmental factors. The TDM requisition forms, which comprise the information source in studies III and IV, and partly study II, contain details regarding age, sex, comedication, smoking habits, daily dose, treatment duration, and time between last dose intake and sampling. In cases where information about the patient's dose, smoking habits and sampling time lacked, the respective patients were excluded. However, regarding other factors, such as comedication, it was not possible to assure that all relevant details were actually provided.

Variable conditions (temperature, light etc) with respect to storage and handling of serum samples between sampling at the physician's office and reception at the laboratory also represent potential limitations of TDM studies. However, the methods used for analyses of drug and metabolite concentrations are fully validated and certified in accordance with the ISO15189 standard. It should be mentioned though, that the validation of the methods is based on spiked serum samples from healthy individuals not using any drugs or herbal remedies. This is in contrast to the included patients, who often use other drugs, which theoretically may affect mass-spectrometric detection in terms of signal suppression etc.

The methodological limitations of TDM-based studies might be outweighed by inclusion of large data materials, as in the present thesis. Moreover, many view the application of naturalistic data as an advantage in projects aimed to identify significant determinants of individual variability. Due to the large degree of 'noise' in the data material, these studies decrease the risk of false positive findings (type 1 error).

The use of TDM data for research purposes is important in identification of factors causing pharmacokinetic variability in naturalistic settings. Pharmacokinetic studies are often performed in healthy volunteers, and in limited number of patients. Without having to expose patients to the burden of participating in a prospective clinical study, it is possible to enrol large number of patients to TDM studies.

5 CONCLUSION

The present work has determined the impact of several factors on the pharmacokinetic variability of olanzapine in psychiatric patients. Smoking habits, advanced age, female gender and comedication with valproic acid or carbamazepine were all found to be significantly associated with the dose-adjusted serum concentration of olanzapine. Together, these factors explained about one third of the overall variability. Thus, if they are taken into account as a basis for individualized dosing of olanzapine during treatment initiation, it will be possible to substantially reduce interpatient variability in olanzapine exposure. The potential clinical benefits of a more individualized dosing strategy, which implies that more patients will reach the target serum concentration range of olanzapine, are reduced frequencies of adverse effects and inadequate therapeutic response. This latter could be translated into better health for the patients and reduced health care costs for the society.

6 REFERENCES

(1) McGrath J, Saha S, Chant D, Welham J. Schizophrenia: a concise overview of incidence, prevalence, and mortality. *Epidemiol Rev* 2008;30:67-76.

(2) Keller WR, Fischer BA, Carpenter WT, Jr. Revisiting the diagnosis of schizophrenia: where have we been and where are we going? *CNS Neurosci Ther* 2011;17:83-88.

(3) van OJ, Rutten BP, Poulton R. Gene-environment interactions in schizophrenia: review of epidemiological findings and future directions. *Schizophr Bull* 2008;34:1066-1082.

(4) van OJ, Kapur S. Schizophrenia. *Lancet* 2009;374:635-645.

(5) Menezes NM, Milovan E. First-episode psychosis: a comparative review of diagnostic evolution and predictive variables in adolescents versus adults. *Can J Psychiatry* 2000;45:710-716.

(6) Picchioni MM, Murray RM. Schizophrenia. *BMJ* 2007;335:91-95.

(7) Lipkovich IA, Deberdt W, Csernansky JG et al. Defining "good" and "poor" outcomes in patients with schizophrenia or schizoaffective disorder: a multidimensional data-driven approach. *Psychiatry Res* 2009;170:161-167.

(8) Boden R, Haenni A, Lindstrom L, Sundstrom J. Biochemical risk factors for development of obesity in first-episode schizophrenia. *Schizophr Res* 2009;115:141-145.

(9) Leboyer M, Kupfer DJ. Bipolar disorder: new perspectives in health care and prevention. *J Clin Psychiatry* 2010;71:1689-1695.

(10) Lin PI, Mitchell BD. Approaches for unraveling the joint genetic determinants of schizophrenia and bipolar disorder. *Schizophr Bull* 2008;34:791-797.

(11) Leucht S, Tardy M, Komossa K, Heres S, Kissling W, Davis JM. Maintenance treatment with antipsychotic drugs for schizophrenia. *Cochrane Database Syst Rev* 2012;5:CD008016.

(12) Miyamoto S, Miyake N, Jarskog LF, Fleischhacker WW, Lieberman JA. Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. *Mol Psychiatry* 2012.

(13) Norwegian Institute of Public Health, Norwegian Prescription Database: Use of antipsychotics in Norway. http://www.norpd.no/. . 16-12-2011. 16-12-2011.
Ref Type: Online Source

(14) WHO DDD index 2012. http://www.whocc.no/atc_ddd_index/. . 25-5-2012.
Ref Type: Online Source

(15) Norwegian medicines agency SPC olanzapine. http://www.legemiddelverket.no/custom/Preparatsok/prepSearch____80333.aspx?mainSearch=N05ah03&onlyheading=. . 20-5-2012.
Ref Type: Online Source

(16) Citrome L. Olanzapine-fluoxetine combination for the treatment of bipolar depression. *Expert Opin Pharmacother* 2011;12:2751-2758.

(17) Leucht S, Corves C, Arbter D, Engel RR, Li C, Davis JM. Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. *Lancet* 2009;373:31-41.

(18) Citrome L. A systematic review of meta-analyses of the efficacy of oral atypical antipsychotics for the treatment of adult patients with schizophrenia. *Expert Opin Pharmacother* 2011.

(19) Lieberman JA, Stroup TS, McEvoy JP et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *N Engl J Med* 2005;353:1209-1223.

(20) Kassahun K, Mattiuz E, Nyhart E Jr et al. Disposition and biotransformation of the antipsychotic agent olanzapine in humans. *Drug Metab Dispos* 1997;25:81-93.

(21) Callaghan JT, Bergstrom RF, Ptak LR, Beasley CM. Olanzapine. Pharmacokinetic and pharmacodynamic profile. *Clin Pharmacokinet* 1999;37:177-193.

(22) Delco F, Tchambaz L, Schlienger R, Drewe J, Krahenbuhl S. Dose adjustment in patients with liver disease. *Drug Saf* 2005;28:529-545.

(23) Nelson D. http://drnelson.uthsc.edu/human.P450.table.html. . 5-6-2012.
Ref Type: Online Source

(24) Daly AK. Pharmacogenetics of the cytochromes P450. *Curr Top Med Chem* 2004;4:1733-1744.

(25) Fer M, Dreano Y, Lucas D et al. Metabolism of eicosapentaenoic and docosahexaenoic acids by recombinant human cytochromes P450. *Arch Biochem Biophys* 2008;471:116-125.

(26) Stingl JC, Brockmoller J, Viviani R. Genetic variability of drug-metabolizing enzymes: the dual impact on psychiatric therapy and regulation of brain function. *Mol Psychiatry* 2012.

(27) Rendic S, Guengerich FP. Update information on drug metabolism systems--2009, part II: summary of information on the effects of diseases and environmental factors on human cytochrome P450 (CYP) enzymes and transporters. *Curr Drug Metab* 2010;11:4-84.

(28) Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. *Pharmacol Ther* 2007;116:496-526.

(29) Meech R, Miners JO, Lewis BC, Mackenzie PI. The glycosidation of xenobiotics and endogenous compounds: versatility and redundancy in the UDP glycosyltransferase superfamily. *Pharmacol Ther* 2012;134:200-218.

(30) Mackenzie PI, Bock KW, Burchell B et al. Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. *Pharmacogenet Genomics* 2005;15:677-685.

(31) Ohno S, Nakajin S. Determination of mRNA expression of human UDP-glucuronosyltransferases and application for localization in various human tissues by real-time reverse transcriptase-polymerase chain reaction. *Drug Metab Dispos* 2009;37:32-40.

(32) Mackenzie PI, Rogers A, Treloar J, Jorgensen BR, Miners JO, Meech R. Identification of UDP glycosyltransferase 3A1 as a UDP N-acetylglucosaminyltransferase. *J Biol Chem* 2008;283:36205-36210.

(33) Kadakol A, Ghosh SS, Sappal BS, Sharma G, Chowdhury JR, Chowdhury NR. Genetic lesions of bilirubin uridine-diphosphoglucuronate glucuronosyltransferase (UGT1A1) causing Crigler-Najjar and Gilbert syndromes: correlation of genotype to phenotype. *Hum Mutat* 2000;16:297-306.

(34) Tukey RH, Strassburg CP. Human UDP-glucuronosyltransferases: metabolism, expression, and disease. *Annu Rev Pharmacol Toxicol* 2000;40:581-616.

(35) Anderson GD. Children versus adults: pharmacokinetic and adverse-effect differences. *Epilepsia* 2002;43 Suppl 3:53-59.

(36) Chen G, Blevins-Primeau AS, Dellinger RW, Muscat JE, Lazarus P. Glucuronidation of nicotine and cotinine by UGT2B10: loss of function by the UGT2B10 Codon 67 (Asp>Tyr) polymorphism. *Cancer Res* 2007;67:9024-9029.

(37) Kassahun K, Mattiuz E, Franklin R, Gillespie T. Olanzapine 10-N-glucuronide. A tertiary N-glucuronide unique to humans. *Drug Metab Dispos* 1998;26:848-855.

(38) Kaivosaari S, Finel M, Koskinen M. N-glucuronidation of drugs and other xenobiotics by human and animal UDP-glucuronosyltransferases. *Xenobiotica* 2011;41:652-669.

(39) Krueger SK, Williams DE. Mammalian flavin-containing monooxygenases: structure/function, genetic polymorphisms and role in drug metabolism. *Pharmacol Ther* 2005;106:357-387.

(40) Cashman JR, Zhang J. Human flavin-containing monooxygenases. *Annu Rev Pharmacol Toxicol* 2006;46:65-100.

(41) Zhang J, Cashman JR. Quantitative analysis of FMO gene mRNA levels in human tissues. *Drug Metab Dispos* 2006;34:19-26.

(42) Koukouritaki SB, Simpson P, Yeung CK, Rettie AE, Hines RN. Human hepatic flavin-containing monooxygenases 1 (FMO1) and 3 (FMO3) developmental expression. *Pediatr Res* 2002;51:236-243.

(43) Koukouritaki SB, Hines RN. Flavin-containing monooxygenase genetic polymorphism: impact on chemical metabolism and drug development. *Pharmacogenomics* 2005;6:807-822.

(44) Bickel MH. Liver metabolic reactions: tertiary amine N-dealkylation, tertiary amine N-oxidation, N-oxide reduction, and N-oxide N-dealkylation. I. Tricyclic tertiary amine drugs. *Arch Biochem Biophys* 1972;148:54-62.

(45) Ring BJ, Catlow J, Lindsay TJ et al. Identification of the human cytochromes P450 responsible for the in vitro formation of the major oxidative metabolites of the antipsychotic agent olanzapine. *J Pharmacol Exp Ther* 1996;276:658-666.

(46) Linnet K. Glucuronidation of olanzapine by cDNA-expressed human UDP-glucuronosyltransferases and human liver microsomes. *Hum Psychopharmacol* 2002;17:233-238.

(47) Erickson-Ridout KK, Zhu J, Lazarus P. Olanzapine metabolism and the significance of UGT1A448V and UGT2B1067Y variants. *Pharmacogenet Genomics* 2011;21:539-551.

(48) Desta Z, Zhao X, Shin JG, Flockhart DA. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. *Clin Pharmacokinet* 2002;41:913-958.

(49) Kirchheiner J, Seeringer A. Clinical implications of pharmacogenetics of cytochrome P450 drug metabolizing enzymes. *Biochim Biophys Acta* 2007;1770:489-494.

(50) Zhou SF. Polymorphism of human cytochrome P450 2D6 and its clinical significance: Part I. *Clin Pharmacokinet* 2009;48:689-723.

(51) Zhou SF. Polymorphism of human cytochrome P450 2D6 and its clinical significance: part II. *Clin Pharmacokinet* 2009;48:761-804.

(52) Sauer JM, Ponsler GD, Mattiuz EL et al. Disposition and metabolic fate of atomoxetine hydrochloride: the role of CYP2D6 in human disposition and metabolism. *Drug Metab Dispos* 2003;31:98-107.

(53) Mega JL, Close SL, Wiviott SD et al. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009;360:354-362.

(54) Gunes A, Dahl ML. Variation in CYP1A2 activity and its clinical implications: influence of environmental factors and genetic polymorphisms. *Pharmacogenomics* 2008;9:625-637.

(55) Seeringer A, Kirchheiner J. Pharmacogenetics-guided dose modifications of antidepressants. *Clin Lab Med* 2008;28:619-626.

(56) Kirchheiner J, Nickchen K, Bauer M et al. Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response. *Mol Psychiatry* 2004;9:442-473.

(57) Swen JJ, Nijenhuis M, de BA et al. Pharmacogenetics: from bench to byte--an update of guidelines. *Clin Pharmacol Ther* 2011;89:662-673.

(58) Amstutz U, Carleton BC. Pharmacogenetic testing: time for clinical practice guidelines. *Clin Pharmacol Ther* 2011;89:924-927.

(59) Erickson-Ridout KK, Sun D, Lazarus P. Glucuronidation of the second-generation antipsychotic clozapine and its active metabolite N-desmethylclozapine. Potential importance of the UGT1A1 A(TA)7TAA and UGT1A4 L48V polymorphisms. *Pharmacogenet Genomics* 2012.

(60) Gulcebi MI, Ozkaynakci A, Goren MZ, Aker RG, Ozkara C, Onat FY. The relationship between UGT1A4 polymorphism and serum concentration of lamotrigine in patients with epilepsy. *Epilepsy Res* 2011;95:1-8.

(61) Ghotbi R, Mannheimer B, Aklillu E et al. Carriers of the UGT1A4 142T>G gene variant are predisposed to reduced olanzapine exposure--an impact similar to male gender or smoking in schizophrenic patients. *Eur J Clin Pharmacol* 2010;66:465-474.

(62) Mao M, Skogh E, Scordo MG, Dahl ML. Interindividual variation in olanzapine concentration influenced by UGT1A4 L48V polymorphism in serum and upstream FMO polymorphisms in cerebrospinal fluid. *J Clin Psychopharmacol* 2012;32:287-289.

(63) Mori A, Maruo Y, Iwai M, Sato H, Takeuchi Y. UDP-glucuronosyltransferase 1A4 polymorphisms in a Japanese population and kinetics of clozapine glucuronidation. *Drug Metab Dispos* 2005;33:672-675.

(64) Wang YH, Trucksis M, McElwee JJ et al. UGT2B17 Genetic Polymorphisms Dramatically Affect the Pharmacokinetics of MK-7246 in Healthy Subjects in a First-in-Human Study. *Clin Pharmacol Ther* 2012.

(65) Guillemette C, Levesque E, Harvey M, Bellemare J, Menard V. UGT genomic diversity: beyond gene duplication. *Drug Metab Rev* 2010;42:24-44.

(66) Overby LH, Carver GC, Philpot RM. Quantitation and kinetic properties of hepatic microsomal and recombinant flavin-containing monooxygenases 3 and 5 from humans. *Chem Biol Interact* 1997;106:29-45.

(67) Cashman JR, Zhang J, Nelson MR, Braun A. Analysis of flavin-containing monooxygenase 3 genotype data in populations administered the anti-schizophrenia agent olanzapine. *Drug Metab Lett* 2008;2:100-114.

(68) de LJ, Santoro V, D'Arrigo C, Spina E. Interactions between antiepileptics and second-generation antipsychotics. *Expert Opin Drug Metab Toxicol* 2012;8:311-334.

(69) Linnet K, Olesen OV. Free and glucuronidated olanzapine serum concentrations in psychiatric patients: influence of carbamazepine comedication. *Ther Drug Monit* 2002;24:512-517.

(70) Zhou SF, Wang B, Yang LP, Liu JP. Structure, function, regulation and polymorphism and the clinical significance of human cytochrome P450 1A2. *Drug Metab Rev* 2010;42:268-354.

(71) Ehmer U, Vogel A, Schutte JK, Krone B, Manns MP, Strassburg CP. Variation of hepatic glucuronidation: Novel functional polymorphisms of the UDP-glucuronosyltransferase UGT1A4. *Hepatology* 2004;39:970-977.

(72) Zhou J, Argikar UA, Remmel RP. Functional analysis of UGT1A4(P24T) and UGT1A4(L48V) variant enzymes. *Pharmacogenomics* 2011;12:1671-1679.

(73) Court MH. Interindividual variability in hepatic drug glucuronidation: studies into the role of age, sex, enzyme inducers, and genetic polymorphism using the human liver bank as a model system. *Drug Metab Rev* 2010;42:209-224.

(74) Kiang TK, Ensom MH, Chang TK. UDP-glucuronosyltransferases and clinical drug-drug interactions. *Pharmacol Ther* 2005;106:97-132.

(75) Chen H, Yang K, Choi S, Fischer JH, Jeong H. Up-regulation of UDP-glucuronosyltransferase (UGT) 1A4 by 17beta-estradiol: a potential mechanism of increased lamotrigine elimination in pregnancy. *Drug Metab Dispos* 2009;37:1841-1847.

(76) Ma Q, Lu AY. Origins of individual variability in P4501A induction. *Chem Res Toxicol* 2003;16:249-260.

(77) Pal D, Mitra AK. MDR- and CYP3A4-mediated drug-herbal interactions. *Life Sci* 2006;78:2131-2145.

(78) Fujita K. Food-drug interactions via human cytochrome P450 3A (CYP3A). *Drug Metabol Drug Interact* 2004;20:195-217.

(79) Morgan ET, Goralski KB, Piquette-Miller M et al. Regulation of drug-metabolizing enzymes and transporters in infection, inflammation, and cancer. *Drug Metab Dispos* 2008;36:205-216.

(80) Patsalos PN, Berry DJ, Bourgeois BF et al. Antiepileptic drugs--best practice guidelines for therapeutic drug monitoring: a position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies. *Epilepsia* 2008;49:1239-1276.

(81) Alnaim L. Therapeutic drug monitoring of cancer chemotherapy. *J Oncol Pharm Pract* 2007;13:207-221.

(82) Arns W, Cibrik DM, Walker RG et al. Therapeutic drug monitoring of mycophenolic acid in solid organ transplant patients treated with mycophenolate mofetil: review of the literature. *Transplantation* 2006;82:1004-1012.

(83) Wijeratne C, Draper B. Reformulation of current recommendations for target serum lithium concentration according to clinical indication, age and physical comorbidity. *Aust N Z J Psychiatry* 2011;45:1026-1032.

(84) Hiemke C, Baumann P, Bergemann N et al. AGNP Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry: Update 2011. *Pharmacopsychiatry* 2011;44:195-235.

(85) Bengtsson F. Therapeutic drug monitoring of psychotropic drugs. TDM "nouveau". *Ther Drug Monit* 2004;26:145-151.

(86) Lesko LJ. The critical path of warfarin dosing: finding an optimal dosing strategy using pharmacogenetics. *Clin Pharmacol Ther* 2008;84:301-303.

(87) FDA Drug Safety Communication. http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm203888.htm. . 2012. 6-8-2012.
Ref Type: Online Source

(88) Melkersson KI, Dahl ML. Relationship between levels of insulin or triglycerides and serum concentrations of the atypical antipsychotics clozapine and olanzapine in patients on treatment with therapeutic doses. *Psychopharmacology (Berl)* 2003;170:157-166.

(89) Perry PJ, Lund BC, Sanger T, Beasley C. Olanzapine plasma concentrations and clinical response: acute phase results of the North American Olanzapine Trial. *J Clin Psychopharmacol* 2001;21:14-20.

(90) Perry PJ, Argo TR, Carnahan RM et al. The association of weight gain and olanzapine plasma concentrations. *J Clin Psychopharmacol* 2005;25:250-254.

(91) Zullino DF, Delessert D, Eap CB, Preisig M, Baumann P. Tobacco and cannabis smoking cessation can lead to intoxication with clozapine or olanzapine. *Int Clin Psychopharmacol* 2002;17:141-143.

(92) Laika B, Leucht S, Heres S, Schneider H, Steimer W. Pharmacogenetics and olanzapine treatment: CYP1A2*1F and serotonergic polymorphisms influence therapeutic outcome. *Pharmacogenomics J* 2010;10:20-29.

(93) Bigos KL, Bies RR, Pollock BG, Lowy JJ, Zhang F, Weinberger DR. Genetic variation in CYP3A43 explains racial difference in olanzapine clearance. *Mol Psychiatry* 2011;16:620-625.

(94) Skogh E, Reis M, Dahl ML, Lundmark J, Bengtsson F. Therapeutic drug monitoring data on olanzapine and its N-demethyl metabolite in the naturalistic clinical setting. *Ther Drug Monit* 2002;24:518-526.

(95) Olesen OV, Linnet K. Olanzapine serum concentrations in psychiatric patients given standard doses: the influence of comedication. *Ther Drug Monit* 1999;21:87-90.

(96) Bigos KL, Pollock BG, Coley KC et al. Sex, race, and smoking impact olanzapine exposure. *J Clin Pharmacol* 2008;48:157-165.

(97) Bachmann CJ, Haberhausen M, Heinzel-Gutenbrunner M, Remschmidt H, Theisen FM. Large intraindividual variability of olanzapine serum concentrations in adolescent patients. *Ther Drug Monit* 2008;30:108-112.

(98) Leucht S, Barnes TR, Kissling W, Engel RR, Correll C, Kane JM. Relapse prevention in schizophrenia with new-generation antipsychotics: a systematic review and exploratory meta-analysis of randomized, controlled trials. *Am J Psychiatry* 2003;160:1209-1222.

(99) Lowe EJ, Ackman ML. Impact of tobacco smoking cessation on stable clozapine or olanzapine treatment. *Ann Pharmacother* 2010;44:727-732.

(100) Ray WA, Meredith S, Thapa PB, Meador KG, Hall K, Murray KT. Antipsychotics and the risk of sudden cardiac death. *Arch Gen Psychiatry* 2001;58:1161-1167.

(101) Gex-Fabry M, Balant-Gorgia AE, Balant LP. Therapeutic drug monitoring of olanzapine: the combined effect of age, gender, smoking, and comedication. *Ther Drug Monit* 2003;25:46-53.

(102) Weiss U, Marksteiner J, Kemmler G, Saria A, Aichhorn W. Effects of age and sex on olanzapine plasma concentrations. *J Clin Psychopharmacol* 2005;25:570-574.

(103) FDA Public Health Advisory. http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/DrugSafetyInformationforHeathcareProfessionals/PublicHealthAdvisories/ucm053171.htm. . 4-11-2005.
Ref Type: Online Source

(104) Waade RB, Molden E, Refsum H, Hermann M. Serum concentrations of antidepressants in the elderly. *Ther Drug Monit* 2012;34:25-30.

(105) Patel MX, Bowskill S, Couchman L et al. Plasma olanzapine in relation to prescribed dose and other factors: data from a therapeutic drug monitoring service, 1999-2009. *J Clin Psychopharmacol* 2011;31:411-417.

(106) Pollock BG, Wylie M, Stack JA et al. Inhibition of caffeine metabolism by estrogen replacement therapy in postmenopausal women. *J Clin Pharmacol* 1999;39:936-940.

(107) Wu TH, Chiu CC, Shen WW et al. Pharmacokinetics of olanzapine in Chinese male schizophrenic patients with various smoking behaviors. *Prog Neuropsychopharmacol Biol Psychiatry* 2008;32:1889-1893.

(108) Nozawa M, Ohnuma T, Matsubara Y et al. The relationship between the response of clinical symptoms and plasma olanzapine concentration, based on pharmacogenetics: Juntendo University Schizophrenia Projects (JUSP). *Ther Drug Monit* 2008;30:35-40.

(109) Carrillo JA, Herraiz AG, Ramos SI, Gervasini G, Vizcaino S, Benitez J. Role of the smoking-induced cytochrome P450 (CYP)1A2 and polymorphic CYP2D6 in steady-state concentration of olanzapine. *J Clin Psychopharmacol* 2003;23:119-127.

(110) Tantcheva-Poor I, Zaigler M, Rietbrock S, Fuhr U. Estimation of cytochrome P-450 CYP1A2 activity in 863 healthy Caucasians using a saliva-based caffeine test. *Pharmacogenetics* 1999;9:131-144.

(111) Lam TK, Gallicchio L, Lindsley K et al. Cruciferous vegetable consumption and lung cancer risk: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2009;18:184-195.

(112) Fontana RJ, Lown KS, Paine MF et al. Effects of a chargrilled meat diet on expression of CYP3A, CYP1A, and P-glycoprotein levels in healthy volunteers. *Gastroenterology* 1999;117:89-98.

(113) Djordjevic N, Ghotbi R, Bertilsson L, Jankovic S, Aklillu E. Induction of CYP1A2 by heavy coffee consumption in Serbs and Swedes. *Eur J Clin Pharmacol* 2008;64:381-385.

(114) Djordjevic N, Ghotbi R, Jankovic S, Aklillu E. Induction of CYP1A2 by heavy coffee consumption is associated with the CYP1A2 -163C>A polymorphism. *Eur J Clin Pharmacol* 2010;66:697-703.

(115) Greil W, Haberle A, Haueis P, Grohmann R, Russmann S. Pharmacotherapeutic trends in 2231 psychiatric inpatients with bipolar depression from the International AMSP Project between 1994 and 2009. *J Affect Disord* 2012;136:534-542.

(116) Haw C, Stubbs J. A survey of the off-label use of mood stabilizers in a large psychiatric hospital. *J Psychopharmacol* 2005;19:402-407.

(117) Wolfsperger M, Greil W, Rossler W, Grohmann R. Pharmacological treatment of acute mania in psychiatric in-patients between 1994 and 2004. *J Affect Disord* 2007;99:9-17.

(118) Citrome L, Volavka J. Pharmacological management of acute and persistent aggression in forensic psychiatry settings. *CNS Drugs* 2011;25:1009-1021.

(119) Hiemke C, Peled A, Jabarin M et al. Fluvoxamine augmentation of olanzapine in chronic schizophrenia: pharmacokinetic interactions and clinical effects. *J Clin Psychopharmacol* 2002;22:502-506.

(120) Markowitz JS, DeVane CL. Suspected ciprofloxacin inhibition of olanzapine resulting in increased plasma concentration. *J Clin Psychopharmacol* 1999;19:289-291.

(121) Pelkonen O, Turpeinen M, Hakkola J, Honkakoski P, Hukkanen J, Raunio H. Inhibition and induction of human cytochrome P450 enzymes: current status. *Arch Toxicol* 2008;82:667-715.

(122) Weigmann H, Gerek S, Zeisig A, Muller M, Hartter S, Hiemke C. Fluvoxamine but not sertraline inhibits the metabolism of olanzapine: evidence from a therapeutic drug monitoring service. *Ther Drug Monit* 2001;23:410-413.

(123) Cohen AF, Land GS, Breimer DD, Yuen WC, Winton C, Peck AW. Lamotrigine, a new anticonvulsant: pharmacokinetics in normal humans. *Clin Pharmacol Ther* 1987;42:535-541.

(124) Perucca E. Pharmacological and therapeutic properties of valproate: a summary after 35 years of clinical experience. *CNS Drugs* 2002;16:695-714.

(125) Morris RG, Black AB, Lam E, Westley IS. Clinical study of lamotrigine and valproic acid in patients with epilepsy: using a drug interaction to advantage? *Ther Drug Monit* 2000;22:656-660.

(126) Weintraub D, Buchsbaum R, Resor SR, Jr., Hirsch LJ. Effect of antiepileptic drug comedication on lamotrigine clearance. *Arch Neurol* 2005;62:1432-1436.

(127) Bergemann N, Kress KR, Abu-Tair F, Frick A, Kopitz J. Valproate lowers plasma concentration of olanzapine. *J Clin Psychopharmacol* 2006;26:432-434.

(128) Spina E, D'Arrigo C, Santoro V et al. Effect of valproate on olanzapine plasma concentrations in patients with bipolar or schizoaffective disorder. *Ther Drug Monit* 2009;31:758-763.

(129) Cerveny L, Svecova L, Anzenbacherova E et al. Valproic acid induces CYP3A4 and MDR1 gene expression by activation of constitutive androstane receptor and pregnane X receptor pathways. *Drug Metab Dispos* 2007;35:1032-1041.

(130) Boulton DW, DeVane CL, Liston HL, Markowitz JS. In vitro P-glycoprotein affinity for atypical and conventional antipsychotics. *Life Sci* 2002;71:163-169.

(131) Lin YC, Ellingrod VL, Bishop JR, Miller DD. The relationship between P-glycoprotein (PGP) polymorphisms and response to olanzapine treatment in schizophrenia. *Ther Drug Monit* 2006;28:668-672.

(132) Granfors MT, Backman JT, Laitila J, Neuvonen PJ. Oral contraceptives containing ethinyl estradiol and gestodene markedly increase plasma concentrations and effects of tizanidine by inhibiting cytochrome P450 1A2. *Clin Pharmacol Ther* 2005;78:400-411.

(133) Harden CL, Herzog AG, Nikolov BG et al. Hormone replacement therapy in women with epilepsy: a randomized, double-blind, placebo-controlled study. *Epilepsia* 2006;47:1447-1451.

(134) Sabers A, Ohman I, Christensen J, Tomson T. Oral contraceptives reduce lamotrigine plasma levels. *Neurology* 2003;61:570-571.

(135) Reimers A, Helde G, Brodtkorb E. Ethinyl estradiol, not progestogens, reduces lamotrigine serum concentrations. *Epilepsia* 2005;46:1414-1417.

(136) Benoit-Biancamano MO, Adam JP, Bernard O et al. A pharmacogenetics study of the human glucuronosyltransferase UGT1A4. *Pharmacogenet Genomics* 2009.

(137) Hagg S, Spigset O, Lakso HA, Dahlqvist R. Olanzapine disposition in humans is unrelated to CYP1A2 and CYP2D6 phenotypes. *Eur J Clin Pharmacol* 2001;57:493-497.

(138) Skogh E, Sjodin I, Josefsson M, Dahl ML. High correlation between serum and cerebrospinal fluid olanzapine concentrations in patients with schizophrenia or schizoaffective disorder medicating with oral olanzapine as the only antipsychotic drug. *J Clin Psychopharmacol* 2011;31:4-9.

(139) Ghotbi R, Christensen M, Roh HK, Ingelman-Sundberg M, Aklillu E, Bertilsson L. Comparisons of CYP1A2 genetic polymorphisms, enzyme activity and the genotype-phenotype relationship in Swedes and Koreans. *Eur J Clin Pharmacol* 2007;63:537-546.

I

Paper I

The effect of variable cigarette consumption on the interaction with clozapine and olanzapine.

Eur J Clin Pharmacol. 2006 Dec;62(12):1049-53.

Paper II

The effect of ethinylestradiol-containing contraceptives on the serum concentration of olanzapine and *N*-desmethyl olanzapine.

Br J Clin Pharmacol. 2011 Apr;71(4):611-5.

II

Paper III

*UGT1A4*3* encodes significantly increased glucuronidation of olanzapine in patients on maintenancetreatment and in recombinant systems.

Clin Pharmacol Ther. 2012 2012 Aug;92(2):221-7

Paper III published Clin Pharmacol Ther. 2012 Aug; 92 (2): 221-7.

III

Paper IV

Valproic acid significantly lowers serum concentration of olanzapine – an interaction effect comparable to smoking.

Submitted Therapeutic Drug Monitoring, 04 May 2012

Paper IV accepted for publication

Update August 2012: Revised version accepted for publication in Therapeutic Drug Monitoring, 11 Jul 2012.

IV