Department of Clinical Pharmacology
University of Helsinki
Finland

STUDIES ON CYP2C8-MEDIATED DRUG INTERACTIONS

Aleksi Tornio

ACADEMIC DISSERTATION

To be presented, with the permission of the Medical Faculty of the University of Helsinki, for public examination in Lecture hall 2 of Biomedicum Helsinki, Haartmaninkatu 8, on August 29th, 2008, at 12 noon.

Helsinki 2008

Supervisors: Professor Pertti Neuvonen, MD
Department of Clinical Pharmacology
University of Helsinki
Helsinki, Finland

Docent Janne Backman, MD
Department of Clinical Pharmacology
University of Helsinki
Helsinki, Finland

Reviewers: Professor Risto Juvonen, PhD
Department of Pharmacology and Toxicology
University of Kuopio
Kuopio, Finland

Docent Kari Aranko, MD
Finnish Red Cross Blood Service
Helsinki, Finland

Opponent: Docent Kari Laine, MD
Department of Pharmacology, Drug Development and Therapeutics
University of Turku
Turku, Finland

ISBN 978-952-92-4126-2 (paperback)
ISBN 978-952-10-4769-5 (PDF, http://ethesis.helsinki.fi)
Helsinki 2008
Yliopistopaino

To my family

CONTENTS

ABBREVIATIONS **6**

LIST OF ORIGINAL PUBLICATIONS **8**

ABSTRACT **10**

INTRODUCTION **12**

REVIEW OF THE LITERATURE **14**

1. Drug-metabolising enzymes and transporters 14
   1.1. Principles of drug metabolism 14
   1.2. CYP enzymes and their nomenclature 14
   1.3. CYP2C8 15
      1.3.1. Substrates 15
      1.3.2. Inhibitors and inducers 16
      1.3.3. Pharmacogenetics 17
   1.4. Other CYP enzymes 18
      1.4.1. CYP1A subfamily 18
      1.4.2. CYP2A6 18
      1.4.3. CYP2B6 18
      1.4.4. CYP2C subfamily 18
      1.4.5. CYP2D6 19
      1.4.6. CYP2E1 19
      1.4.7. CYP3A subfamily 20
   1.5. Other phase I enzymes 20
   1.6. Conjugating enzymes 22
   1.7. Drug transporters 22
      1.7.1 Uptake transporters 23
      1.7.2. Efflux transporters 23
   1.8. Interaction mechanisms involving drug-metabolising enzymes and transporters 24
      1.8.1. Inhibition 24
      1.8.2. Induction 25
2. CYP2C8 substrates studied 26
   2.1. Loperamide 26
   2.2. Zopiclone 27
   2.3. Ibuprofen 28
   2.4. Pioglitazone 29
   2.5. Repaglinide 30
3. CYP inhibitors used in this study 32
   3.1. Gemfibrozil 32
   3.2. Itraconazole 33
   3.3. Trimethoprim 34

AIMS OF THE STUDY **35**

MATERIALS AND METHODS 36
1. Subjects........ 36
2. Study designs........ 36
3. Blood and urine sampling........ 37
4. *In vitro* studies........ 38
5. Determination of plasma and urine drug concentrations........ 38
6. Pharmacokinetic calculations........ 39
7. Pharmacodynamic measurements........ 40
   7.1. Psychomotor performance (Studies I and II)........ 40
   7.2. Blood glucose (Study V)........ 40
8. Genotyping........ 41
9. *In vitro-in vivo* correlations........ 41
10. Statistical analysis........ 42
11. Ethical considerations........ 42

RESULTS........ 43
1. Effects of gemfibrozil on CYP2C8 substrates (Studies I, II and III)........ 43
   1.1. Loperamide (Study I)........ 43
   1.2. Zopiclone (Study II)........ 44
   1.3. Ibuprofen (Study III)........ 44
2. Effects of trimethoprim and the *CYP2C8* genotype on pioglitazone (Study IV)........ 46
   2.1. Effect of trimethoprim........ 46
   2.2. Effect of the *CYP2C8*3* allele........ 47
3. Effect of dosing interval on gemfibrozil-repaglinide interaction (Study V)........ 48

DISCUSSION........ 50
1. Methodological considerations........ 50
   1.1. *In vitro* studies........ 50
   1.2. *In vivo* studies........ 51
2. Effect of gemfibrozil on loperamide, zopiclone and ibuprofen........ 52
3. Effects of trimethoprim and the *CYP2C8* genotype on pioglitazone........ 53
4. Effect of dosing interval on gemfibrozil-repaglinide interaction........ 54
5. General discussion........ 55

CONCLUSIONS........ 59

ACKNOWLEDGEMENTS........ 60

REFERENCES........ 62

ORIGINAL PUBLICATIONS........ 77

ABBREVIATIONS

Ae	amount excreted in urine
AhR	aryl hydrocarbon receptor
ANOVA	analysis of variance
Arnt	AhR nuclear translocator
AUC	area under the concentration-time curve
AUEC	area under the effect-time curve
BCRP	breast cancer resistance protein
BMI	body mass index
CAR	constitutive androstane receptor
Cl_{renal}	renal clearance
C_{max}	peak concentration
CoA	coenzyme A
COX	cyclooxygenase
COMT	catechol O-methyltransferase
CV	coefficient of variation
CYP	cytochrome P450
DNA	deoxyribonucleic acid
DSST	Digit Symbol Substitution Test
EDTA	ethylenediaminetetraacetic acid
FMO	flavin-containing monooxygenase
GABA	γ-aminobutyric acid
GST	glutathione transferase
HIV	human immunodeficiency virus
HLM	human liver microsomes
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HPLC	high-performance liquid chromatography
IC_{50}	inhibitor concentration producing 50% decrease in activity
k_e	elimination rate constant
k_f	apparent formation rate constant
K_i	inhibition constant
K_m	Michaelis-Menten constant
LC-MS-MS	liquid chromatography-tandem mass spectrometry
MAO	monoamine oxidase
mRNA	messenger ribonucleic acid
MRP	multidrug resistance-associated protein
NADPH	nicotinamide adenine dinucleotide phosphate
NAT	arylamine N-acetyltransferase
NSAID	non-steroidal anti-inflammatory drug
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OCT	organic cation transporter
OCTN	organic cation/carnitine transporter
PAH	polycyclic aromatic hydrocarbon

PCR	polymerase chain reaction
PEPT	peptide transporter
P-gp	P-glycoprotein (also MDR1)
pK_a	acid dissociation constant
PPAR	peroxisome proliferator activated receptor
PXR	pregnane X receptor
rhCYP	recombinant human CYP
RXR	retinoid X receptor
SD	standard deviation
SE	standard error
SNP	single nucleotide polymorphism
SSRI	selective serotonin reuptake inhibitor
SULT	sulfotransferase
$t_{½}$	elimination half-life
t_{max}	time to peak concentration
TPMT	thiopurine methyltransferase
UGT	UDP-glucuronosyltransferase
VAS	visual analogue scale
V_{max}	maximum reaction velocity

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which will be referred to in the text by the Roman numerals I to V.

I Niemi M, Tornio A, Pasanen MK, Fredrikson H, Neuvonen PJ, Backman JT. Itraconazole, gemfibrozil and their combination markedly raise the plasma concentrations of loperamide. *Eur J Clin Pharmacol* 2006;62:463-72.

II Tornio A, Neuvonen PJ, Backman JT. The CYP2C8 inhibitor gemfibrozil does not increase the plasma concentrations of zopiclone. *Eur J Clin Pharmacol* 2006;62:645-51.

III Tornio A, Niemi M, Neuvonen PJ, Backman JT. Stereoselective interaction between the CYP2C8 inhibitor gemfibrozil and racemic ibuprofen. *Eur J Clin Pharmacol* 2007;63:463-9.

IV Tornio A, Niemi M, Neuvonen PJ, Backman JT. Trimethoprim and the *CYP2C8*3* allele have opposite effects on the pharmacokinetics of pioglitazone. *Drug Metab Dispos* 2008;36:73-80.

V Tornio A, Niemi M, Neuvonen M, Laitila J, Kalliokoski A, Neuvonen PJ, Backman JT. The effect of gemfibrozil on repaglinide pharmacokinetics persists for at least 12 h after the dose: Evidence for mechanism-based inhibition of CYP2C8 *in vivo*. *Clin Pharmacol Ther* (in press).

ABSTRACT

Background and aims

Cytochrome P450 (CYP) 2C8 is involved in the metabolism of several clinically used drugs, including paclitaxel, repaglinide and rosiglitazone. Drug interactions caused by inhibition or induction of CYP2C8 can cause considerable variation in the effective exposure to its substrates. The aim of this work was to investigate the effect of model inhibitors of CYP2C8 on the pharmacokinetics of loperamide, zopiclone, ibuprofen and pioglitazone, in order to characterise the role of CYP2C8 in their metabolism. Gemfibrozil and trimethoprim were used as model inhibitors of CYP2C8. In addition, the effect of the *CYP2C8*3* allele on the pharmacokinetics of pioglitazone was investigated. Finally, the effect of dosing interval between gemfibrozil and repaglinide was studied in relation to the gemfibrozil-repaglinide interaction.

Methods

Studies I to V were randomised crossover studies with 2 to 5 phases. 10 to 16 healthy volunteers participated in each study. Pre-treatment with a clinically relevant dose of inhibitor (gemfibrozil, itraconazole or trimethoprim) was followed by a single oral dose of the study drug (loperamide, zopiclone, ibuprofen, pioglitazone or repaglinide). Thereafter, blood and urine samples were collected for the determination of drug concentrations. The pharmacodynamics of loperamide and zopiclone were assessed by psychomotor tests and subjective evaluations, and that of repaglinide by blood glucose measurements. Additionally, the metabolism of zopiclone and pioglitazone was studied *in vitro* in studies II and IV.

Results

Gemfibrozil, itraconazole and their combination raised the area under the concentration-time curve (AUC) of loperamide 2.2- ($P < 0.05$), 3.8- ($P < 0.001$) and 12.6-fold ($P < 0.001$), respectively, compared to placebo. Gemfibrozil had no effect on the pharmacokinetics of parent zopiclone. On the other hand, gemfibrozil raised the AUC of R-ibuprofen by 34% ($P < 0.001$) and increased its elimination half-life ($t_{½}$) from 2.9 to 4.5 hours ($P < 0.001$), with only minor effects on the S-enantiomer of ibuprofen. Trimethoprim raised the AUC of pioglitazone by 42% ($P < 0.001$) and prolonged its dominant $t_{½}$ from 3.9 to 5.1 hours ($P < 0.001$), but had no effect on its peak plasma concentration (C_{max}). The *CYP2C8*3* allele was associated with a decreased AUC of pioglitazone compared to the subjects with the reference genotype (*CYP2C8*1/*1*), and after correction for weight, this difference was statistically significant ($P < 0.05$). The gemfibrozil-repaglinide interaction persisted up to a 12 hour dosing interval between gemfibrozil and repaglinide. Gemfibrozil ingested simultaneously with or 3, 6, or 12 hours before repaglinide increased repaglinide $AUC_{0-\infty}$ 7.0-, 6.5-, 6.2- and 5.0-fold, respectively ($P < 0.001$), and the C_{max} of repaglinide increased about two-fold in all gemfibrozil phases ($P < 0.001$), compared to control. During repaglinide administration, the mean blood glucose concentration from 0 to 9 hours decreased in each of the gemfibrozil phases, compared to control ($P < 0.005$), whereas the pharmacodynamics of loperamide and zopiclone were not affected by the pre-treatment drugs.

In vitro, zopiclone (500 nM) elimination was not affected by the CYP2C8 inhibitors montelukast and gemfibrozil, but the CYP3A4 inhibitors itraconazole and ketoconazole markedly inhibited its elimination. Pioglitazone metabolite M-IV formation was inhibited by trimethoprim in pooled human liver microsomes (HLM) and recombinant human CYP2C8 (rhCYP2C8). At clinically relevant concentrations of pioglitazone, CYP2C8 was predominantly responsible for M-IV formation, whereas at higher concentrations the role of CYP3A4 increased.

Conclusions
These studies clarify the role of CYP2C8 in the metabolism of several drugs. The concentrations of loperamide and R-ibuprofen were found to be increased by the CYP2C8 inhibitor gemfibrozil, indicating that CYP2C8 participates in the metabolism of these drugs *in vivo*. On the other hand, the metabolism of zopiclone at clinically relevant concentrations was not affected *in vivo* or *in vitro* by CYP2C8 inhibition. Trimethoprim moderately raised the plasma concentrations of pioglitazone by inhibiting its CYP2C8-mediated biotransformation. In addition, the *CYP2C8*3* allele was associated with increased metabolic clearance of pioglitazone *in vivo*, which is in line with the results of pharmacogenetic studies on repaglinide and rosiglitazone. The inhibitory effect of gemfibrozil on CYP2C8 persists at least 12 hours, strongly suggesting that the main mechanism of the gemfibrozil-repaglinide interaction is irreversible mechanism-based inhibition of CYP2C8.

INTRODUCTION

Drug interactions can cause considerable variation in drug responses and increase the risk of adverse drug reactions. Many drug-drug interactions occur during metabolic processing, as most drugs need to be metabolised in order to be eliminated from the body. Cytochrome P450 (CYP) enzymes are of major importance in the biotransformation of a great number of drugs. The liver is the main organ of CYP-mediated metabolism (Meyer 1996). However, CYP enzymes are expressed in other tissues as well, and extrahepatic metabolism can be significant for many drugs (Krishna and Klotz 1994).

Drugs that inhibit CYP enzymes can affect the elimination of other drugs, increasing their plasma concentrations, and leading to enhanced effects or increased toxicity. In the case of prodrugs, which are activated by CYP-mediated metabolism, inhibition can lead to lower concentrations of the active compound and lack of efficacy (Lin and Lu 1998).

The importance of CYP2C8 in drug metabolism has only recently been recognised. To date, CYP2C8 has been found to play a major role in the metabolism of several drugs, including paclitaxel, cerivastatin, repaglinide, rosiglitazone and pioglitazone (Rahman et al. 1994, Wang et al. 2002b, Bidstrup et al. 2003, Baldwin et al. 1999, Jaakkola et al. 2006c). At first, however, the importance of CYP2C8 was greatly underestimated, in many cases based on *in vitro* studies, and only later drug interaction studies revealed the importance of CYP2C8 in the metabolism of drugs such as cerivastatin, repaglinide and pioglitazone (Backman et al. 2002, Niemi et al. 2003b, Jaakkola et al. 2005).

Many drugs, including loperamide, zopiclone and ibuprofen, have been reported to be metabolised by CYP2C8 *in vitro* (Kim et al. 2004, Becquemont et al. 1999, Hamman et al. 1997). However, no *in vivo* data has been available on the role of CYP2C8 in the metabolism of loperamide and zopiclone, and pharmacogenetic studies have provided only indirect evidence of the participation of CYP2C8 in the metabolism of ibuprofen (Totah and Rettie 2005). Pioglitazone is metabolised by CYP2C8 both *in vitro* and *in vivo*, but the effect of the model CYP2C8 inhibitor trimethoprim on the pharmacokinetics of pioglitazone has not been documented *in vivo*. In addition, several allelic variants of the *CYP2C8* gene have been recognised, but the evidence regarding the effect of the most common polymorphism leading to amino acid changes in Caucasians, *CYP2C8*3*, has been somewhat contradictory. As this polymorphism has been reported to cause both increased and decreased metabolic activity, it has not been clear how it affects the pharmacokinetics of different substrates of CYP2C8, e.g. pioglitazone.

Although the lipid-lowering drug gemfibrozil is only a weak inhibitor of CYP2C8 *in vitro*, it greatly increases the plasma concentrations of several drugs metabolised by CYP2C8. The *in vivo* inhibition of CYP2C8 by gemfibrozil has been proposed to be mediated by its glucuronide metabolite, which is a mechanism-based inhibitor of CYP2C8 (Ogilvie et al. 2006). However, the clinical relevance of the irreversible mechanism-based inhibition that has been observed *in vitro* with gemfibrozil glucuronide is not known. In addition, gemfibrozil can also inhibit other CYP enzymes and drug transporters, and the contribution of the different mechanisms to drug interactions caused by gemfibrozil is somewhat uncertain.

The purpose of this work was to investigate CYP2C8-mediated drug interactions both *in vitro* and *in vivo*. The effects of gemfibrozil on the pharmacokinetics of loperamide, zopiclone and ibuprofen, and the effect of trimethoprim on pioglitazone were studied. In addition, this work investigated the impact of the *CYP2C8*3* allele on pioglitazone pharmacokinetics and the effect of dosing interval on the gemfibrozil-repaglinide interaction.

REVIEW OF THE LITERATURE

1. Drug-metabolising enzymes and transporters

1.1. Principles of drug metabolism

Drugs are eliminated from the body via metabolism and excretion. Most drugs are lipophilic compounds, and thus in order to be excreted they must be biotransformed into more water soluble forms (Meyer 1996). The liver is the major organ of drug metabolism, but other organs, e.g. gastrointestinal tract, kidneys, lung and skin, can also contribute to drug metabolism (Krishna and Klotz 1994). When a drug is administered orally, it already begins to be biotransformed in the gastrointestinal tract by enterocytes, and in the liver after passing through the portal vein, before it enters systemic circulation. This phenomenon is called first-pass metabolism, and it can considerably decrease the oral bioavailability of many drugs (Shen et al. 1997).

Drug biotransformation has been traditionally classified into phase I functionalisation and phase II conjugation reactions. The phase I enzymes insert a functional group on their substrate, and the phase II enzymes conjugate their substrates with endogenous molecules. Most phase I metabolism reactions are catalysed by CYP enzymes in the endoplasmic reticulum. The resulting metabolites are usually inactive or less active than the parent drug. However, there are also examples of toxic metabolites, and metabolism-activated drugs (prodrugs) (Meyer 1996). The phase II enzymes, including for example UDP-glucuronosyltransferases (UGT), sulfotransferases (SULT) and glutathione transferases (GST), in general facilitate the excretion of their substrates by biotransforming them into more water-soluble forms, and they are located in both cytoplasm and endoplasmic reticulum (Cribb et al. 2005). Despite the nomenclature, phase II metabolism can occur on some unchanged drugs and phase I enzymes can also metabolise conjugated drugs (Josephy et al. 2005).

The inter-individual variability in drug disposition is high, and can be affected by environmental, genetic and disease determinants. A major part of the individual differences in drug response can be caused by drug interactions, including inhibition and induction, and genetic variability in drug-metabolising enzymes and transporters. (Wilkinson 2005, Ho and Kim 2005).

1.2. CYP enzymes and their nomenclature

The cytochrome P450 enzymes (CYP enzymes) are a superfamily of heme-containing monooxygenases (Wrighton and Stevens 1992). The name originates from their distinct peak absorption wavelength (450 nm) when the reduced form of the enzyme has bound carbon monoxide (Omura and Sato 1962).

The most common and well-known type of reaction catalysed by CYP enzymes is insertion of oxygen onto the substrate. The first step in the catalytical cycle is the binding of the substrate to the apoprotein moiety of the ferric (Fe^{3+}) haemoprotein. In the second step, this complex undergoes one-electron reduction to the ferrous (Fe^{2+}) state by accepting an

electron from nicotinamide adenine dinucleotide phosphate (NADPH) via the NADPH cytochrome P450 reductase. The third step is the binding of molecular oxygen to the ferrous ion of the complex, followed by a second one-electron reduction. After this, one atom of the oxygen is reduced to water, and the other atom is inserted into the substrate. Finally, the complex dissociates to the product (oxidised metabolite) and free enzyme to allow the catalytical cycle to be repeated (Sheweita 2000, Werck-Reichhart and Feyereisen 2000). The reactions catalysed by CYP enzymes include hydroxylation, epoxidation, dealkylation, dehalogenation, N- and S-oxidation, as well as oxidative deamination (Gibson and Skett 2001, Parkinson 2001).

Cytochrome P450s are named by a number indicating the gene family with >40% amino acid sequence identity (e.g. CYP3), a letter indicating the subfamily with >55% amino acid sequence identity (e.g. CYP3A), and a number for the gene (e.g. CYP3A4) (Nebert and Russell 2002). The names of CYP genes are presented in italics (e.g. *CYP2C8*), and the specific alleles are indicated with an asterisk followed by an Arabic numeral (e.g. *CYP2C8*3*). The name of the protein encoded by the gene is non-italicised, with a period between the gene product and number (e.g. CYP2C8.3). The reference sequence (i.e. **1*) is the first allele sequenced, and thus not necessarily the most common one in all ethnic populations (Ingelman-Sundberg et al. 2000). An up-to-date listing of the human CYP alleles can be found at http://www.cypalleles.ki.se.

In humans, 57 CYP enzymes have been identified. Of the 18 CYP families, the CYP1, CYP2 and CYP3 families predominantly participate in the metabolism of xenobiotics, whereas the other CYP families have mainly endogenous roles (Nebert and Russell 2002, Lewis 2004).

1.3. CYP2C8

The importance of CYP2C8 in drug metabolism has become recognised only recently partly due to a lack of diagnostic inhibitors (Totah and Rettie 2005). CYP2C8 accounts for about 6% of the total liver CYP content (Rowland-Yeo et al. 2003). In addition to hepatocytes, CYP2C8 protein has been detected in salivary ducts, intestine, kidney and adrenal cortical cells (Enayetallah et al. 2004). However, the protein expression of CYP2C8 in the intestine is low (Läpple et al. 2003). The crystal structure of the CYP2C8 enzyme has been determined to 2.7 Å resolution (Schoch et al. 2004). It has a relatively large active site cavity (1438 $Å^3$), similar in size to that of CYP3A4 (1386 $Å^3$), but the shapes of the cavities differ considerably (Yano et al. 2004). This provides an explanation for why CYP2C8 and CYP3A4 share many substrates, but often catalyse the formation of different metabolites.

1.3.1. Substrates

CYP2C8 plays a major role in the metabolism of several drugs, including amodiaquine, cerivastatin, paclitaxel, pioglitazone, repaglinide and rosiglitazone (Table 1) (Li et al. 2002, Wang et al. 2002b, Rahman et al. 1994, Jaakkola et al. 2006c, Bidstrup et al. 2003, Kajosaari et al. 2005a, Baldwin et al. 1999). In addition, certain endogenous agents such as arachidonic acid and retinoic acid can be metabolised by CYP2C8 (Rifkind et al. 1995, Nadin and Murray 1999). Notably, CYP2C8 shares a number of substrates with CYP3A4, but less with

other CYPs. In addition, CYP2C8 has been shown to metabolise some glucuronide conjugates of drugs, e.g. diclofenac acyl glucuronide (Kumar et al. 2002).

Table 1. Examples of CYP2C8 substrates, inhibitors and inducers.

Substrates	Reference	Inhibitors	Reference
Amiodarone (3A4)	Ohyama et al. 2000	Clotrimazole	Ong et al. 2000
Amodiaquine	Li et al. 2002	Gemfibrozil	Wang et al. 2002b
Arachidonic acid (1A2, 2C9, 2E1)	Rifkind et al. 1995	Ketoconazole	Ong et al. 2000
Buprenorfin (3A4)	Picard et al. 2005	Mometasone furoate	Walsky et al. 2005a
Carbamazepine (3A4)	Kerr et al. 1994	Montelukast	Walsky et al. 2005b
Cerivastatin (3A4)	Wang et al. 2002b	Trimethoprim	Wen et al. 2002
Chloroquine (3A4)	Projean et al. 2003a	Quercetin	Rahman et al. 1994
Diclofenac (2C9)	Bort et al. 1999	Zafirlukast	Walsky et al. 2005a
Ibuprofen (2C9)	Hamman et al. 1997		
Loperamide (3A4)	Kim et al. 2004	**Inducers**	
Methadone (3A4, 2D6)	Wang and DeVane 2003	Dexamethasone	Gerbal-Chaloin et al. 2001
Morphine (3A4)	Projean et al. 2003b	Phenobarbital	
Paclitaxel (3A4)	Rahman et al. 1994	Rifampicin	
Pioglitazone (3A4)	Jaakkola et al. 2006c		
Repaglinide (3A4)	Bidstrup et al. 2003		
Retinoic acid (2C9)	Nadin and Murray 1999		
Rosiglitazone (2C9)	Baldwin et al. 1999		
Simvastatin acid (3A4)	Prueksaritanont et al. 2003		
Troglitazone (3A4)	Yamazaki et al. 1999		
Zopiclone (3A4)	Becquemont et al. 1999		

Other CYP enzymes participating in the biotransformation of the substrates are given in parenthesis.

1.3.2. Inhibitors and inducers

The inhibitory potential of a large number of xenobiotics to CYP2C8 has been characterised *in vitro* (Table 1) (Ong et al. 2000, Walsky et al. 2005a, Tornio et al. 2005). Initially, quercetin was used as a diagnostic *in vitro* inhibitor despite its low selectivity. *In vitro*, trimethoprim and especially montelukast are selective inhibitors of CYP2C8 (Wen et al. 2002, Walsky et al. 2005b). *In vivo*, trimethoprim is a moderately potent inhibitor of CYP2C8 (Niemi et al. 2004b, Niemi et al. 2004a). However, montelukast has been found to have no effect on the CYP2C8 substrates repaglinide and pioglitazone *in vivo*, probably due to the extensive plasma protein binding of montelukast (Kajosaari et al. 2006b, Jaakkola et al. 2006b). Gemfibrozil is a strong *in vivo* inhibitor of CYP2C8 (Backman et al. 2002, Niemi et al. 2003a, Niemi et al. 2003b), which seems to be explained to a great extent by its 1-O-β-glucuronide metabolite, which is a mechanism-based inhibitor of CYP2C8 (Shitara et al. 2004, Ogilvie et al. 2006). Several other drugs, such as clotrimazole and mometasone furoate, are potent inhibitors of CYP2C8 *in vitro* (Walsky et al. 2005a). They are, however, topically applied drugs and thus unlikely to cause clinically relevant drug interactions, since their systemic concentrations are low. In addition, the CYP3A4 inhibitor ketoconazole has been shown to inhibit CYP2C8, and the inhibitory effect is observed at a concentration of 1 μM *in vitro* (Ong et al. 2000). *In vivo*, CYP2C8 is induced by rifampicin, and *in vitro* evidence suggests inducibility by phenobarbital and dexamethasone (Niemi et al. 2000,

Gerbal-Chaloin et al. 2001). In addition, CYP2C8 has been reported to be induced in human hepatocytes by fibrates, including fenofibric acid, clofibric acid and gemfibrozil (Prueksaritanont et al. 2005).

1.3.3. Pharmacogenetics

The *CYP2C8* gene spans a 31 kilobase region with 9 exons (Klose et al. 1999). To date, several nucleotide sequence variations have been identified in *CYP2C8*. *CYP2C8*3* (c.416G>A, p.Arg139Lys and c.1196A>G, p.Lys399Arg) is the most frequent variant allele changing the amino acid sequence of CYP2C8 in the Caucasian population, with an allele frequency of about 10-20%. On the other hand, the most common amino acid changing polymorphism in black populations is *CYP2C8*2* (c.805A>T, p.Ile269Phe), but it is very rare in Caucasians. The *CYP2C8*4* (c.792C>G, p.Ile264Met) allele is present, at least in Caucasian populations, with an allele frequency of about 8% (Totah and Rettie 2005). *In vitro* studies have suggested that CYP2C8.3 has reduced activity for metabolising paclitaxel, arachidonic acid and amodiaquine, but amidarone N-deethylation was not affected by this polymorphism (Table 2) (Dai et al. 2001, Soyama et al. 2001, Bahadur et al. 2002, Parikh et al. 2007, Soyama et al. 2002). *In vivo*, the *CYP2C8*3* allele has been associated with decreased plasma concentrations of repaglinide and rosiglitazone (Niemi et al. 2003c, Kirchheiner et al. 2006). However, no effect was evident on the pharmacokinetics of paclitaxel in relation to the *CYP2C8* genotype (Henningsson et al. 2005). On the other hand, the clearance of ibuprofen was markedly decreased in carriers of the *CYP2C8*3* allele (García-Martín et al. 2004). Thus, the general *in vivo* effect of the *CYP2C8*3* allele is unclear. Additionally, there is a strong linkage disequilibrium between *CYP2C8*3* and *CYP2C9*2* (c.430C>T, p.Arg144Cys) variant alleles, and more than 95% of individuals with the *CYP2C8*3* allele have also been reported to be carriers of the *CYP2C9*2* allele (Yasar et al. 2002). Recently, two novel haplotypes, characterised by non-coding single nucleotide polymorphisms (SNP) with the dbSNP identifiers rs7909236 and rs1113129/rs3216029, have been identified with frequencies of about 20% in Caucasians (Rodríguez-Antona et al. 2007). The former was associated with increased activity for metabolising paclitaxel *in vitro* and repaglinide *in vivo* and the latter with reduced activity both *in vitro* and *in vivo*. Due to the role of CYP2C8 in the biosynthesis of vasoactive substances from arachidonic acid, the *CYP2C8* genotype has been investigated as a risk factor for cardiovascular morbidity, but conclusive evidence for this is still lacking (Yasar et al. 2003, Lee et al. 2007).

Table 2. CYP2C8.3 activity toward substrates compared to CYP2C8.1.

Substrate	*In vitro*	*In vivo*	Reference
Amiodarone	↔		Soyama et al. 2002
Amodiaquine	↓		Parikh et al. 2007
Arachidonic acid	↓		Dai et al. 2001
Ibuprofen		↓	García-Martín et al. 2004, Martínez et al. 2005
Paclitaxel	↓	↔	Dai et al. 2001, Henningsson et al. 2005
Repaglinide		↑	Niemi et al. 2003c, Niemi et al. 2005b
Rosiglitazone		↑	Kirchheiner et al. 2006

↑ increased activity, ↓ decreased activity, ↔ no change in activity.

1.4. Other CYP enzymes

1.4.1. CYP1A subfamily

The CYP1A subfamily has two members: CYP1A1 and CYP1A2. CYP1A1 is mostly extrahepatic, and participates in the metabolism of endogenous substrates (Nebert and Russell 2002). On the other hand, CYP1A2 is mainly found in the liver, and accounts for 10-20% of the total CYP content in the liver (Rowland-Yeo et al. 2003, Klein et al. 2006). CYP1A2 metabolises several drugs, such as caffeine, theophylline, clozapine and tizanidine (Table 3) (Bertilsson et al. 1994, Pelkonen et al. 1998, Granfors et al. 2004a). CYP1A2 inhibitors include ciprofloxacin and fluvoxamine (Fuhr et al. 1992, Brøsen et al. 1993). Polyaromatic hydrocarbons (PAH), found for instance in cigarette smoke and grilled food, can induce the expression of CYP1A enzymes via the aryl hydrocarbon receptor (AhR) (Nebert and Russell 2002).

1.4.2. CYP2A6

CYP2A6 represents 1-10% of the total liver CYP content (Rowland-Yeo et al. 2003, Klein et al. 2006), and is involved in the metabolism of at least nicotine and coumarin. Methoxsalen and pilocarpine are potent inhibitors of CYP2A6 and it seems to be induced by phenobarbital (Kimonen et al. 1995, Pelkonen et al. 2000). The CYP2A6 enzyme is also polymorphic, and the *CYP2A6* genotype affecting nicotine metabolism can have an influence on smoking habits (Malaiyandi et al. 2005).

1.4.3. CYP2B6

CYP2B6 constitutes on average only about 3% of the total hepatic CYP content, but the inter-individual variability in its expression is very high (Rowland-Yeo et al. 2003), probably due to both genetic polymorphisms and inductive effects of various xenobiotics (Wang and Negishi 2003, Zanger et al. 2007). Until recently, it had been regarded to be of minor importance in drug metabolism. However, CYP2B6 has been found to play a major role in the metabolism of drugs such as bupropion, propofol and efavirenz (Table 3). Thiotepa, ticlopidine and clopidogrel are potent inhibitors of CYP2B6 (Turpeinen et al. 2006). CYP2B6 is also highly polymorphic, with functional consequences, e.g. altered efavirenz concentrations *in vivo* (Zanger et al. 2007).

1.4.4. CYP2C subfamily

In addition to CYP2C8, there are three other members of the CYP2C subfamily: CYP2C9, CYP2C18 and CYP2C19. These enzymes are of clinical importance, with the exception of CYP2C18, which has not been found to be expressed in any tissue (Table 3). The other CYP2C enzymes are expressed mainly in the liver, but also in the intestine (Obach et al. 2001, Läpple et al. 2003). All of the CYP2C enzymes are genetically polymorphic (Daly 2003).

CYP2C9 is the most abundant CYP2C isoform in both the liver (about 17% of the total liver CYP content) and intestine (Läpple et al. 2003). It metabolises a number of drugs, including S-warfarin, tolbutamide and several non-steroidal anti-inflammatory drugs (NSAIDs). Sulphaphenazole, amiodarone and several azole antifungals inhibit CYP2C9, and it is induced by rifampicin and barbiturates (Miners and Birkett 1998). The *CYP2C9* polymorphisms have clinical implications e.g. in warfarin treatment (Aithal et al. 1999, Wadelius and Pirmohamed 2007).

CYP2C19, constituting about 3% of the total hepatic CYP content (Rowland-Yeo et al. 2003), metabolises proton pump inhibitors, diazepam, citalopram and tricyclic antidepressants. A prototypical substrate for CYP2C19 is S-mephenytoin, and omeprazole is its inhibitor both *in vitro* and *in vivo*, but is rather unselective. Up to 6% of Caucasians are poor metabolisers of substrates of CYP2C19 (Desta et al. 2002). The *CYP2C19* genotype can affect, for example, the efficacy of proton pump inhibitor treatment (Furuta et al. 2004).

1.4.5. CYP2D6

The relative CYP2D6 content in the liver is only about 4%, but it has been estimated to be involved in the metabolism of up to 25% of all drugs (Zanger et al. 2001, Ingelman-Sundberg 2005). Most CYP2D6 substrates are distinct in that they have a basic nitrogen located close to their site of oxidation (Zanger et al. 2004). Its substrates include for example several beta-blockers, tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), antipsychotics and other drugs such as codeine and dextromethorphan (Table 3) (Pelkonen et al. 1998). Many drugs, including quinidine, flecainide, paroxetine, moclobemide and fluoxetine, inhibit CYP2D6 (Zanger et al. 2004). In contrast to other drug-metabolising CYP enzymes, CYP2D6 is not known to be inducible (Ingelman-Sundberg 2005). CYP2D6 exhibits considerable genetic variation, ranging from no expression to multiple copies of functional *CYP2D6*. Individuals can be divided into poor, intermediate, extensive and ultrarapid metabolisers based on their metabolic capacity (Zanger et al. 2004).

1.4.6. CYP2E1

Although CYP2E1 corresponds to about 15% of the total CYP content in the liver (Rowland-Yeo et al. 2003, Klein et al. 2006), it participates in the metabolism of relatively few drugs. Its substrates include paracetamol, chlorzoxazone and several halogenated anaesthetics such as halothane, enflurane and sevoflurane (Tanaka et al. 2000). However, it is of major toxicological relevance, as it is involved in the formation of many reactive metabolites (e.g. the hepatotoxic metabolite of paracetamol) and carcinogens (Tanaka et al. 2000). Ethanol is also a substrate and inducer of this enzyme (Pelkonen et al. 1998).

1.4.7. CYP3A subfamily

The CYP3A subfamily consists of CYP3A4, CYP3A5, CYP3A7 and CYP3A43, accounting for about 30% of the total liver CYP content (Rowland-Yeo et al. 2003, Klein et al. 2006). The CYP3A subfamily has been estimated to be involved in the metabolism of over 50% of all drugs (Pelkonen et al. 1998, Wrighton et al. 2000, Gonzalez and Tukey 2006).

CYP3A4 is the most important drug-metabolising enzyme in humans. It is also the most abundant CYP enzyme in both the intestine and the liver (Zhang et al. 1999, Rowland-Yeo et al. 2003). Its substrates include atorvastatin, simvastatin, midazolam, triazolam and several calcium channel blockers (Table 3) (Dresser et al. 2000). Inhibitors of CYP3A4 include erythromycin, itraconazole, ketoconazole, grapefruit juice and human immunodeficiency virus (HIV) protease inhibitors (Dresser et al. 2000). CYP3A4 is induced by several drugs, such as carbamazepine, phenytoin, rifampicin and St John's wort (*Hypericum perforatum*) (Backman et al. 1996a, Backman et al. 1996b, Dürr et al. 2000). There is a high degree of inter-individual variability in CYP3A4 activity. A number of variant *CYP3A4* alleles have been found, but their frequencies are low, and they do not adequately explain the variability in CYP3A4 activity (Lamba et al. 2002).

CYP3A5 has a similar spectrum of substrates as CYP3A4. However, there appear to be certain differences in affinity for particular substrates between the two enzymes (Wrighton et al. 1990). CYP3A5 is genetically polymorphic, and is expressed in only about 20% of adult livers in Caucasians. CYP3A7 is the predominant CYP isoform in the foetal liver, but it is also expressed in some adult livers. A fourth member of the CYP3A subfamily has been identified, CYP3A43, which in addition to the liver is expressed in the prostate and testis, but its function is currently not known (Daly 2006).

1.5. Other phase I enzymes

In addition to CYP enzymes, phase I metabolism of xenobiotics can be catalysed by other drug-metabolising enzymes. Their major difference compared to CYP enzymes is that their catalytic action is more limited with respect to structure of substrates, and they typically catalyse only a single type of reaction. Among the most important of these non-CYP enzymes are flavin-containing monooxygenases (FMOs), monoamine oxidases (MAOs), alcohol and aldehyde dehydrogenases, and xanthine oxidase. FMOs have overlapping substrate specificity with CYP enzymes, and they can metabolise e.g. chlorpromazine, nortriptyline and verapamil (Beedham 1997). MAOs can metabolise both exogenous monoamines and endogenous monoamine neurotransmitters, and MAO-inhibiting drugs can be used in the treatment of depression and neurodegenerative conditions such as Parkinson's disease (Youdim and Bakhle 2006). Alcohol dehydrogenase is the major metaboliser of ethanol, producing acetaldehyde, which is further metabolised by aldehyde dehydrogenase. Xanthine oxidase metabolises xanthine-containing drugs, such as caffeine and theophylline, and purine analogues (Gibson and Skett 2001). In addition, several esterases can play a role in the biotransformation of different drugs. For example, carboxylesterases play a major role in the metabolism of several ester-containing drugs and prodrugs (Imai et al. 2006).

Table 3. Examples of substrates, inhibitors and inducers of some drug-metabolising CYP enzymes.

CYP1A2	CYP2B6	CYP2C9	CYP2C19	CYP2D6	CYP3A4
			SUBSTRATES		
Caffeine[1] Clozapine[2] Rofecoxib[3] Theophylline[1] Tizanidine[4]	Bupropion[6] Cyclophosphamide[6] Efavirenz[6] Pethidine[6] Propofol[6]	Diclofenac[7] Ibuprofen[7] Losartan[7] Phenytoin[7] S-warfarin[7] Tolbutamide[7]	Citalopram[10] Diazepam[10] Proguanil[10] Proton pump inhibitors[10] S-mephenytoin[10]	Amitriptyline[11] Codeine[11] Fluoxetine[11] Fluvoxamine[11] Haloperidol[11] Risperidone[11]	Atorvastatin[13] Cyclosporine[13] Felodipine[13] HIV protease inhibitors[13] Midazolam[13] Simvastatin[13] Triazolam[13] Verapamil[13]
			INHIBITORS		
Ciprofloxacin[5] Fluvoxamine[1] Furafylline[1]	Clopidogrel[6] Thiotepa[6] Ticlopidine[6]	Amiodarone[7] Fluconazole[7] Sulphaphenazole[7] Sulphamethoxazole[7] Voriconazole[8]	Fluvoxamine[10] Omeprazole[10]	Flecainide[11] Fluoxetine[11] Moclobemide[11] Paroxetine[11] Quinidine[11] Terbinafine[12]	Clarithromycin[13] Erythromycin[13] Grapefruit juice[13] HIV protease inhibitors[13] Ketoconazole[13] Itraconazole[13] Voriconazole[8]
			INDUCERS		
Tobacco smoke[1]	Rifampicin[6] Phenobarbital[6]	Rifampicin[9] Phenobarbital[9]	Rifampicin[9] Phenobarbital[9]	Not known[11]	Carbamazepine[1] Phenytoin[1] Rifampicin[1]

[1]Pelkonen et al. 1998, [2]Bertilsson et al. 1994, [3]Karjalainen et al. 2006, [4]Granfors et al. 2004a, [5]Fuhr et al. 1992, [6]Turpeinen et al. 2006, [7]Miners and Birkett 1998, [8]Theuretzbacher et al. 2006, [9]Gerbal-Chaloin et al. 2001, [10]Desta et al. 2002, [11]Zanger et al. 2001, [12]Abdel-Rahman et al. 1999, [13]Dresser et al. 2000.

1.6. Conjugating enzymes

Conjugating enzymes attach additional molecules to the nucleophilic or electrophilic sites of their substrates. Conjugation can occur on functional groups already present on the parent compound or on groups added by e.g. CYP enzymes. Conjugation reactions generally lead to more water-soluble products which can be readily excreted in bile or urine (Meyer 1996, Gibson and Skett 2001).

Glucuronidation is the most common form of conjugation for drugs and endogenous compounds. UDP-glucuronosyltransferases are a superfamily of enzymes that catalyse the conjugation of glucuronic acid to their substrates. There are three subfamilies of UGTs in humans: UGT1A, UGT2A and UGT2B. The UGT isoforms are variably expressed in many tissues, including the liver, intestine and kidney, and they are located in the endoplasmic reticulum of the cell. The substrate specificity of UGTs is broad and overlapping, and includes endogenous steroids, thyroid hormone, bilirubin and retinoic acid, in addition to xenobiotics (Fisher et al. 2001).

Cytosolic sulfotransferases metabolise a large number of xenobiotics and small endogenous compounds such as steroids, bile acids and neurotransmitters. SULTs insert a sulfonate group onto their substrates, with a large overlap in specificity with UGTs. Three SULT families, SULT1, SULT2 and SULT4 have been identified in humans (Gamage et al. 2006).

Methyltransferases are important in the metabolism of many endogenous compounds and various drugs. Examples of methyltransferases are thiopurine methyltransferase (TPMT) which metabolises e.g. 6-mercaptopurine, and catechol O-methyltransferase (COMT) which metabolises catecholamine neurotransmitters as well as catechol drugs such as L-dopa and methyldopa (Weinshilboum et al. 1999).

Arylamine N-acetyltransferases (NATs) catalyse the conjugation of an acetyl group from acetyl coenzyme A (CoA) to their substrates. In humans, there are two NAT isoforms, NAT1 and NAT2. Acetylation is important in the metabolism of aromatic and heterocyclic amine drugs (Westwood et al. 2006).

Glutathione conjugation is an important mechanism in detoxification of electrophilic xenobiotics and inactivation of endogenous metabolites during oxidative stress. Cytosolic glutathione transferases insert a glutathione group onto substrates, resulting in water-soluble metabolites that are either transported out of the cell or metabolised further (Hayes et al. 2005).

1.7. Drug transporters

The importance of transporters in the pharmacokinetics of drugs has been increasingly recognised in recent years. In addition to the physiochemical properties of a drug, such as the acid dissociation constant (pK_a), size and lipophilicity, transporter-mediated processes play an important role in drug disposition. There are two types of drug transporters, uptake and efflux transporters, and they can affect the extent and direction of drug movement across organs such as the liver, kidney and brain. In addition to facilitating drug disposition,

transporters have important roles in transporting endogenous substrates such as sugars, lipids, amino acids, bile acids, steroids and hormones (Ho and Kim 2005, Shitara et al. 2006).

1.7.1 Uptake transporters

Uptake transporters act by transporting drugs into cells. This class of transporters includes the organic anion transporting polypeptide (OATP), organic anion transporter (OAT), organic cation transporter (OCT), organic cation/carnitine transporter (OCTN) and peptide transporter (PEPT) families (Ho and Kim 2005).

The OATPs are expressed in the intestine, liver, kidney and brain. Humans have 11 OATPs, of which OATP1B1, OATP1B3 and OATP2B1 are expressed in the sinusoidal membrane of hepatocytes, and participate in the hepatic uptake of drugs and endogenous substrates. These three transporters have overlapping selectivity with several clinically important substrates, such as fexofenadine, methotrexate and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins). OATPs can be inhibited by, for example, cyclosporine, rifampicin, gemfibrozil and macrolides (Niemi 2007). Numerous genetic polymorphisms in these transporters have been found (König et al. 2006), and for example the genetic variability in *SLCO1B1* (the gene encoding OATP1B1) has been associated with changes in the pharmacokinetics of several statins (Niemi et al. 2006, Pasanen et al. 2006, Pasanen et al. 2007).

OATs are expressed at least in the kidney, brain and placenta. Four members of this transporter family have been identified, and their substrates include several NSAIDs, para-aminohippuric acid, cimetidine and tetracycline (You 2004, Ho and Kim 2005). OCTs and OCTNs transport organic cations such as endogenous amines and several drugs. Their messenger ribonucleic acid (mRNA) has been found in the kidney, liver, intestine, brain and placenta (You 2004). Organic anion and organic cation transport systems play a major role in the renal elimination of many drugs (Dresser et al. 2001).

1.7.2. Efflux transporters

Efflux transporters export drugs from the cell, often against pronounced concentration gradients. They can limit entry of drugs into the cell or facilitate their removal from the cell. The best known drug efflux transporter is the P-glycoprotein (P-gp, MDR1). Other efflux transporters include the multidrug resistance-associated protein (MRP) family and the breast cancer resistance protein (BCRP) (Ho and Kim 2005, Fischer et al. 2005).

P-gp can affect the absorption of drugs in the intestine, drug distribution across the blood-brain barrier and placenta, and biliary and renal excretion of drugs. P-gp has a large number of substrates, including cyclosporine, digoxin, diltiazem, loperamide, paclitaxel, verapamil and HIV protease inhibitors. Many, but not all, of the substrates of P-gp are also substrates for CYP3A4. P-gp is inhibited by cyclosporine, erythromycin, itraconazole and quinidine. P-gp is also inducible, and induction by rifampicin and St John's Wort has been reported (Kim 2002, Lin and Yamazaki 2003).

MRPs are located in the intestine, brain, kidney and liver. They are involved in the transport of bile salts, steroid hormones, and both unconjugated and conjugated drugs. MRP1, which is located in various tissues, and MRP3, which is mainly expressed in the liver, transport their substrates into the bloodstream. Their substrates include methotrexate, etoposide and vincristine. On the other hand, MRP2 is found in the liver, kidney and intestine, and has important roles in biliary and renal excretion of endogenous and xenobiotic substrates. MRP2 has been shown to transport vinca alkaloids, HIV-protease inhibitors, pravastatin, methotrexate and cisplatin. BCRP is expressed in the intestine, liver and placenta, and its substrates include doxorubicin, topotecan and zidovudine (Chan et al. 2004, Fischer et al. 2005, Ho and Kim 2005).

1.8. Interaction mechanisms involving drug-metabolising enzymes and transporters

Inhibition of a drug-metabolising enzyme can result in decreased clearance and increased plasma concentrations of its substrate drug, and concurrent elevated pharmacological effects or increased toxicity. On the other hand, induction increases the metabolic capacity of the affected enzyme. Thus, induction leads to decreased plasma concentrations of the substrate drug, and often reduced pharmacological activity (Lin and Lu 1998). Inhibition of drug transporters in the liver, intestine or kidney can cause similar changes, but the effect of inhibition varies, largely depending on the type (uptake or efflux) and the localisation of the transporter inhibited. In addition, transporter inhibition can affect the tissue distribution of drugs, e.g. it can decrease the concentration in the liver. However, interactions involving drug transporters are still poorly characterised (Ho and Kim 2005).

1.8.1. Inhibition

The mechanisms of enzyme inhibition can be classified roughly into three categories: reversible, quasi-irreversible and irreversible inhibition. Reversible inhibition is further categorised into competitive, uncompetitive and mixed type inhibition. In competitive inhibition, the binding of the inhibitor to the enzyme prevents the binding of the substrate to the active site of the enzyme. It is believed that inhibitors of this kind share some structural similarity with the substrates of the inhibited enzyme. Indeed, some inhibitors are also substrates of the inhibited enzyme, but this is not always the case. On the other hand, uncompetitive inhibition is characterised by the binding of the inhibitor to the enzyme-substrate complex, which results in a non-productive complex. However, in practice, mixed type (competitive-uncompetitive) inhibition is commonly observed in which an inhibitor can bind both to the free enzyme and to the enzyme-substrate complex. A special case of mixed type inhibition is noncompetitive inhibition in which the inhibitor does not bind to the active site of the enzyme and has no effect on substrate binding, but the enzyme-substrate-inhibitor complex is unable to function catalytically (Hollenberg 2002, Madan et al. 2002).

Irreversible inhibition is characterised by the formation of tightly bound inhibitory complexes. The distinction between quasi-irreversible and irreversible inhibition is that quasi-irreversible inhibitors can be removed from the enzyme under specific experimental conditions, but for irreversible inhibitors this is not possible. Irreversible inhibition requires

metabolic activation by the enzyme, and is thus often called mechanism-based or suicide inhibition. As the inhibition is permanent, new enzyme must be formed to regain the catalytical function. In general, mechanism-based inhibition is considered rare, but in the case of CYP enzymes it is relatively common, presumably due to the formation of reactive intermediates during the oxidative process (Hollenberg 2002, Ghanbari et al. 2006). Compared to reversible inhibition, which is only concentration-dependent, mechanism-based inhibition is both concentration and time-dependent, and it can be studied by pre-incubating the inhibitor prior to incubation with the substrate of the index reaction in the *in vitro* assay (Venkatakrishnan and Obach 2007). Examples of mechanism-based CYP inhibitors are rofecoxib for CYP1A2, clopidogrel and ticlopidine for CYP2B6, and erythromycin for CYP3A4 (Karjalainen et al. 2006, Richter et al. 2004, Turpeinen et al. 2005, Periti et al. 1992).

Although inhibition of drug transporters is a recognised cause of many drug-drug interactions, the mechanisms of transporter inhibition are not understood in detail (Ho and Kim 2005). The inhibition of transporters is a complex process and can involve multiple mechanisms. For example, different drugs can inhibit P-gp by competing for the drug binding sites of the transporter, by blocking the ATP hydrolysis process, or by both of these mechanisms (Lin and Yamazaki 2003). In addition, studying transporter inhibition is more complicated than studying metabolism, because cell-based systems are required and passive transport must be taken into account (Ho and Kim 2005).

1.8.2. Induction

The net effect of enzyme induction is an increase in the protein levels. Induction of drug-metabolising enzymes is mediated for the most part by intracellular receptors. The most important of these receptors are Ah receptor, pregnane X receptor (PXR) and constitutive androstane receptor (CAR) (Fuhr 2000).

The cytosolic Ah receptor binds to the inducing agent, and forms a heterodimer with the Ah receptor nuclear translocator (Arnt). The CYP1A subfamily, including the clinically important CYP1A2, is induced via this mechanism, but other enzymes, such as certain GSTs and UGTs, can also be affected (Dickins 2004).

The nuclear receptors PXR and CAR mediate the induction of members of the CYP2 and CYP3 families. They must first bind to the inducing ligand, and thereafter form a heterodimer with the retinoid X receptor (RXR), which in turn binds to specific deoxyribonucleic acid (DNA) sequences resulting in increased expression (Willson and Kliewer 2002). Induction via PXR is caused for example by rifampicin and hyperforin (the principal constituent in St John’s wort), whereas phenobarbital, phenytoin and carbamazepine are activators of CAR (Fuhr 2000). In addition to CYP enzymes, many drug-conjugating enzymes and drug transporters can also be affected by this type of induction (Tompkins and Wallace 2007). For example, P-gp is induced by rifampicin via PXR (Geick et al. 2001).

A distinct type of induction is the ability of ethanol to increase CYP2E1 levels by binding to the active site of the enzyme itself, which leads to the stabilisation of the enzyme protein.

However, in this case the induction coincides with inhibition, and the induction is only evident for a short period of time after the exposure to the inducer has stopped. Ethanol-type induction of CYP2E1 also occurs with many other organic solvents (e.g. acetone and benzene) and isoniazid (Fuhr 2000).

2. CYP2C8 substrates studied

2.1. Loperamide

Loperamide is a peripherally acting μ opioid receptor agonist which is widely used in the symptomatic treatment of diarrhoea. It lacks the typical central nervous system effects of opioids, since it has a low oral bioavailability and low penetration through the blood-brain barrier (Killinger et al. 1979, Dagenais et al. 2004). Loperamide is thought to undergo considerable first-pass metabolism as the plasma concentrations of parent loperamide are very low. It has an elimination half-life ($t_{½}$) of about 10 to 20 hours (Killinger et al. 1979, Doser et al. 1995). The recommended initial dose of loperamide is 4 mg and the maximum daily dose is 16 mg (Imodium label information).

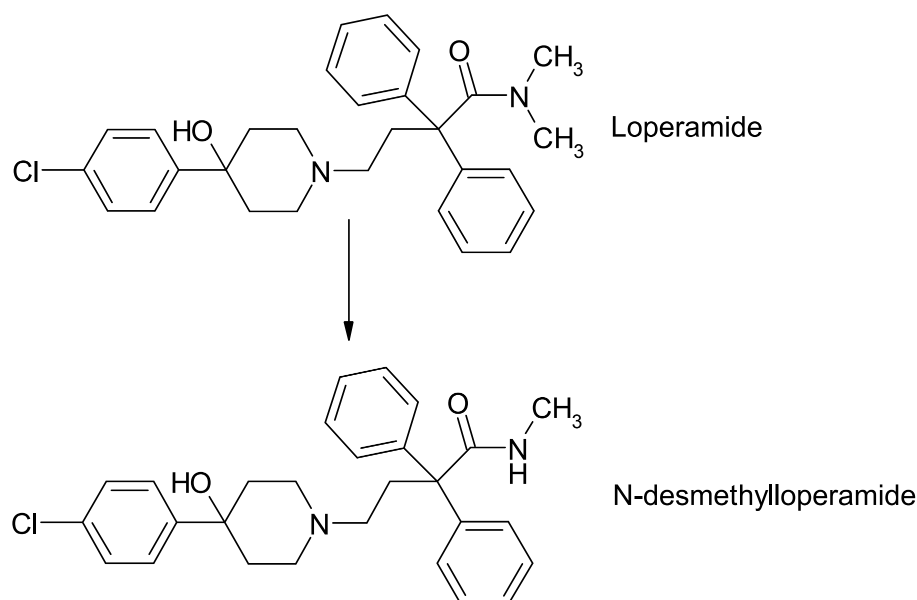


Figure 1. Chemical structures of loperamide and its major metabolite.

In vitro, loperamide was recently shown to be metabolised predominantly by CYP2C8 and CYP3A4. The metabolism of loperamide to its main metabolite, N-desmethylloperamide, in human liver microsomes (HLM) was inhibited by quercetin and ketoconazole, which are nonselective inhibitors of CYP2C8 and CYP3A4, respectively. In addition, CYP2C8, CYP3A4, and to a lesser extent CYP2B6, appeared to contribute to loperamide N-demethylation according to experiments with recombinant human CYP enzymes (rhCYPs) and a correlation study in HLM (Kim et al. 2004). In addition, loperamide is a substrate of P-

gp (Dagenais et al. 2004). Concomitant use of loperamide and quinidine, an inhibitor of P-gp, has resulted in opioid-like central nervous system effects, while resulting in only moderately raised plasma concentrations of loperamide (Skarke et al. 2003). However, ritonavir, a strong inhibitor of both CYP3A4 and P-glycoprotein, increased the area under the concentration-time curve (AUC) of loperamide 2.7-fold without evidence for enhanced central nervous system effects (Tayrouz et al. 2001). Furthermore, the combination of the CYP2C8 inhibitor trimethoprim and the CYP2C9 inhibitor sulfamethoxazole has been found to raise the AUC of loperamide 1.9-fold (Kamali and Huang 1996).

2.2. Zopiclone

Zopiclone is a type A γ-aminobutyric acid (GABA) receptor agonist used as a hypnotic agent. It is chemically unrelated to benzodiazepines, but has a similar pharmacological profile (Noble et al. 1998). Zopiclone is eliminated mainly by metabolism in the liver with a $t_{½}$ of about 4-6 hours. In humans, its main metabolites are N-desmethylzopiclone and zopiclone-N-oxide (Fernandez et al. 1995). The most common adverse effect of zopiclone is a bitter aftertaste (Allain et al. 1991). The recommended hypnotic dose of zopiclone is 7.5 mg, or 3.75 mg in elderly patients, but it can be increased to 15 mg in patients not responding to the lower dose (Noble et al. 1998).

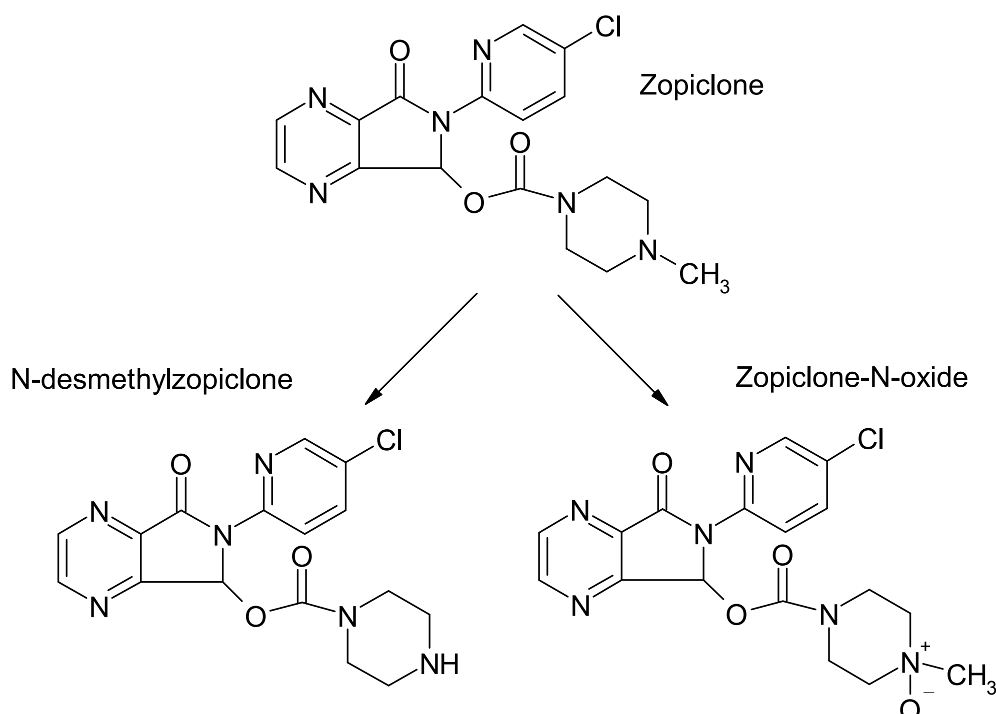


Figure 2. Chemical structures of zopiclone and its major metabolites.

Zopiclone has been reported to be metabolised to N-desmethylzopiclone and zopiclone-N-oxide by CYP3A4 and CYP2C8 *in vitro*. CYP2C8 has been found to be the predominant enzyme catalysing the formation of zopiclone metabolites in yeast-expressed rhCYPs *in vitro*. However, taking into account the relative content of each CYP isoform in the liver,

CYP2C8, CYP2C9 and CYP3A4 would all seem to participate in the metabolism. Furthermore, the N-desmethylation of zopiclone correlated with CYP3A4 and CYP2C8 activity, whereas the N-oxidation correlated with CYP3A4 activity in different HLM samples (Becquemont et al. 1999). In humans, the CYP3A4 inhibitors erythromycin and itraconazole have been reported to raise the AUC of zopiclone by 1.8- and 1.7-fold, respectively, whereas the concentrations of zopiclone are drastically reduced by the CYP inducer rifampicin (Aranko et al. 1994, Jalava et al. 1996, Villikka et al. 1997).

2.3. Ibuprofen

Ibuprofen is a widely used NSAID, which is usually administered as a racemic mixture of R-(−)-ibuprofen and S-(+)-ibuprofen. *In vivo*, about 60% of R-ibuprofen is unidirectionally converted to S-ibuprofen, which is primarily responsible for the inhibition of cyclooxygenases 1 and 2 (COX-1 and COX-2) (Adams et al. 1976, Lee et al. 1985, Evans 1992). Ibuprofen is rapidly absorbed, and extensively (>98%) bound to plasma proteins. It has a $t_{½}$ of about 2 hours. Racemic ibuprofen is clinically used up to four times daily, with a maximum total daily dose of up to 2400-3200 mg (Davies 1998). Gastrointestinal toxicity is a common concern for all NSAIDs, but ibuprofen is regarded as relatively safe compared to other drugs of this class, at least when low doses are used (Henry et al. 1996).

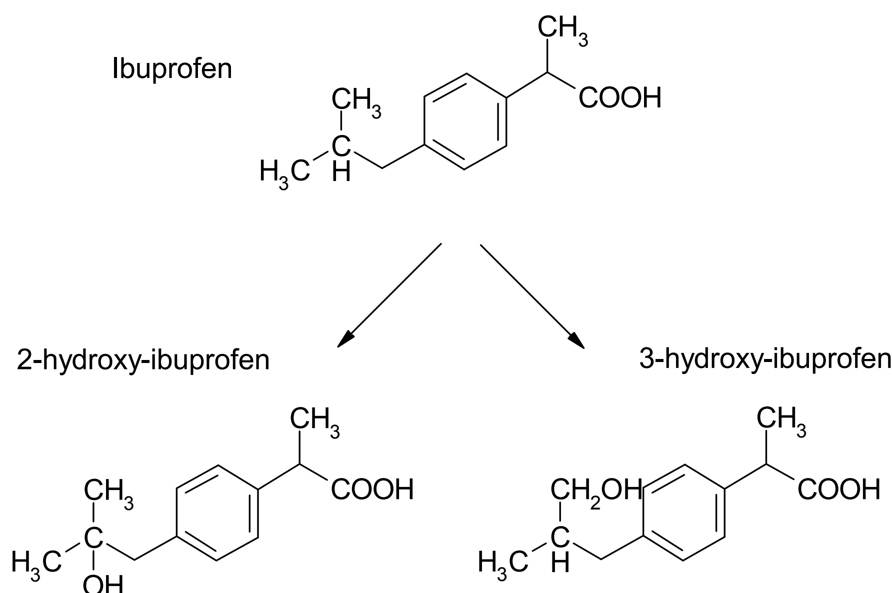


Figure 3. Chemical structures of ibuprofen and its major metabolites.

In vitro, ibuprofen is predominantly metabolised by CYP2C9, but CYP2C8 also contributes to the metabolism of R-ibuprofen. RhCYP2C8 and rhCYP2C9 have been found to contribute equally to the R-2-hydroxylation of ibuprofen, whereas the R-3-hydroxylation and S-hydroxylation of ibuprofen were catalysed by rhCYP2C9. At an ibuprofen concentration of 100 µM, other CYP isoforms did not metabolise ibuprofen (Hamman et al. 1997). In healthy volunteers, the CYP2C9 inhibitors voriconazole and fluconazole have been found to raise the concentration of S-ibuprofen markedly, but only slightly increase the concentration of R-

ibuprofen (Hynninen et al. 2006). The *CYP2C9*3* allele has been shown to be associated with a reduced S-ibuprofen clearance (Kirchheiner et al. 2002). On the other hand, the *CYP2C8*3* allele has been found to be associated with a decreased clearance of both R- and S-ibuprofen (García-Martín et al. 2004, Martínez et al. 2005).

2.4. Pioglitazone

The thiazolidinedione pioglitazone is a peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist used in the treatment of type 2 diabetes. The oral bioavailability of pioglitazone is over 80%, and it is highly (>97%) bound to plasma proteins. Pioglitazone is extensively metabolised in the liver. The main metabolites in humans are M-IV and M-III (a secondary metabolite formed from M-IV), which are also pharmacologically active, contributing significantly to the therapeutic effect. The terminal $t_{½}$ of pioglitazone ranges from 3 to 9 hours, while the $t_{½}$ of M-IV and M-III are considerably longer (Eckland and Danhof 2000). Adverse effects of pioglitazone are weight gain, peripheral oedema and fluid retention, which can lead to or exacerbate heart failure. Pioglitazone is administered once daily at a dosage of 15 or 30 mg, with titration to 45 mg if necessary (Waugh et al. 2006).

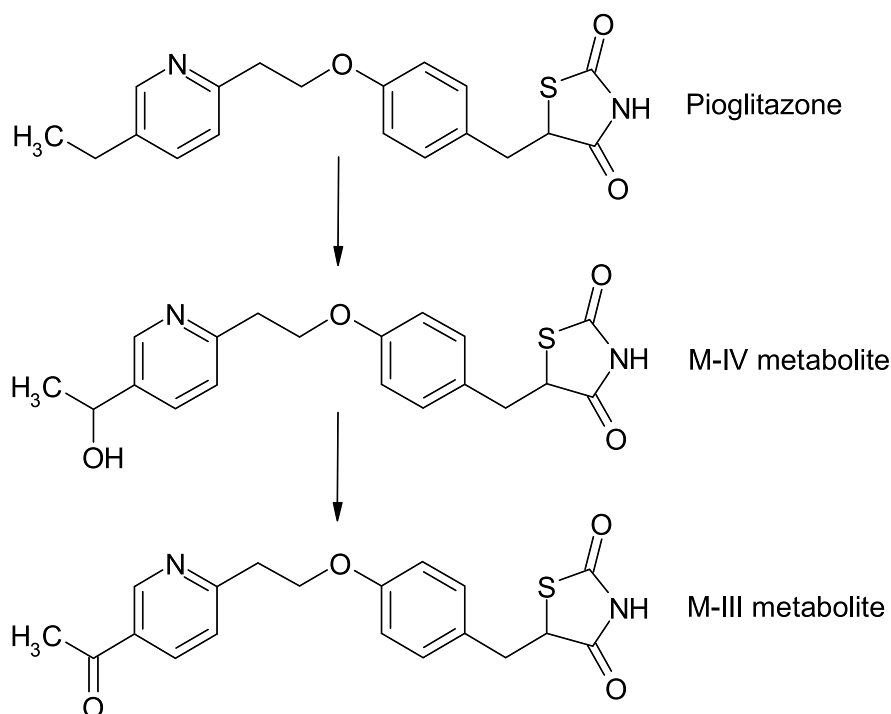


Figure 4. Chemical structures of pioglitazone and its major metabolites.

In vitro, pioglitazone is metabolised predominantly by CYP2C8 and CYP3A4. The metabolism of pioglitazone is inhibited by CYP2C8 and CYP3A4 inhibitors in HLM, and recombinant CYP2C8 and CYP3A4 are the main isoforms that catalyse the metabolism of pioglitazone *in vitro* (Hanefeld 2001, Jaakkola et al. 2006c). *In vivo*, the CYP3A4 inhibitor itraconazole has been shown to have virtually no effect on the pharmacokinetics of pioglitazone, while the AUC of pioglitazone has been found to be raised 3.2-fold by the

CYP2C8 inhibitor gemfibrozil (Jaakkola et al. 2005). The CYP inducer rifampicin substantially decreases plasma concentrations of pioglitazone (Jaakkola et al. 2006a). Pioglitazone has been reported to inhibit CYP2C8 and CYP3A4 *in vitro* (Sahi et al. 2003, Walsky et al. 2005a), but it does not increase plasma concentrations of the CYP2C8 and CYP3A4 substrate repaglinide in healthy volunteers (Kajosaari et al. 2006a).

Pioglitazone is a competitive inhibitor of OATP1B1 *in vitro*, and thus could be a substrate (Nozawa et al. 2004). However, the pharmacokinetics of pioglitazone are not affected by the *SLCO1B1* c.521T>C polymorphism, suggesting that OATP1B1 does not play a significant role in the pharmacokinetics of pioglitazone (Kalliokoski et al. 2007). The effects of other genetic polymorphisms on pioglitazone metabolism had not been characterised prior to this thesis work.

2.5. Repaglinide

Repaglinide is a short-acting meglitinide-analogue antidiabetic drug. It is used to reduce postprandial glucose levels in patients with type 2 diabetes. Repaglinide has a considerable first-pass metabolism and an oral bioavailability of about 60%. Repaglinide is extensively (>98%) protein bound, and it is eliminated rapidly with a $t_{½}$ of about 1 hour (Hatorp et al. 1999, Hatorp 2002). Repaglinide is administered before meals in a dose range of 0.5-4 mg, and the recommended maximum daily dose is 16 mg (NovoNorm product information). The most common adverse effect of repaglinide is hypoglycaemia (Culy and Jarvis 2001).

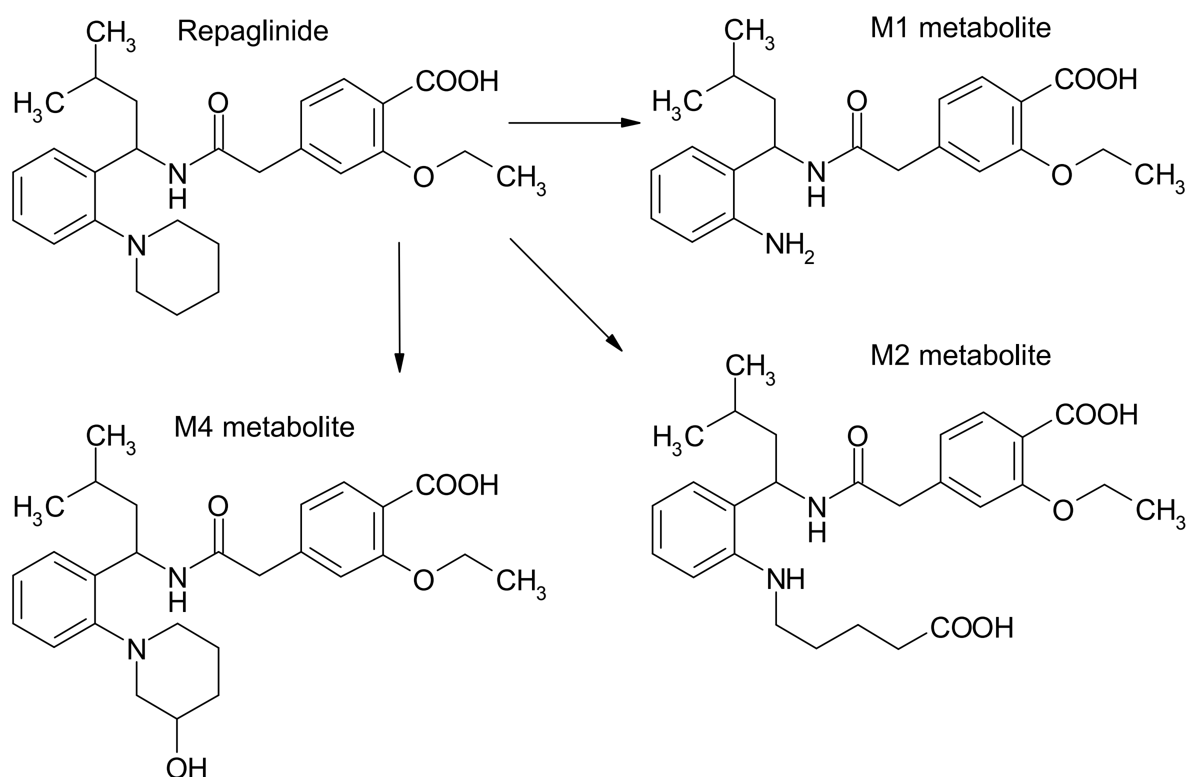


Figure 5. Chemical structures of repaglinide and its major metabolites.

Repaglinide is extensively metabolised in the liver, and less than 2% of the dose is excreted unchanged. In humans, the main metabolites of repaglinide are M1, M2 and M4 (van Heiningen et al. 1999). *In vitro*, repaglinide is metabolised predominantly by CYP2C8 and CYP3A4 (Bidstrup et al. 2003, Kajosaari et al. 2005a). The inhibition of CYP3A4 *in vitro* by cyclosporine and *in vivo* by telithromycin decreases the formation of M1 more than M2 and M4, indicating that CYP3A4 is important in the formation of M1 (Kajosaari et al. 2005b, Kajosaari et al. 2006b). On the other hand, metabolism of repaglinide to M4 seems to be primarily catalysed by CYP2C8 (Bidstrup et al. 2003). In pharmacogenetic studies, the *CYP2C8*3* allele has been found to be associated with decreased plasma concentrations of repaglinide compared to the reference genotype (*CYP2C8*1/*1*) (Niemi et al. 2003c, Niemi et al. 2005b). In addition, repaglinide pharmacokinetics are affected by the *SLCO1B1* c.521T>C polymorphism, strongly suggesting that repaglinide is a substrate of OATP1B1 (Niemi et al. 2005b). This could also explain why no effect of the *CYP2C8* genotype on repaglinide pharmacokinetics was seen in another study where the *SLCO1B1* genotype of the subjects was not determined (Bidstrup et al. 2006).

The CYP3A4 inhibitors clarithromycin, grapefruit juice, itraconazole, ketoconazole and telithromycin have been found to moderately raise the plasma concentrations of repaglinide, with less than 2-fold changes in its AUC (Niemi et al. 2001a, Bidstrup et al. 2006, Niemi et al. 2003b, Hatorp et al. 2003, Kajosaari et al. 2006b). On the other hand, the CYP2C8 inhibitors gemfibrozil and trimethoprim have been reported to increase the AUC of repaglinide 8.1-fold and 1.6-fold, respectively (Niemi et al. 2003b, Niemi et al. 2004b). Additionally, the CYP3A4 and OATP1B1 inhibitor cyclosporine has been found to increase the AUC of repaglinide 2.4-fold (Kajosaari et al. 2005b). Concentrations of repaglinide are decreased by rifampicin, but the extent of this interaction is related to the dosing interval between rifampicin and repaglinide, as rifampicin can both induce and inhibit CYP2C8 and CYP3A4, as well as OATP1B1, and the inhibitory effect lasts only few hours after rifampicin dosing (Bidstrup et al. 2004, Kajosaari et al. 2005a, Lau et al. 2007).

3. CYP inhibitors used in this study

3.1. Gemfibrozil

Gemfibrozil is a fibric acid derivative (fibrate) used in the treatment of hypertriglyceridaemia. Gemfibrozil is rapidly and completely absorbed from the gastrointestinal tract. The elimination of gemfibrozil is fast, with a $t_{½}$ of about 1.5 to 2 hours, although a terminal $t_{½}$ of up to 7.6 hours has been reported. The principal metabolite of gemfibrozil is a benzoic acid derivative, M3 (Todd and Ward 1988). Both the parent gemfibrozil and its metabolites form glucuronide conjugates, and the primary enzyme responsible for gemfibrozil glucuronidation in humans seems to be UGT2B7 (Mano et al. 2007). Gemfibrozil is used clinically at a dose of 600 mg twice daily.

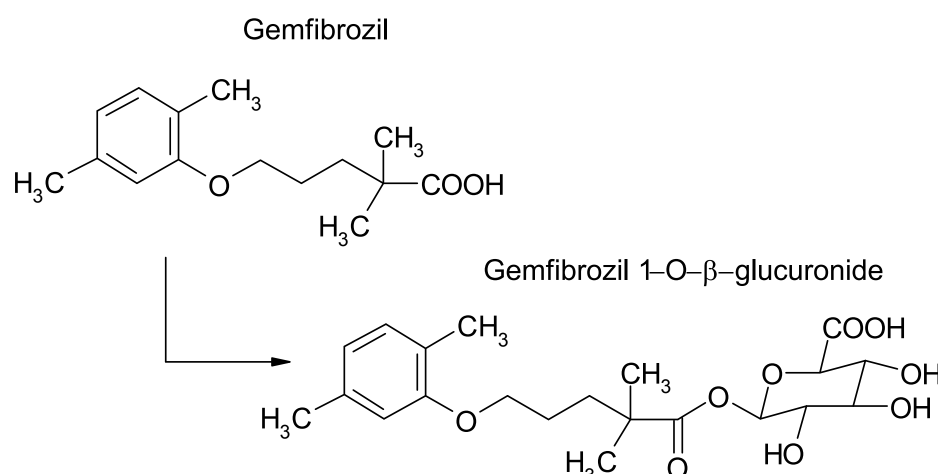


Figure 6. Chemical structures of gemfibrozil and gemfibrozil 1-O-β-glucuronide.

Gemfibrozil is the most potent known inhibitor of CYP2C8 *in vivo*. It raises the plasma concentrations of several CYP2C8 substrates, such as repaglinide (8.1-fold increase in AUC), cerivastatin (5.6-fold increase in AUC), pioglitazone (3.2- and 3.4-fold increase in AUC) and rosiglitazone (2.3-fold increase in AUC) (Niemi et al. 2003b, Backman et al. 2002, Jaakkola et al. 2005, Deng et al. 2005, Niemi et al. 2003a). The strong *in vivo* inhibitory potential of gemfibrozil is possibly explained by its 1-O-β-glucuronide, which is a mechanism-based inhibitor of CYP2C8 (Shitara et al. 2004, Ogilvie et al. 2006). On the other hand, gemfibrozil markedly raises the plasma concentrations of other statins, such as simvastatin, lovastatin, atorvastatin, pravastatin and rosuvastatin, that are not significantly metabolised by CYP2C8 (Neuvonen et al. 2006). Indeed, gemfibrozil and its metabolites have been reported to also inhibit other drug-metabolising enzymes and transporters (Table 4). This can partially explain some of the drug interactions caused by gemfibrozil, but the exact contribution of the different interaction mechanisms is not clear. In addition, gemfibrozil has been reported to induce CYP2C8 and CYP3A4 in human hepatocytes, but the clinical relevance of this finding has not been clarified (Prueksaritanont et al. 2005).

Table 4. Comparison of the inhibition potency of gemfibrozil and its metabolites on different CYPs and drug transporters ranked by *in vitro* IC_{50} values.

	CYP2C8	CYP2C9	CYP3A4	OATP1B1	OAT3	P-gp
Gemfibrozil	+ [1-3]	++ [3, 4]	- [2,4]	+(+) [2, 5, 6]	++ [7]	- [6]
with preincubation	+ [3]	++ [3]	- [3]			
Gemfibrozil 1-O-β-glucuronide	++ [2, 3]	- [3]	- [3]	++ [2, 5]	++ [7]	
with preincubation	+++ [3]	- [3]	- [3]			
M3	- [2]		- [2]	- [2, 5]	++ [7]	

[1]Wang et al. 2002b, [2]Shitara et al. 2004, [3]Ogilvie et al. 2006, [4]Wen et al. 2001, [5]Nakagomi-Hagihara et al. 2007b, [6]Yamazaki et al. 2005, [7]Nakagomi-Hagihara et al. 2007a.
IC_{50}, inhibitor concentration producing 50% decrease in activity
- no inhibition
+ weak inhibition (IC_{50} about 100 μM)
++ moderate inhibition (IC_{50} about 10 μM)
+++ strong inhibition (IC_{50} about 1 μM)

3.2. Itraconazole

Itraconazole is a triazole antifungal agent that inhibits the fungal P450 enzyme lanosterol-14α-demethylase, thereby interfering with fungal cell membrane ergosterol synthesis. Itraconazole is extensively bound to plasma proteins and only about 0.2% is unbound in plasma. Itraconazole accumulates in tissues such as the skin, nails, kidney and liver. Itraconazole is metabolised to several metabolites in the liver, and the major metabolite in humans is hydroxyitraconazole, which reaches higher plasma concentrations than the parent itraconazole, and has considerable antifungal activity as well. Elimination of itraconazole is biphasic, with a terminal $t_{½}$ of 20 to 24 hours after a single dose, which increases to 30 hours at steady state (Grant and Clissold 1989, De Beule and Van Gestel 2001). The recommended daily dose range of itraconazole is 100-400 mg.

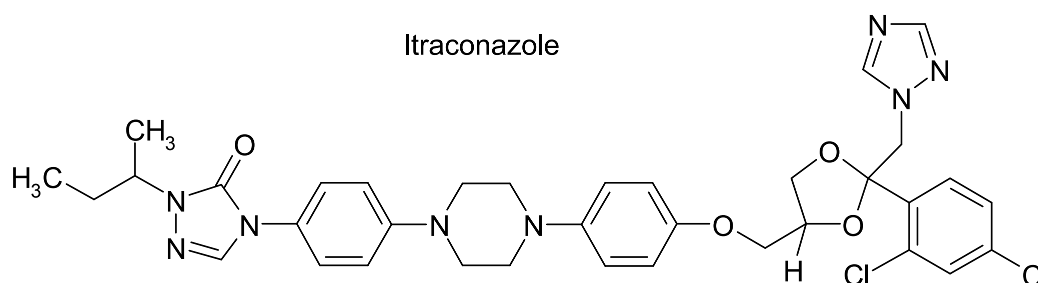


Figure 7. Chemical structure of itraconazole.

Itraconazole potently inhibits CYP3A4 *in vitro* (Back and Tjia 1991). *In vivo*, it greatly increases the concentrations of drugs that are predominantly metabolised by CYP3A4, such as midazolam, triazolam and simvastatin (Olkkola et al. 1994, Neuvonen et al. 1998, Varhe et al. 1994). The *in vitro* and *in vivo* inhibitory potential of itraconazole seems to be partly explained by its metabolites, including hydroxyitraconazole (Isoherranen et al. 2004, Templeton et al. 2007). Itraconazole also inhibits P-gp, affecting the plasma concentrations

and renal excretion of its substrates, e.g. digoxin (Jalava et al. 1997, Wang et al. 2002a). On the other hand, the plasma concentrations of itraconazole can be affected by concomitant use of drugs that inhibit or induce CYP3A4 (Albengres et al. 1998). In addition, gemfibrozil has been found to decrease the plasma concentrations of itraconazole (Niemi et al. 2003b, Jaakkola et al. 2005).

3.3. Trimethoprim

Trimethoprim is a dihydrofolate reductase inhibitor, which inhibits the synthesis of bacterial DNA. Trimethoprim alone and in combination with sulphonamides has been widely used to treat urinary and respiratory tract infections (Smilack 1999, Masters et al. 2003). Trimethoprim is well absorbed from the gastrointestinal tract, and is excreted mostly unchanged in the urine, with a $t_{½}$ of 8-14 hours. About 10-20% of trimethoprim is metabolised, but the enzymes involved in its biotransformation are not known (Masters et al. 2003). Trimethoprim is typically used at a dose of 160 mg twice daily.

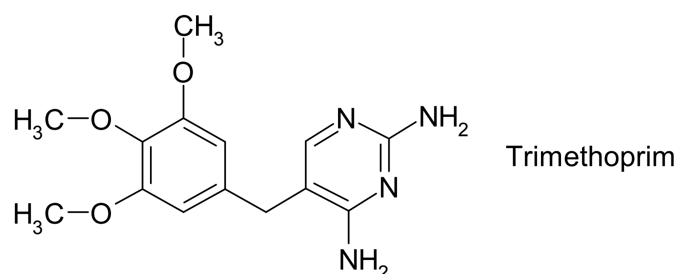


Figure 8. Chemical structure of trimethoprim.

Trimethoprim is a selective and moderately potent inhibitor of CYP2C8 *in vitro*, with an inhibition constant (K_i) of 32 μM and little effect on other CYP isoforms at concentrations <100 μM (Wen et al. 2002). In humans, it has been reported to raise the concentrations of the CYP2C8 substrates rosiglitazone and repaglinide by 1.4- and 1.6-fold, respectively (Niemi et al. 2004a, Niemi et al. 2004b). In addition, rifampicin has been reported to reduce the plasma concentrations of trimethoprim (Ribera et al. 2001).

AIMS OF THE STUDY

The aim of this thesis work was to investigate the effects of CYP2C8-inhibiting drugs on the pharmacokinetics of drugs metabolised by CYP2C8 in order to clarify its role in drug metabolism. Furthermore, the pharmacogenetics of CYP2C8 and the inhibition mechanism of gemfibrozil on CYP2C8 were studied.

Specific aims of the studies were:

I To investigate the effects of the CYP2C8 inhibitor gemfibrozil, the CYP3A inhibitor itraconazole and their combination on the pharmacokinetics and pharmacodynamics of loperamide, to determine the role of these enzymes in the metabolism of loperamide *in vivo*.

II To investigate the effect of gemfibrozil on the pharmacokinetics and pharmacodynamics of zopiclone *in vivo*, and the effect of different CYP inhibitors on the metabolism of zopiclone *in vitro*, to determine the importance of CYP2C8 in the metabolism of zopiclone.

III To investigate the effect of gemfibrozil on the pharmacokinetics of racemic ibuprofen *in vivo*, in order to determine the role of CYP2C8 in the metabolism of ibuprofen enantiomers *in vivo*.

IV To investigate the effects of the CYP2C8 inhibitor trimethoprim and the *CYP2C8*3* allele on the pharmacokinetics of pioglitazone *in vivo*, and the effect of trimethoprim on the metabolism of pioglitazone *in vitro*.

V To investigate the effect of dosing interval on the extent of gemfibrozil-repaglinide interaction, in order to clarify the mechanism of the interaction and to determine if the interaction could be avoided by increasing the interval between administration of gemfibrozil and repaglinide.

MATERIALS AND METHODS

1. Subjects

A total of 56 (36 male, 20 female) healthy volunteers participated in the studies (Table 5). Two of the male subjects participated in two of the studies. The volunteers were ascertained to be healthy by medical history, physical examination, and routine laboratory tests before entering the study. None of the subjects was using continuous medication, oral contraceptives or was a frequent smoker. The use of any other drugs was prohibited one week prior to each study. Participation in any other trial and blood donation within 4 weeks before and after the study were also prohibited.

Table 5. Characteristics of the subjects.

Study	Subjects (male/female)	Age (y)	Weight (kg)	BMI (kg/m^2)
I	12 (6/6)	21 ± 2	70 ± 14	22 ± 3
II	10 (7/3)	23 ± 2	74 ± 10	23 ± 2
III	10 (8/2)	21 ± 2	77 ± 5	24 ± 2
IV	16 (8/8)	21 ± 1	68 ± 12	22 ± 3
V	10 (9/1)	23 ± 3	79 ± 12	24 ± 1

Age, weight and body mass index (BMI) data are mean ± SD (standard deviation).

2. Study designs

The studies were carried out in the Department of Clinical Pharmacology, University of Helsinki. Studies I to IV had a randomised, placebo-controlled, crossover design (Table 6). Study V had a randomised, open, crossover design. The studies consisted of 2 to 5 phases. In each phase, a pre-treatment period with the inhibitor drug under study or placebo (no pre-treatment in study V) was followed by the study day, during which a single oral dose of the study drug was ingested.

The pre-treatment medications, placebos and the study drugs were supplied, packed and labelled according to a randomisation list for each subject by the Helsinki University Central Hospital Pharmacy. Repaglinide tablets were halved and weighed by the investigators. Drug doses were chosen to reflect current clinical use. A low dose of repaglinide was chosen for safety reasons.

The study drugs were administered orally with 150 ml water after an overnight fast at 09:00 on the study day. In studies I to IV, the subjects received a standard warm meal 3 hours, and light meals 7 and 11 hours (7 and 9 hours in study I) after the study drug intake. In study V, the subjects received a standardised breakfast (eaten in 10 minutes) precisely 15 minutes after repaglinide administration, 2 standardised snacks (eaten in 5 minutes) after 1 and 2 hours, a standard warm meal after 3 hours, and snacks after 7 and 9 hours. Food intake was identical in the different study phases.

Table 6. Structure of the studies.

Study	Pre-treatment medication and dose	Duration of pre-treatment (number of doses)	Washout period (weeks)	Study drug and dose
I	Gemfibrozil 600 mg x 2 Itraconazole 100 mg x 2 (first dose 200 mg) Gemfibrozil and itraconazole Placebo x 2	10 doses	4	Loperamide 4 mg on day 3 at 09:00
II	Gemfibrozil 600 mg x 2 Placebo x 2	6 doses	2	Zopiclone 7.5 mg on day 3 at 09:00
III	Gemfibrozil 600 mg x 2 Placebo x 2	6 doses	2	Ibuprofen 400 mg on day 3 at 09:00
IV	Trimethoprim 160 mg x 2 Placebo x 2	12 doses	4	Pioglitazone 15 mg on day 3 at 09:00
V	Gemfibrozil 600 mg x 2 No pre-treatment	5 doses	3	Repaglinide 0.25 mg at 09:00 either 0, 3, 6 or 12 h after last gemfibrozil dose

During the study days the subjects were under direct medical supervision. During the days of loperamide and zopiclone administration, naloxone and flumazenil, the antidotes for the drugs, respectively, were available for use. During the days of repaglinide administration, blood glucose levels were monitored throughout the day. Additional carbohydrates were given for symptomatic hypoglycaemia, or if blood glucose concentration dropped below 2.0 mmol/l. In addition, glucose solution for intravenous use and glucagon for intramuscular use were available.

3. Blood and urine sampling

On the days of study drug administration, a forearm vein of each subject was cannulated for blood sampling. Timed blood samples (5 or 10 ml each) were collected into tubes containing ethylenediaminetetraacetic acid (EDTA). In study V, blood glucose concentrations were measured immediately after each blood sampling by the glucose oxidase method (Precision G Blood Glucose Testing System; Medisense, Bedford, Mass., USA). Plasma was separated within 30 min of sampling, and samples were stored at −70 °C until analysis.

Urine was collected cumulatively in fractions of −1-12, 12-24, 24-36 and 36-48 hours in study I, and in fractions of 0-12 and 12-24 hours in study II. After each collection period, the volume of urine was measured and an aliquot was stored at −70 °C until analysis.

4. *In vitro* studies

The *in vitro* metabolism of zopiclone and pioglitazone was studied in studies II and IV, respectively. Pooled HLM, rhCYP2C8 and rhCYP3A4 were purchased from BD Biosciences (BD Gentest; Woburn, Mass., USA). According to the producer, human liver tissue had been collected in accordance with all pertinent regulations, and the procedures for organ collection had been reviewed and accepted by the respective institutional Human Subjects Committees. The study drugs and other chemicals were purchased from commercial sources.

Incubations were carried out in 0.1 M sodium phosphate buffer (pH 7.4), containing 5.0 mM $MgCl_2$ and 1.0 mM β-NADPH. The incubations contained 2.0 mg/ml microsomal protein with zopiclone, and 0.3 mg/ml microsomal protein or 20 pmol/ml recombinant CYP with pioglitazone. The other components were premixed, and the incubations were initiated by addition of the β-NADPH. The study drugs were dissolved in methanol and the final methanol concentration was 1% in all incubations. The samples were incubated in duplicate at 37 °C in a shaking water bath. The incubation time was 60 min for zopiclone and 8 min for pioglitazone. The mean values of the duplicates were used in the calculations.

To investigate the effects of different CYP inhibitors on the metabolism of zopiclone in HLM, ketoconazole (0.1 µM and 1 µM) and itraconazole (0.8 µM and 8 µM) were used as inhibitors of CYP3A4, montelukast (1.6 µM and 16 µM) as an inhibitor of CYP2C8, gemfibrozil (20 µM and 200 µM) as an inhibitor of CYP2C8 and CYP2C9, and sulphaphenazole (1 µM and 10 µM) as an inhibitor of CYP2C9. The final concentrations of all inhibitors were chosen based on previously published reports (Baldwin et al. 1995, Wen et al. 2001, Wang et al. 2002b, Isoherranen et al. 2004, Walsky et al. 2005b).

The Michaelis-Menten constant of pioglitazone (K_m) and the maximum reaction velocity (V_{max}) of pioglitazone M-IV metabolite formation were determined by incubating pioglitazone (0.375-50 µM) with HLM, rhCYP2C8, and rhCYP3A4. The K_i for trimethoprim was determined by coincubating pioglitazone (1.25-10 µM) and trimethoprim (0-200 µM) with either HLM or rhCYP2C8. The K_m, V_{max} and K_i values were calculated by fitting different kinetic models to the data using nonlinear regression analysis with the program SigmaPlot for Windows, version 9.01 (Systat Software Inc., San Jose, Calif., USA)

5. Determination of plasma and urine drug concentrations

The concentrations of the study drugs, pre-treatment drugs, and their metabolites were determined using PE SCIEX liquid chromatography-tandem mass spectrometry (LC-MS-MS) systems (Sciex Division of MDS, Toronto, Ontario, Canada) or high-performance liquid chromatography (HPLC) with ultraviolet detection (Table 7). When no authentic reference compound was available, the concentrations of the metabolites were measured as arbitrary units (u/ml) based on the ratio of the peak height of each metabolite to that of the internal standard in the chromatogram.

Table 7. The quantification of analytes in pharmacokinetic studies I to V.

Study	Analytes quantified	Method used	Limit of quantification	Interday CV [b]
I	Loperamide and N-desmethylloperamide [a]	LC-MS-MS	0.05 ng/ml	<11%
	Gemfibrozil	HPLC-UV	0.1 µg/ml	<7%
	Itraconazole and hydroxyitraconazole	HPLC-UV	10 ng/ml	<6%
II	Zopiclone and N-desmethylzopiclone [a]	LC-MS-MS	0.025 ng/ml	<5%
	Zopiclone-N-oxide	LC-MS-MS	0.1 ng/ml	<5%
	Gemfibrozil	HPLC-UV	0.1 µg/ml	<7%
III	R-(−)- and S-(+)-ibuprofen	HPLC-UV	0.1 µg/ml	<4%
	Gemfibrozil	HPLC-UV	0.1 µg/ml	<7%
IV	Pioglitazone	LC-MS-MS	0.1 ng/ml	<11%
	M-III	LC-MS-MS	0.1 ng/ml	<8%
	M-IV	LC-MS-MS	0.1 ng/ml	<14%
	Trimethoprim	HPLC-UV	0.1 µg/ml	<5%
V	Repaglinide, M1, M2 and M4 [a]	LC-MS-MS	0.02 ng/ml	<6%
	Gemfibrozil	LC-MS-MS	0.02 ng/ml	<9%
	Gemfibrozil 1-O-β-glucuronide	LC-MS-MS	0.1 µg/ml	<15%

[a] Limit of quantification and CV is for the parent drug, no reference compound for the metabolite
[b] Interday coefficient of variation (CV) of the assay at relevant concentrations.

6. Pharmacokinetic calculations

The pharmacokinetics of the study drugs, and their metabolites if applicable, were characterised by peak concentration (C_{max}), time to peak concentration (t_{max}), $t_{½}$, AUC from time 0 to infinity ($AUC_{0-\infty}$) and to the last point of measurement (AUC_{0-t}). The C_{max} and t_{max} values were taken directly from the original data. The terminal log-linear part of each concentration-time curve was identified visually for each subject. The elimination rate constant (k_e) was determined by linear regression analysis of the log-linear part of the plasma concentration-time curve. The $t_{½}$ was calculated by the equation $t_{½} = \ln 2/k_e$. The AUC values were calculated by use of the linear trapezoidal rule for the rising phase of the plasma concentration-time curve and the log-linear trapezoidal rule for the descending phase, with extrapolation to infinity, when appropriate, by dividing the last measured concentration by k_e. In studies I and II, the amounts of loperamide and zopiclone excreted into urine (Ae) within 48 hours and 24 hours, respectively, were calculated. The renal clearances (Cl_{renal}) of loperamide and zopiclone were calculated as Cl_{renal} = Ae/AUC for the corresponding time interval. In study IV, apparent formation rate constants (k_f) were calculated for pioglitazone metabolites M-IV and M-III by individual modelling with a 1-compartment first-order formation model. The pharmacokinetic calculations were performed with the program MK-model, version 5.0 (Biosoft, Cambridge, UK).

7. Pharmacodynamic measurements

7.1. Psychomotor performance (Studies I and II)

In studies I and II, psychomotor performance of each subject was assessed before the administration of the study drug and immediately after each blood sampling, up to 12 hours.

The Digit Symbol Substitution Test (DSST) has been demonstrated to be sensitive for measuring both cognitive and motor effects of psychoactive drugs (Stone 1984). In this test, the volunteer substitutes digits (1-9) for simple coded symbols. The number of correctly substituted digits in 2 minutes was recorded. Before the study started, the volunteers were properly trained to perform the test.

Subjective drowsiness and drug effect were evaluated on a 100-mm-long horizontal visual analogue scale (VAS) with adjectives with opposite meanings at both ends. The scale ranged from Alert to Drowsy, and No drug effect to Maximal drug effect, respectively, expressed in Finnish. The subject puts a mark on this scale as a self-rating of his or her feelings at the moment.

For each pharmacodynamic variable, the area under the effect-time curve from 0 to 12 hours ($AUEC_{0-12\ h}$) was calculated by the linear trapezoidal rule. In addition, the maximum response for each pharmacodynamic variable was recorded.

7.2. Blood glucose (Study V)

In study V, the pharmacodynamic effects of repaglinide were characterised by baseline blood glucose concentration, minimum blood glucose concentration and mean blood glucose concentration from 0 to 9 hours. The mean concentrations were calculated by dividing the area under the blood glucose concentration-time curve by the corresponding time interval.

8. Genotyping

For genotyping in studies III, IV and V, an EDTA blood sample was drawn from each subject and stored at −20 °C prior to genomic DNA extraction using standard methods (Qiaamp DNA Blood Mini Kit; Qiagen, Hilden, Germany). The genotyping was performed using validated Taqman allelic discrimination assays in an Applied Biosystems 7300 Real-Time polymerase chain reaction (PCR) system (Foster City, Calif., USA).

In studies III and V, the subjects were retrospectively genotyped for selected polymorphisms. In study III, the *CYP2C8* and *CYP2C9* genotypes were determined by investigating the presence of *CYP2C8*3* (c.416G>A and c.1196A>G), *CYP2C8*4* (c.792C>G), and *CYP2C9*2* (c.430C>T) and *CYP2C9*3* (c.1075A>C) alleles. In study V, the presence of *CYP2C8*3* and *CYP2C8*4* alleles, as well as the c.521T>C SNP in the *SLCO1B1* gene, was investigated.

In study IV, the subjects were recruited from a pool of more than 400 pharmacogenetically characterised subjects genotyped for *CYP2C8* alleles. The participants were prospectively selected on the basis of their *CYP2C8* genotype and allocated into three groups: *CYP2C8*1/*1* (i.e., non-carriers of *CYP2C8*3*; n=8), *CYP2C8*1/*3* (n=5), and *CYP2C8*3/*3* (n=3). Only non-carriers of the *CYP2C8*4* allele were recruited.

9. *In vitro-in vivo* correlations

In study IV, the relationship between the *in vitro* inhibition of pioglitazone metabolism and the increase in the AUC of pioglitazone by trimethoprim ($AUC_{inhibited}$ / $AUC_{control}$) was investigated by fitting the following equation to the observed individual data using linear regression analysis:

$$\frac{AUC_{inhibited}}{AUC_{control}} = 1 + \frac{C_{liver} / C_{plasma}\, ratio \cdot C_{avg,trimethoprim}}{K_i}$$

where C_{liver} / C_{plasma} *ratio* = concentration of trimethoprim in liver (unbound, available to enzyme) / concentration (total) of trimethoprim in plasma
K_i = the observed K_i of trimethoprim
$C_{avg,trimethoprim}$ = the observed average (total) plasma concentration of trimethoprim, which was calculated as follows:

$$C_{avg,trimethoprim} = \frac{AUC_{0-13h}}{13\,h}$$

where AUC_{0-13h} = the AUC of trimethoprim during the study day

The regression analysis was performed with SPSS for Windows, version 15.0.1. (SPSS Inc, Chicago, Ill., USA).

10. Statistical analysis

The results are expressed as mean (range) values, unless otherwise specified. In studies II and III, the pharmacokinetic and pharmacodynamic variables between the phases were compared with the paired t-test, or in the case of t_{max}, with the Wilcoxon signed-rank test. In studies I and V, the variables were compared with repeated measures analysis of variance (ANOVA), followed by a posteriori testing with the paired t-test. Bonferroni correction was applied to multiple comparisons in study I, but uncorrected *P* values were used in study V to avoid false negative conclusions and because the direction of the interaction had been documented previously. The t_{max} values were compared with Friedman's ANOVA followed by the Wilcoxon signed-rank test. In study IV, the pharmacokinetic variables between the placebo and trimethoprim phases were compared with repeated measures ANOVA with the *CYP2C8* genotype as a between-subjects factor. The differences between genotype groups were tested with 1-way ANOVA and post hoc comparisons with least significant differences.

When an interaction was observed, the Pearson correlation coefficient was used to investigate the possible relationships between pharmacokinetic variables of the pre-treatment drug and the extent of interaction. The differences were considered statistically significant when P was < 0.05. The statistical analysis was performed with SPSS for Windows (SPSS Inc, Chicago, Ill., USA).

11. Ethical considerations

The study protocols were approved by the Ethics Committee for Studies in Healthy Subjects and Primary Care (studies I and II) or by the Coordinating Ethics Committee (studies III, IV and V) of the Helsinki and Uusimaa Hospital District, as well as the Finnish National Agency for Medicines. Before entering the studies, all subjects had received both oral and written information, and had given their written informed consent.

RESULTS

1. Effects of gemfibrozil on CYP2C8 substrates (Studies I, II and III)

1.1. Loperamide (Study I)

Gemfibrozil raised the $AUC_{0-\infty}$ and C_{max} of loperamide 2.2-fold (*P* < 0.05) and 1.6-fold (*P* < 0.05), respectively, and prolonged the $t_{½}$ of loperamide from 11.9 to 16.7 hours (*P* < 0.01; Figures 9 and 10). Itraconazole raised the $AUC_{0-\infty}$ and C_{max} of loperamide 3.8-fold (*P* < 0.001) and 2.9-fold (*P* < 0.001), respectively, and prolonged the $t_{½}$ of loperamide to 18.7 hours (*P* < 0.001). The combination of itraconazole and gemfibrozil raised the $AUC_{0-\infty}$ and C_{max} of loperamide 12.6-fold (*P* < 0.001) and 4.2-fold (*P* < 0.001), respectively, and prolonged the $t_{½}$ of loperamide to 36.9 hours (*P* < 0.001).

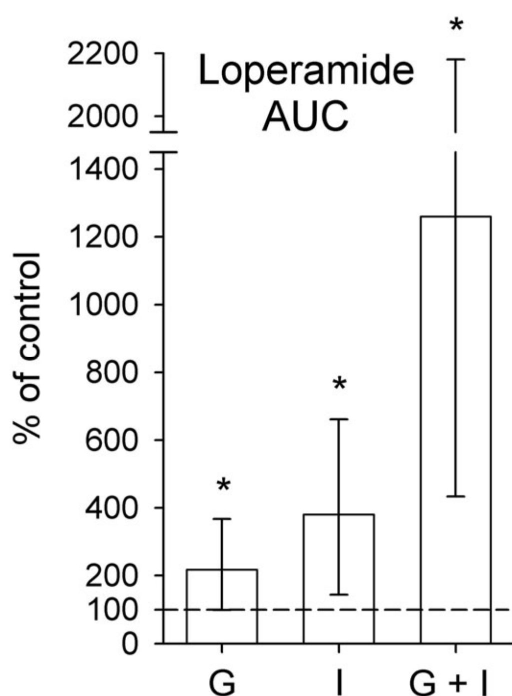


Figure 9. Effect of gemfibrozil (G), Itraconazole (I) and their combination (G+I) on the $AUC_{0-\infty}$ of loperamide in study I. The mean $AUC_{0-\infty}$ values for loperamide during the inhibitor phases are given as percentages of the mean values from the control (placebo) phase. The error bars depict the ranges of individual percentages of control values.

* *P* < 0.05 versus control.

Gemfibrozil and the combination of gemfibrozil and itraconazole reduced the Cl_{renal} of loperamide by 28% (*P* < 0.05) and 34% (*P* < 0.01), respectively. Gemfibrozil, itraconazole and their combination reduced the plasma $AUC_{0-72\ h}$ ratio of N-desmethylloperamide to loperamide by 46%, 65% and 88%, respectively (*P* < 0.001).

No significant differences between the phases were seen in the effects of loperamide on performance in DSST, or in subjective drowsiness.

The plasma concentrations of itraconazole and hydroxyitraconazole were markedly lower during the gemfibrozil-itraconazole phase than during the itraconazole phase. Gemfibrozil reduced the mean C_{max} and $AUC_{0-13\ h}$ of itraconazole by 57% and 54%, respectively (*P* < 0.001), and those of hydroxyitraconazole by 37% and 41%, respectively (*P* < 0.001). Itraconazole had no significant effect on the plasma concentrations of gemfibrozil on the

study day. There were no significant correlations between the pharmacokinetic variables of gemfibrozil, itraconazole and hydroxyitraconazole, and the extent of their interaction with loperamide.

1.2. Zopiclone (Study II)

Gemfibrozil had no significant effect on any of the pharmacokinetic variables of the parent zopiclone (Figure 10). The $AUC_{0-\infty}$ and C_{max} of zopiclone during the gemfibrozil phase were 99% and 94% of control, respectively. The $t_{½}$ values of zopiclone were 4.7 and 4.8 hours during the control and gemfibrozil phases, respectively.

Gemfibrozil raised the $AUC_{0-\infty}$ of the zopiclone metabolites, zopiclone-N-oxide and N-desmethylzopiclone, by 97% ($P < 0.001$) and 19% ($P < 0.01$), respectively, and increased their C_{max} by 62% ($P < 0.001$) and 16% ($P < 0.001$), respectively. The Cl_{renal} of zopiclone-N-oxide was also decreased 48% by gemfibrozil ($P < 0.001$).

No significant differences between the phases were seen in the effects of zopiclone on performance in DSST, or in subjective drowsiness or overall drug effect.

The C_{max} and $AUC_{0-10\ h}$ of gemfibrozil were 28.5 µg/ml (range 12.9-40.3 µg/ml) and 92.1 µg·h/ml (range 61.8-124 µg·h/ml), respectively, in the gemfibrozil phase.

In vitro, CYP3A4 inhibitors markedly, and in a concentration dependent manner, inhibited the depletion of zopiclone (500 nM) in HLM. On the other hand, neither montelukast (CYP2C8 inhibitor) nor gemfibrozil (CYP2C8 and CYP2C9 inhibitor) had any significant effect on zopiclone metabolism.

1.3. Ibuprofen (Study III)

Gemfibrozil raised the $AUC_{0-\infty}$ of R-ibuprofen by 34% ($P < 0.001$), and prolonged its $t_{½}$ from 2.9 to 4.5 hours ($P < 0.001$; Figure 10). Its C_{max} and t_{max} values were not affected by gemfibrozil.

Gemfibrozil prolonged the $t_{½}$ of S-ibuprofen from 2.6 to 3.5 hours ($P < 0.001$), but had no statistically significant effect on the other pharmacokinetic variables of S-ibuprofen. The $AUC_{0-\infty}$ ratio of R-ibuprofen to S-ibuprofen was increased from 0.79 to 0.99 by gemfibrozil ($P < 0.001$).

The C_{max} and $AUC_{0-13\ h}$ of gemfibrozil were 26.6 µg/ml (range 19.6-35.9 µg/ml) and 78.6 µg·h/ml (range 65.2-99.9 µg·h/ml), respectively, in the gemfibrozil phase.

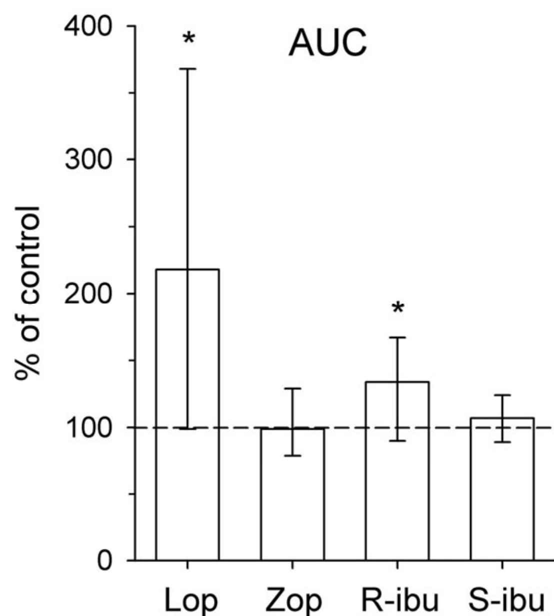
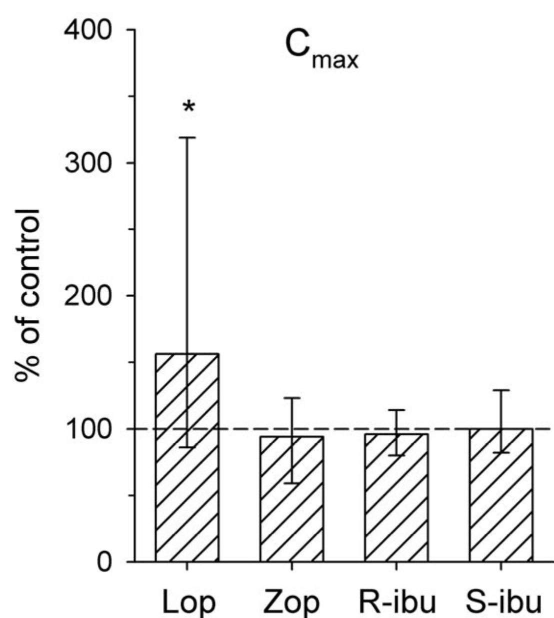
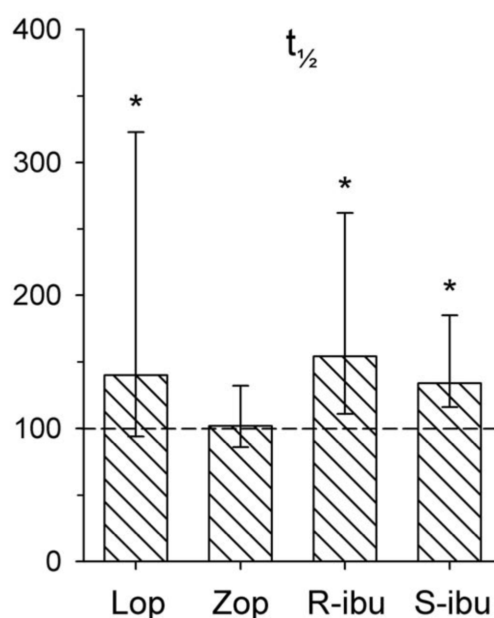


Figure 10. Effect of gemfibrozil on the pharmacokinetics of loperamide (Lop), zopiclone (Zop), R-ibuprofen (R-ibu) and S-ibuprofen (S-ibu) in studies I to III. The mean $AUC_{0-\infty}$, C_{max} and $t_{½}$ values during the gemfibrozil phase are given as percentages of the mean values from the control (placebo) phase. The error bars depict the ranges of individual percentages of control values.

* $P < 0.05$ versus control.





2. Effects of trimethoprim and the *CYP2C8* genotype on pioglitazone (Study IV)

2.1. Effect of trimethoprim

Trimethoprim raised the $AUC_{0-\infty}$ of parent pioglitazone by 42% ($P < 0.001$) and prolonged its dominant $t_{½}$ from 3.9 to 5.1 hours ($P < 0.001$; Figure 11). However, the C_{max} of pioglitazone was not statistically significantly changed by trimethoprim.

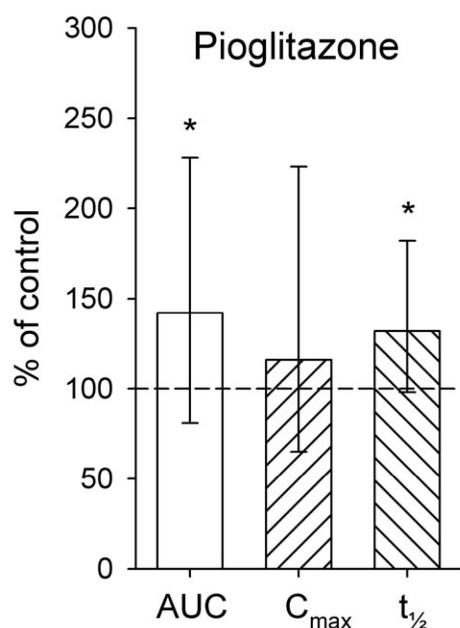


Figure 11. Effect of trimethoprim on the pharmacokinetics of pioglitazone in study IV. The mean $AUC_{0-\infty}$, C_{max} and $t_{½}$ of pioglitazone during the trimethoprim phase are given as percentages of the mean values from the control (placebo) phase. The error bars depict the ranges of individual percentages of control values.

* $P < 0.05$ versus control.

The $AUC_{0-\infty}$ of pioglitazone metabolite M-IV was slightly raised (by 10%; $P = 0.048$), while the C_{max} and $t_{½}$ were unaffected by trimethoprim. The k_f of M-IV decreased from 0.17 to 0.10 h^{-1} ($P < 0.001$), and the M-IV to pioglitazone $AUC_{0-\infty}$ ratio was also lowered by trimethoprim.

Trimethoprim C_{max} and $AUC_{0-13\ h}$ were 3.1 µg/ml (range 1.2-4.7 µg/ml) and 28.6 mg·h/ml (range 7.8-41.9 mg·h/ml), respectively, in the trimethoprim phase. One of the subjects had exceptionally low concentrations and a short $t_{½}$ for trimethoprim, and no increase in the AUC of pioglitazone was observed in this subject.

The extent of trimethoprim-pioglitazone interaction (pioglitazone $AUC_{inhibited}$ / pioglitazone $AUC_{control}$) could be predicted on the basis of the *in vitro* K_i of trimethoprim and its average plasma concentration using a competitive inhibition model with a C_{liver}/C_{plasma} ratio of 2.35 for trimethoprim. The equation obtained using regression analysis explained 24% of the variability in the extent of interaction.

In vitro, the M-IV metabolite was formed by both rhCYP2C8 and rhCYP3A4. The metabolite formation by CYP2C8 was best described by a model for Michaelis-Menten kinetics with substrate inhibition, whereas the formation by CYP3A4 was best described by

sigmoidal (Hill) kinetics. At clinically relevant pioglitazone concentrations (<2 μM), the formation rate of M-IV by CYP2C8 was 5 to 10 times higher than that of CYP3A4. Trimethoprim inhibited M-IV formation in HLM and recombinant CYP2C8 in a concentration dependent manner, and the inhibitory effect was best described by the competitive inhibition model, with K_i values of 38.2 ± 3.9 (mean ± SE, standard error) and 34.1 ± 4.0 μM, respectively.

2.2. Effect of the *CYP2C8*3* allele

During the placebo phase, the subjects heterozygous and homozygous for the *CYP2C8*3* allele had lower plasma concentrations of pioglitazone than the subjects with the *CYP2C8*1/*1* genotype. The weight-adjusted $AUC_{0-\infty}$ of pioglitazone was 34% smaller ($P <$ 0.05) in the *CYP2C8*3/*3* group and 26% smaller ($P < 0.05$) in the *CYP2C8*1/*3* group than in the *CYP2C8*1/*1* group (Figure 12). This difference was also maintained in the trimethoprim phase for the *CYP2C8*3/*3* group ($P < 0.05$ versus the *CYP2C8*1/*1* group). The extent of the interaction did not appear to depend on the *CYP2C8* genotype.

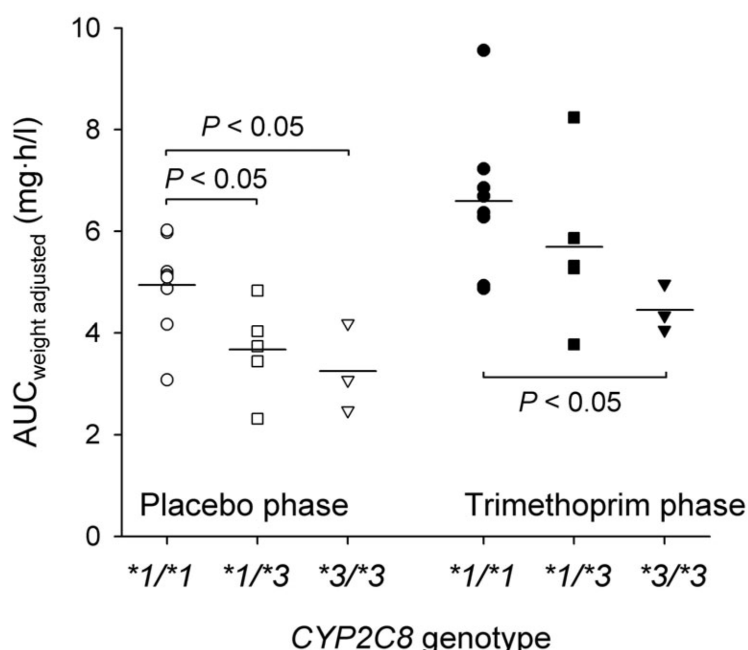


Figure 12. The individual $AUC_{0-\infty}$ values of pioglitazone adjusted for body weight in each genotype group during placebo (open symbols) and trimethoprim (closed symbols) treatment in study IV. The horizontal lines depict the mean of each group.

Pioglitazone metabolite M-IV and M-III $AUC_{0-\infty}$ ratios to the parent pioglitazone were higher in the variant allele-carrier groups than in the control group. However, there were no statistically significant differences between the genotype groups in the weight-adjusted $AUC_{0-\infty}$ values of M-IV and M-III.

3. Effect of dosing interval on gemfibrozil-repaglinide interaction (Study V)

Gemfibrozil raised the $AUC_{0-\infty}$ of repaglinide 7.0-, 6.5-, 6.2-, and 5.0-fold ($P < 0.001$) when repaglinide was taken either simultaneously with, or 3, 6 or 12 hours after the last dose of the gemfibrozil pre-treatment, respectively (Figure 13). The C_{max} in each gemfibrozil phase was about 2-fold greater than in the control phase. The $t_{½}$ of repaglinide was prolonged from 1.2 hours to 3.0, 2.7, 2.4, and 2.0 hours ($P < 0.001$) by gemfibrozil with the 0, 3, 6 and 12 hour dosing intervals, respectively.

The $AUC_{0-3\ h}$ of the repaglinide metabolite M4 was reduced by 79% ($P < 0.001$), 86% ($P < 0.001$), 77% ($P < 0.001$) and 38% ($P < 0.05$) by gemfibrozil with a 0, 3, 6 or 12 hour interval between gemfibrozil and repaglinide administration, respectively. In contrast, the $AUC_{0-\infty}$ values of M1 and M2 were higher during each of the gemfibrozil phases than in the control phase. The C_{max} of M4 was greatly, and that of M2 slightly, decreased by gemfibrozil, whereas the C_{max} of M1 was increased. All metabolite to repaglinide AUC ratios were lower during all gemfibrozil phases than in the control phase ($P < 0.001$ for M2 and M4, $P < 0.05$ for M1).

The mean blood glucose concentration from 0 to 9 hours was lower in each of the gemfibrozil phases than in the control phase ($P < 0.005$). The minimum blood glucose concentration was also lowered by gemfibrozil up to the 6 hour dosing interval between gemfibrozil and repaglinide, compared to control ($P < 0.001$).

The C_{max} and $AUC_{0-9\ h}$ of gemfibrozil were 42.4 μg/ml (range 26.3-64.4 μg/ml) and 105 μg·h/ml (range 58.2-143 μg·h/ml), and the corresponding values of gemfibrozil 1-O-β-glucuronide were 29.9 μg/ml (range 20.1-48.7 μg/ml) and 116 μg·h/ml (range 82.8-205 μg·h/ml), when the last gemfibrozil dose was ingested simultaneously with repaglinide. The observed C_{max} values (during the 9 hour period after the administration of repaglinide) of gemfibrozil and gemfibrozil 1-O-β-glucuronide declined along with the increasing dosing interval, and during the 12 hour interval phase, these values were only about 5% and 10% of the respective (actual) C_{max} values during simultaneous administration with repaglinide. There were no significant correlations between the pharmacokinetic variables of gemfibrozil and its 1-O-β-glucuronide and the extent of interaction with repaglinide.

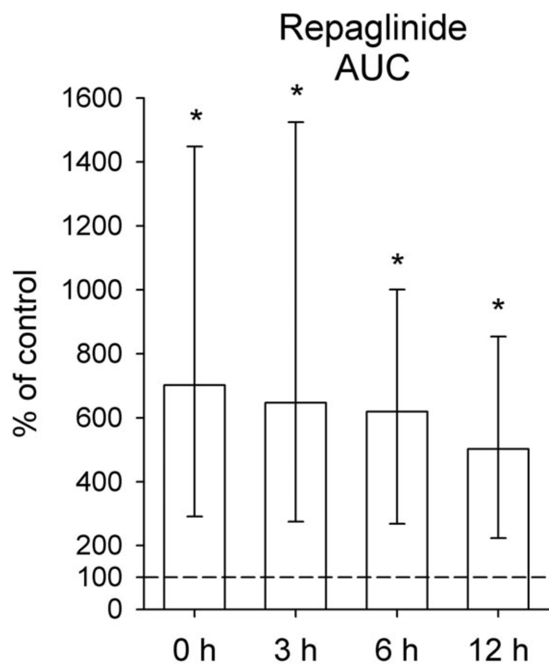
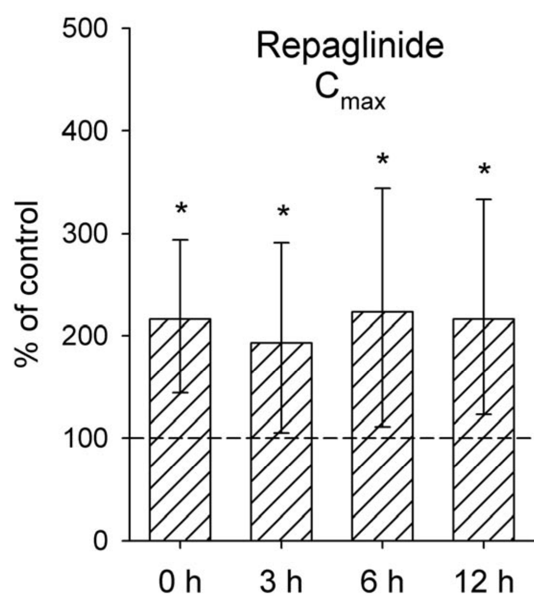
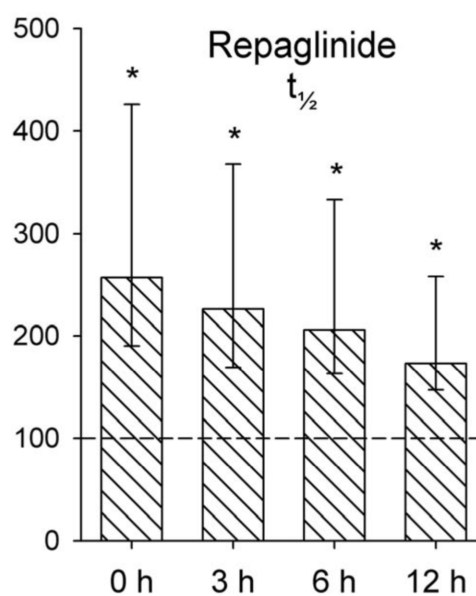


Figure 13. The effect of gemfibrozil on the pharmacokinetics of repaglinide when repaglinide was administered either simultaneously with, or 3 h, 6 h or 12 h after gemfibrozil in study V. The mean $AUC_{0-\infty}$, C_{max} and $t_{½}$ of repaglinide during the gemfibrozil phases are given as percentages of the mean values from the control (placebo) phase. The error bars depict the ranges of individual percentages of control values.

* $P < 0.05$ versus control.





DISCUSSION

1. Methodological considerations

1.1. *In vitro* studies

Human liver microsomes (HLM) and recombinant CYP enzymes are well-validated tools for the investigation of drug metabolism and possible drug-drug interactions *in vitro* (Rodrigues 1999). HLMs contain all the enzymes present in the endoplasmic reticulum of the hepatocytes, with their relative abundances conserved. To avoid inter-individual variability, pooled HLMs were used since they represent the population average more precisely than single liver samples. On the other hand, rhCYP enzymes contain only one specific CYP isoform, and are useful in identifying the role of certain CYP isoforms in the metabolism of a drug.

The aim in study II was to investigate the effect of inhibition of different CYP enzymes on the elimination of zopiclone. Therefore, a single (clinically relevant) concentration of zopiclone was incubated with HLM with different selective CYP enzyme inhibitors. In addition, the metabolism of pioglitazone and the effect of trimethoprim on it were characterised. For this, the metabolism of pioglitazone was studied in HLM and rhCYP2C8 and rhCYP3A4. The formation rate of the pioglitazone metabolite M-IV was measured since it is the major primary metabolite of pioglitazone (Eckland and Danhof 2000).

Most drugs are poorly soluble in water, and thus organic solvents are often needed in *in vitro* studies. They can, however, affect the enzymatic activities of different CYP isoforms to a varying degree (Chauret et al. 1998). In this work, methanol was used as a solvent in all (including control) incubations, and its concentrations were kept sufficiently low (1% v/v) to avoid significant effect on CYP enzyme activity (Busby et al. 1999). Other incubation conditions, such as ionic strength and the pH of the incubation medium, can also affect the results of *in vitro* studies (Venkatakrishnan et al. 2001), and thus, 0.1 M sodium phosphate buffer (pH 7.4) was used as the incubation medium to provide conditions that are equivalent to those in the hepatic cytoplasm *in vivo*.

The use of relevant substrate concentration is critical in *in vitro* studies, especially when only a single concentration is used. If a drug is metabolised by multiple CYP enzymes, the contribution of different CYP isoforms to the metabolism can vary greatly depending on the concentration used (Venkatakrishnan et al. 2001). The plasma concentrations of zopiclone typically peak at about 60-90 ng/ml (Fernandez et al. 1995). The *in vitro* zopiclone concentration of 500 nM (194 ng/ml) was chosen to be low enough to reflect a clinically relevant level, and high enough to allow reliable quantification of its enantiomer concentrations. However, the plasma concentrations of a drug do not necessarily reflect the actual concentrations at the site of metabolism in the hepatocyte (Venkatakrishnan et al. 2001). A range of pioglitazone (0.375-50 µM) concentrations was chosen to investigate the kinetic parameters of M-IV formation and the relative contribution of CYP2C8 and CYP3A4 in the metabolism of pioglitazone at different concentrations.

The *in vitro* study on zopiclone could have been improved by using variable zopiclone concentrations, or by utilising rhCYPs. However, our aim was to study the role of CYP enzymes, CYP2C8 in particular, in the metabolism of zopiclone at clinically relevant concentrations. Thus, only a single concentration was chosen. The study on pioglitazone would have been more comprehensive if the metabolism of pioglitazone had also been studied with the CYP2C8.3 enzyme. However, this enzyme was not commercially available.

1.2. *In vivo* studies

The studies performed in healthy volunteers had a randomised, balanced, cross-over design. Thus, the subjects served as their own controls, limiting the effects of inter-individual variability, and reducing the number of subjects needed to find a possible clinically significant interaction. The wash-out periods (2, 3 or 4 weeks) were chosen to minimise the risk of possible carry-over effects. The wash-out durations were chosen according to the pharmacokinetic properties of the drugs studied, and taking into account the time necessary for the synthesis of new CYP enzyme in the case of irreversible inhibition (Ghanbari et al. 2006).

In studies I to IV the pre-treatment dose was administered 1 hour before the study drug to ensure adequate absorption of the pre-treatment drug by the time the study drug was ingested. In order to decrease variability in drug absorption, and in study V to decrease variability in blood glucose concentrations, the subjects fasted overnight and received standardised meals during the study days. The pre-treatment drug doses were chosen to reflect normal therapeutic use, and their concentrations were monitored during the study days and ascertained to be at therapeutic levels. Additionally, to monitor compliance, the subjects marked the precise timing of drug intake according to the pre-treatment schedule. In study V, where the effect of variable dosing intervals was investigated, the intake of the last pre-treatment dose (either at 21:00, 03:00 or 06:00) was ensured by contacting the subjects by phone.

In study V, a small dose (0.25 mg) of repaglinide was chosen for safety reasons, because healthy subjects are more sensitive to its hypoglycaemic effect than diabetic patients. In an earlier study, when repaglinide was administered 1 hour after gemfibrozil, gemfibrozil raised the mean AUC of repaglinide 8-fold (Niemi et al. 2003b). Thus, it was estimated that the pharmacokinetic and pharmacodynamic effects would be similar or lesser when repaglinide was administered simultaneously with, or 3, 6 or 12 hours after the last gemfibrozil dose. During the study day, blood glucose concentrations were measured repeatedly, and additional carbohydrates were given for symptomatic hypoglycaemia or when blood glucose decreased below 2.0 mmol/l.

The pharmacodynamic tests in studies I and II were used to examine the clinical relevance of the possible interactions. The VAS scale for studying subjective effects and the DSST test for studying psychomotor effects are well-validated and widely used for these purposes (Aranko et al. 1994, Backman et al. 1996a, Granfors et al. 2004b). Before entering the studies, the subjects were trained to properly perform the DSST test.

2. Effect of gemfibrozil on loperamide, zopiclone and ibuprofen

Gemfibrozil markedly increased the plasma concentrations of loperamide and moderately increased those of R-ibuprofen, whereas no effect was observed on the pharmacokinetics of zopiclone. Each of these drugs has been reported to be metabolised by CYP2C8 *in vitro* (Kim et al. 2004, Hamman et al. 1997, Becquemont et al. 1999). The concentrations of gemfibrozil were similar in each of the three studies, and in line with previous pharmacokinetic studies with gemfibrozil (Miller and Spence 1998). Thus, a lack of compliance in gemfibrozil ingestion or its poor absorption would not explain the different effects of gemfibrozil on these drugs.

Gemfibrozil increased the AUC of loperamide, and reduced the plasma N-desmethylloperamide to loperamide $AUC_{0\text{-}72\ h}$ ratio and the ratio of N-desmethylloperamide to loperamide in urine. Itraconazole had a similar, albeit somewhat stronger, effect on these variables. The combination of gemfibrozil and itraconazole acted synergistically to further raise the plasma concentrations of loperamide. This indicates that both gemfibrozil and itraconazole inhibited the biotransformation of loperamide to its main metabolite, N-desmethylloperamide. These results are in agreement with previous data showing that loperamide is biotransformed to N-desmethylloperamide by CYP3A4 and CYP2C8 *in vitro* (Kim et al. 2004). During the gemfibrozil-itraconazole phase, the plasma concentrations of both itraconazole and hydroxyitraconazole were considerably lower than during the itraconazole phase, in line with two other studies (Niemi et al. 2003b, Jaakkola et al. 2005). This finding could be explained by a reduction in the oral bioavailability of itraconazole caused by gemfibrozil, or by displacement of itraconazole and hydroxyitraconazole from plasma proteins by gemfibrozil, leading to increased clearance or volume of distribution. Although the plasma concentrations of loperamide rose several fold, no changes were observed in the pharmacodynamics of loperamide in this study with a low single dose of 4 mg loperamide. However, an increased risk of adverse effects or increased efficacy should be considered when loperamide is used concomitantly with gemfibrozil, itraconazole or their combination, especially if a higher dose of loperamide is used or it is used on a regular basis.

The pharmacokinetics of zopiclone were unaffected by gemfibrozil. However, the concentrations of zopiclone-N-oxide and N-desmethylzopiclone were increased by gemfibrozil, and the Cl_{renal} of zopiclone-N-oxide was lowered. The effects on zopiclone metabolites could be explained by inhibition of their further metabolism or inhibition of drug transporters by gemfibrozil. On the other hand, another explanation for these findings could be induction of CYP3A4, coinciding with inhibition of CYP2C8 by gemfibrozil (Prueksaritanont et al. 2005). However, as the further elimination pathways of zopiclone metabolites are not known in detail, the contributions of different mechanisms to providing an explanation for these findings are not clear. In a previous *in vitro* study, both CYP3A4 and CYP2C8 contributed to the metabolism of parent zopiclone (Becquemont et al. 1999). However, these experiments were performed at a supratherapeutic zopiclone (50 μM) concentration. In contrast, when 500 nM (194 ng/ml) zopiclone was used in study II, inhibitors of CYP2C8 had only a negligible effect on zopiclone metabolism. This highlights the importance of using clinically relevant concentrations when *in vitro – in vivo* correlations are made. Although parent zopiclone concentrations were unaffected and no changes were observed in the psychomotor tests, both zopiclone-N-oxide and N-desmethylzopiclone

possess some pharmacological activity (Goa and Heel 1986, Carlson et al. 2001), and therefore their increased concentrations could slightly enhance the effects of zopiclone when administered concomitantly with gemfibrozil.

Gemfibrozil moderately increased the AUC of R-ibuprofen and prolonged its $t_{½}$, but had little effect on S-ibuprofen. In humans, both R- and S-ibuprofen are metabolised via 2- and 3-hydroxylation (Tan et al. 2002). *In vitro*, both CYP2C8 and CYP2C9 participate in the formation of R-2-hydroxyibuprofen, whereas the formation of S-2-, S-3- and R-3-hydroxyibuprofen is mainly catalysed by CYP2C9 (Hamman et al. 1997). In healthy volunteers, the CYP2C9 inhibitors voriconazole and fluconazole raise the plasma concentrations of S-ibuprofen, whereas their effects on R-ibuprofen are only minor (Hynninen et al. 2006). The findings that CYP2C8 and CYP2C9 inhibitors only moderately affect the pharmacokinetics of R-ibuprofen could be explained by alternative pathways in the elimination of R-ibuprofen. A possible explanation could be the unidirectional conversion of R-ibuprofen to S-ibuprofen that occurs *in vivo* via an enzymatic pathway (Tracy et al. 1993). The clinical significance of the interaction between gemfibrozil and ibuprofen is likely to be limited, as the therapeutic effects of ibuprofen are mainly mediated by its S-enantiomer, and as it has a wide therapeutic range (Davies 1998). However, the coadministration of inhibitors of both CYP2C8 and CYP2C9 with ibuprofen could have greater effects on the pharmacokinetics of both ibuprofen enantiomers.

3. Effects of trimethoprim and the *CYP2C8* genotype on pioglitazone

Trimethoprim moderately raised the plasma concentrations of pioglitazone. The increase in the $AUC_{0-\infty}$ of pioglitazone by trimethoprim (1.4-fold) is in line with previous trimethoprim interaction studies with the CYP2C8 substrates repaglinide (1.6-fold increase in $AUC_{0-\infty}$) and rosiglitazone (1.4-fold increase in $AUC_{0-\infty}$) (Niemi et al. 2004a, Niemi et al. 2004b). Pioglitazone has been reported to be metabolised by CYP2C8 and CYP3A4 (Hanefeld 2001, Jaakkola et al. 2006c), but the CYP3A4 inhibitor itraconazole has no significant effect on the pharmacokinetics of pioglitazone (Jaakkola et al. 2005). Providing a plausible explanation for this finding, the pioglitazone M-IV metabolite was formed predominantly by CYP2C8 at low, clinically relevant concentrations (<2 µM), whereas the importance of CYP3A4 increased at higher pioglitazone concentrations due to sigmoidal kinetics of M-IV formation by rhCYP3A4.

The *CYP2C8*3* allele was found to be associated with moderately decreased plasma concentrations of pioglitazone. Both the weight-adjusted $AUC_{0-\infty}$ and the M-IV/pioglitazone and M-III/pioglitazone $AUC_{0-\infty}$ ratios showed a gene-dose effect, with the values for the heterozygous *CYP2C8*3* allele carriers in between those of the homozygous carriers and the non-carrier group. Thus, as found previously with repaglinide and rosiglitazone (Niemi et al. 2003c, Kirchheiner et al. 2006), the *CYP2C8*3* allele seems to be associated with an increased metabolic clearance of pioglitazone *in vivo*.

Although the opposing effects of trimethoprim and the *CYP2C8*3* allele on pioglitazone were only moderate, trimethoprim increased the AUC of pioglitazone two-fold in some subjects, and the concentrations of the active metabolite M-IV were also increased by trimethoprim. The adverse effects of pioglitazone, which include weight gain and fluid retention, appear to be dose (concentration) dependent (Waugh et al. 2006). Thus, the efficacy and the occurrence of adverse effects of pioglitazone can be altered by drug interactions and genetic factors that alter CYP2C8 activity.

4. Effect of dosing interval on gemfibrozil-repaglinide interaction

The interaction between gemfibrozil and repaglinide persisted even with an interval of up to 12 hours between gemfibrozil and repaglinide administration, with a 5- to 7-fold mean increase in the AUC of repaglinide. Notably, the formation of M4, which is predominantly formed by CYP2C8 (Bidstrup et al. 2003, Kajosaari et al. 2005b), was almost abolished by gemfibrozil. By the time repaglinide was administered 12 hours after the last dose of gemfibrozil, the plasma concentrations of gemfibrozil and its 1-O-β-glucuronide had declined to very low levels. This supports the proposed mechanism-based inhibition of CYP2C8 by gemfibrozil 1-O-β-glucuronide as the main mechanism of this interaction (Ogilvie et al. 2006). Moreover, taking into account mechanism-based inhibition of CYP2C8 in a metabolic prediction model has markedly improved the predictions of the interactions of gemfibrozil with CYP2C8 substrates, compared to competitive inhibition alone (Hinton et al. 2008). In addition, gemfibrozil is concentrated in the liver, at least in rats (Sallustio et al. 1996, Sabordo et al. 1999), and thus, the concentrations of gemfibrozil and gemfibrozil 1-O-β-glucuronide in the liver can be much higher than those in plasma. The combination of high intracellular concentrations and mechanism-based inhibition of CYP2C8 could be sufficient to explain the high inhibition efficiency of gemfibrozil with CYP2C8 substrates

The results of this study have clinical implications concerning gemfibrozil interactions with repaglinide and CYP2C8 substrates in general. As the degree of interaction remained quite stable during the typical 12 hour dosing interval of gemfibrozil, the interaction cannot be avoided by administering gemfibrozil and repaglinide (or another CYP2C8 substrate) at different times. However, the interactions of gemfibrozil with repaglinide and possibly other drugs metabolised by CYP2C8 could be managed by decreasing the dose of the substrate drug, because similar dose adjustment is needed, regardless of the timing of the drug administration. On the other hand, although the gemfibrozil-repaglinide interaction persisted for at least 12 hours after the last gemfibrozil dose, it is not known how long this inhibition potential is maintained. Thus, care is warranted for at least several days when gemfibrozil is discontinued and a therapy with a drug that is metabolised by CYP2C8 is initiated.

5. General discussion

The significance of CYP2C8 in drug metabolism has been recognised only recently. Previously, research on CYP2C8 was hindered by the lack of selective inhibitors and probe substrates. However, recent findings have provided the means to study CYP2C8-mediated drug metabolism both *in vitro* and *in vivo*.

In vitro, paclitaxel 6-α-hydroxylation has been used as a selective probe reaction to study CYP2C8 activity (Rahman et al. 1994). More recently, amodiaquine N-desethylation has been reported to be a selective high affinity and turnover probe reaction for CYP2C8 (Li et al. 2002). Initially, quercetin was used as a probe *in vitro* inhibitor of CYP2C8. However, it has poor selectivity as it can also inhibit other CYPs, e.g. CYP3A4 (Ha et al. 1995). On the other hand, trimethoprim and especially montelukast are more selective inhibitors of CYP2C8, although the inhibitory effect of montelukast is highly related to protein concentration in the incubation medium (Wen et al. 2002, Walsky et al. 2005b). In addition, inhibitory monoclonal antibodies for CYP2C8 are commercially available (Venkatakrishnan et al. 2001). It is also of note that compounds previously thought to be selective inhibitors of other CYP enzymes can also inhibit CYP2C8. For example, ketoconazole and diethyldithiocarbamate, inhibitors of CYP3A4 and CYP2E1, respectively, cause significant inhibition of CYP2C8 as well (Ong et al. 2000).

Repaglinide and rosiglitazone have been recommended for use as *in vivo* probes for CYP2C8-mediated metabolism (Huang et al. 2007). In addition, pioglitazone can be considered as a possible probe substrate. Repaglinide, unlike pioglitazone and rosiglitazone, easily causes hypoglycaemia when used in healthy volunteers, and therefore, its use necessitates careful monitoring of blood glucose and follow-up of the subjects. On the other hand, repaglinide has been found to be the most sensitive CYP2C8 substrate for the effects of CYP2C8 inhibitors like gemfibrozil and trimethoprim (Niemi et al. 2003b, Niemi et al. 2004b). However, repaglinide is also a substrate of OATP1B1, and its pharmacokinetics is affected by the *SLCO1B1* genotype (Niemi et al. 2005b). In contrast, the pharmacokinetics of pioglitazone and rosiglitazone are unaffected by the *SLCO1B1* genotype (Kalliokoski et al. 2007). *In vitro*, the metabolism of rosiglitazone is catalysed mainly by CYP2C8, with a minor contribution from CYP2C9 (Baldwin et al. 1999), whereas pioglitazone is metabolised mainly by CYP2C8, and to a lesser degree by CYP3A4 (Jaakkola et al. 2006c). *In vivo*, gemfibrozil and trimethoprim raise the AUC of pioglitazone slightly more than that of rosiglitazone, suggesting that the role of CYP2C8 is slightly greater in the metabolism of pioglitazone than in the metabolism of rosiglitazone (Niemi et al. 2003a, Niemi et al. 2004a, Jaakkola et al. 2005). Thus, pioglitazone could serve as a more selective *in vivo* probe for CYP2C8 than rosiglitazone, with a better single dose safety profile than repaglinide. However, reliable monitoring of the pioglitazone active metabolites M-IV and M-III requires longer blood sampling schedules.

Trimethoprim and gemfibrozil are well established *in vivo* inhibitors of CYP2C8 (Niemi et al. 2003b, Niemi et al. 2004b, Huang et al. 2007). To date, many clinically relevant drug interactions involving CYP2C8 have been found to be caused by these two drugs (Table 8). On the other hand, some *in vitro* inhibitors of CYP2C8, such as montelukast and quercetin, have no effect *in vivo* (Kim et al. 2005, Kajosaari et al. 2006b, Jaakkola et al. 2006b). Thus,

other *in vivo* inhibitors of CYP2C8 that could cause clinically relevant drug interactions are currently not known. However, the CYP3A4 inhibitor ketoconazole is also a relatively potent inhibitor of CYP2C8 *in vitro* (Ong et al. 2000). Therefore, the effects of ketoconazole on the metabolism of some drugs *in vitro* and *in vivo* may be partially due to inhibition of CYP2C8. It can be speculated that this may have led to underestimation of the importance of CYP2C8 in drug metabolism, because ketoconazole is a widely used model inhibitor of CYP3A4.

Table 8. Drug interaction studies in humans involving inhibition of CYP2C8-mediated metabolism.

Substrate	Inhibitor	Fold increase in AUC	Reference
Cerivastatin	Gemfibrozil	5.59	Backman et al. 2002
Loperamide	Gemfibrozil	2.18	Study I
	Trimethoprim + Sulfamethoxazole	1.89	Kamali and Huang 1996
Pioglitazone	Gemfibrozil	3.22	Jaakkola et al. 2005
		3.39	Deng et al. 2005
	Montelukast	0.98	Jaakkola et al. 2006b
	Trimethoprim	1.42	Study IV
	Zafirlukast	1.00	Jaakkola et al. 2006b
Repaglinide	Gemfibrozil	8.12	Niemi et al. 2003b
	Gemfibrozil	7.02	Study V
	Montelukast	0.99	Kajosaari et al. 2006b
	Pioglitazone	0.90	Kajosaari et al. 2006a
	Trimethoprim	1.61	Niemi et al. 2004b
R-ibuprofen	Gemfibrozil	1.34	Study III
Rosiglitazone	Gemfibrozil	2.29	Niemi et al. 2003a
	Montelukast	1.02	Kim et al. 2007
	Trimethoprim	1.37	Niemi et al. 2004a
		1.31	Hruska et al. 2005
	Quercetin	0.99	Kim et al. 2005
Zopiclone	Gemfibrozil	0.99	Study II

The most notable drug interactions concerning CYP2C8 have been the gemfibrozil-repaglinide and gemfibrozil-cerivastatin interactions (Niemi et al. 2003b, Backman et al. 2002). Gemfibrozil greatly increased the plasma concentrations of repaglinide and cerivastatin (8.1- and 5.1-fold increases in AUC, respectively), and considerably enhanced and prolonged the blood glucose lowering effect of repaglinide. Cerivastatin, which is predominantly metabolised by CYP2C8 (Wang et al. 2002b), was withdrawn from the market in 2001 after an increase in the occurrence of rhabdomyolysis, including several fatal cases. In many of these incidents, cerivastatin had been used concomitantly with gemfibrozil (Staffa et al. 2002). Although other mechanisms could have contributed to this interaction, the increase in cerivastatin concentrations by gemfibrozil likely played a major role in the occurrence of these serious adverse effects (Backman et al. 2002, Neuvonen et al. 2006).

In vitro, parent gemfibrozil is a more potent inhibitor of CYP2C9 than of CYP2C8 (Wen et al. 2001, Wang et al. 2002b). *In vivo*, however, the effect of gemfibrozil on the pharmacokinetics of CYP2C9 substrate drugs has been found to be only minor (Niemi et al. 2001b, Lilja et al. 2005, Niemi et al. 2005a), while it has been shown to greatly increase the plasma concentrations of several CYP2C8 substrates (Table 8). This discrepancy is possibly explained by a metabolite of gemfibrozil, gemfibrozil 1-O-β-glucuronide, which is a potent and selective mechanism-based inhibitor of CYP2C8 (Ogilvie et al. 2006). Further *in vivo* evidence for the supposed mechanism-based inhibition of CYP2C8 was provided in study V, where the gemfibrozil-repaglinide interaction was maintained at least 12 hours after the last dose of gemfibrozil. However, gemfibrozil and its 1-O-β-glucuronide also inhibit OATP1B1 (Shitara et al. 2004), which could contribute to the gemfibrozil-repaglinide and gemfibrozil-cerivastatin interactions, as well as explain its effects on drugs that are not metabolised by CYP enzymes to a significant degree, such as pravastatin and rosuvastatin (Kyrklund et al. 2003, Schneck et al. 2004). Additionally, gemfibrozil has been found to reduce the Cl_{renal} of pravastatin (Kyrklund et al. 2003), as well as the Cl_{renal} of loperamide and zopiclone-N-oxide in studies I and II. Recently, gemfibrozil and its metabolites were shown to inhibit the renal transporter OAT3, providing a possible explanation for the effect of gemfibrozil on the Cl_{renal} of pravastatin (Nakagomi-Hagihara et al. 2007a).

In this thesis work, using gemfibrozil as a probe inhibitor, the rank order of the importance of CYP2C8-mediated metabolism *in vivo* was found to be repaglinide > loperamide > ibuprofen > zopiclone. The results with loperamide and ibuprofen were in line with previous *in vitro* results (Hamman et al. 1997, Kim et al. 2004). Surprisingly, the plasma concentrations of zopiclone were found not to be affected by gemfibrozil. This is at variance with earlier *in vitro* findings, and suggests that the high zopiclone concentrations used in the earlier *in vitro* study led to overestimation of the role of CYP2C8 in the metabolism of zopiclone (Becquemont et al. 1999). On the other hand, the role of CYP2C8 in the metabolism certain other drugs, such as cerivastatin and repaglinide, has apparently been underestimated on the basis of *in vitro* experiments (Boberg et al. 1997, Guay 1998). Whether this underestimation is due to selection of supratherapeutic substrate concentrations or other experimental issues remains to be elucidated.

The allele frequency of *CYP2C8*3* has been reported to be 10-20% in the Caucasian population (Totah and Rettie 2005). Thus, this quite common polymorphism could be an important factor determining the inter-individual variability in the pharmacokinetics of drugs that are CYP2C8 substrates. However, the results of pharmacogenetic studies on CYP2C8 have been conflicting. *In vitro*, CYP2C8.3 protein has been found to have decreased activity to metabolise its substrates (Dai et al. 2001, Bahadur et al. 2002). *In vivo*, the effect of the *CYP2C8*3* allele seems to be substrate specific, resulting in increased (repaglinide and rosiglitazone) and decreased (ibuprofen) activity phenotypes, compared to those of the reference genotype (*CYP2C8*1/*1*) (Niemi et al. 2003c, Kirchheiner et al. 2006, García-Martín et al. 2004). However, the results with ibuprofen could be confounded by the fact that it is metabolised by both CYP2C8 and CYP2C9 (Hamman et al. 1997), and that there is a linkage disequilibrium between the *CYP2C8*3* and *CYP2C9*2* variant alleles (Yasar et al. 2002). On the other hand, another study showed no significant difference in ibuprofen pharmacokinetics between the heterozygous and homozygous *CYP2C9*2* allele carriers and the subjects with the *CYP2C9*1/*1* genotype (Kirchheiner et al. 2002). This contradicts the

former results, as most of these *CYP2C9*2* carriers likely had the same number of *CYP2C8*3* alleles (either homozygous in both or heterozygous in both). The view that the *CYP2C8*3* allele is associated most often with increased metabolic activity *in vivo* was supported by the findings of study IV with pioglitazone. However, general conclusions about all substrates of CYP2C8 can not yet be made, because there may be substrate-specific effects.

CONCLUSIONS

Study I
Gemfibrozil, itraconazole, and in particular their combination, raised the plasma concentrations of loperamide. This indicates that CYP2C8 and CYP3A4 are important in the elimination of loperamide and that inhibitors of both CYP2C8 and CYP3A4 can increase loperamide concentrations. Care is warranted if loperamide is used together with drugs that inhibit both CYP2C8 and CYP3A4 enzymes.

Study II
Gemfibrozil did not increase plasma concentrations or pharmacodynamic effects of zopiclone, and thus, zopiclone is not significantly metabolised by CYP2C8 *in vivo*. Although the concentrations of the active metabolites of zopiclone were moderately affected, concomitant use of gemfibrozil and zopiclone can be considered safe.

Study III
Gemfibrozil raised the plasma concentrations of R-ibuprofen moderately, but did not affect those of the active enantiomer S-ibuprofen. Thus, CYP2C8 is only of limited importance in the metabolism of ibuprofen. The clinical significance of the interaction between gemfibrozil and ibuprofen is limited, and the inhibition of CYP2C8 is not likely to affect the efficacy or tolerability of ibuprofen.

Study IV
Trimethoprim moderately raised pioglitazone plasma concentrations. *In vitro*, the importance of CYP2C8-mediated metabolism of pioglitazone was high at clinically relevant pioglitazone concentrations. The *CYP2C8*3* allele was associated with decreased plasma concentrations of pioglitazone. Thus, both CYP2C8 inhibition and pharmacogenetic factors can affect the pharmacokinetics of pioglitazone and possibly affect the efficacy and safety of pioglitazone.

Study V
The possibility of a gemfibrozil-repaglinide interaction persisted at least up to 12 hours after the previous gemfibrozil dose. The main mechanism of the interaction is likely to be irreversible mechanism-based inhibition of CYP2C8 by gemfibrozil 1-O-β-glucuronide. A similar long-lasting interaction potential of gemfibrozil with other CYP2C8 substrate drugs can also be expected.

General conclusions
These studies clarified the role of CYP2C8 in the metabolism and interactions of different drugs. However, some of the *in vivo* findings were not directly predictable from previous *in vitro* data. Thus, these results emphasise the need for clinical drug interaction studies to confirm *in vitro* findings. The results also confirm the role of gemfibrozil as a model inhibitor for CYP2C8 *in vivo*, and that this interaction potential is long-lasting. Also, genetic variation can affect the pharmacokinetics of drugs metabolised by CYP2C8, but possible substrate-specific effects require the use of different substrates.

ACKNOWLEDGEMENTS

This work was carried out in the Department of Clinical Pharmacology, University of Helsinki, during the years 2003 to 2008. I am grateful to all those who helped me during this period.

I am especially grateful to my supervisor, Professor Pertti Neuvonen. He always had the time for me whenever I needed it. The working environment he has created is truly exceptional, and it has been a privilege to work under his dedicated supervision. His knowledge and lifelong work in the field of clinical pharmacology are highly impressive.

I am most grateful to my other supervisor, Docent Janne Backman. He has taught me almost everything I know about research methodology, from statistics to pharmacokinetics. His wide knowledge of the field is admirable, and the guidance he has provided has been invaluable to carry out this thesis work.

I would like to thank Professor Risto Juvonen and Docent Kari Aranko for their constructive comments and prompt review of this thesis.

I am thankful to Mary Metzler for editing the language of this thesis.

I am grateful to docent Mikko Niemi for his remarkable input and guidance in study I, and also for his generous help and valuable comments. I am thankful to Annikka Kalliokoski for pleasant company and collaboration in study V.

Thanks are also due to Hanna Fredrikson, Jouko Laitila and Mikko Neuvonen for their skillful drug concentration measurements. I am especially thankful to Jouko for teaching me the basics of laboratory work and for his valuable help in carrying out the *in vitro* experiments. Eija Mäkinen-Pulli, Kerttu Mårtensson and Lisbet Partanen are warmly thanked for their skilful technical assistance and friendly company. Without you, this work would not have been possible.

I wish to thank all my colleagues at the Department of Clinical Pharmacology: Kati Ahonen, Samuel Fanta, Johanna Honkalammi, Kalle Hoppu (and the staff of the Poison Information Center), Mika Isohanni, Tiina Jaakkola, Jenni Keskitalo, Kaisa Kurkinen, Carl Kyrklund, Jari Lilja, Heli Malm, Anna-Riitta Pasanen, Kari Raaska, Tuure Saarinen, Han Yi and Xiang Xiaoqiang. I want to thank Marja Pasanen for her friendship and support; Lauri Kajosaari, Marjo Karjalainen and Marika Schröder (Granfors), for their invaluable advice and friendly company. Furthermore, I am grateful to Tuija Itkonen for assistance in many practical manners. Thanks to all of you for creating the outstanding atmosphere at KliFa!

This work was financially supported by the Clinical Drug Research Graduate School and the Finnish Medical Society Duodecim, which are gratefully acknowledged.

I would like to thank my friends in and out of the medical school for good times spent together. I especially wish to thank my friends in Asikkala for their long-lasting friendship. There would be far too many important people to start naming them all, so I hope these general words will suffice.

Finally, I would like to thank my parents, Tarja and Pekka, and my brother Matti for their support and encouragement.

Helsinki, June 17th, 2008

Aleksi Tornio

REFERENCES

Abdel-Rahman SM, Marcucci K, Boge T, Gotschall RR, Kearns GL, Leeder JS. Potent inhibition of cytochrome P-450 2D6-mediated dextromethorphan O-demethylation by terbinafine. *Drug Metab Dispos* 1999;27:770-5.

Adams SS, Bresloff P, Mason CG. Pharmacological differences between the optical isomers of ibuprofen: evidence for metabolic inversion of the (-)-isomer. *J Pharm Pharmacol* 1976;28:256-7.

Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet* 1999;353:717-9.

Albengres E, Le Louët H, Tillement JP. Systemic antifungal agents. Drug interactions of clinical significance. *Drug Saf* 1998;18:83-97.

Allain H, Delahaye C, Le Coz F, Blin P, Decombe R, Martinet JP. Postmarketing surveillance of zopiclone in insomnia: analysis of 20,513 cases. *Sleep* 1991;14:408-13.

Aranko K, Luurila H, Backman JT, Neuvonen PJ, Olkkola KT. The effect of erythromycin on the pharmacokinetics and pharmacodynamics of zopiclone. *Br J Clin Pharmacol* 1994;38:363-7.

Back DJ, Tjia JF. Comparative effects of the antimycotic drugs ketoconazole, fluconazole, itraconazole and terbinafine on the metabolism of cyclosporin by human liver microsomes. *Br J Clin Pharmacol* 1991;32:624-6.

Backman JT, Olkkola KT, Neuvonen PJ. Rifampin drastically reduces plasma concentrations and effects of oral midazolam. *Clin Pharmacol Ther* 1996a;59:7-13.

Backman JT, Olkkola KT, Ojala M, Laaksovirta H, Neuvonen PJ. Concentrations and effects of oral midazolam are greatly reduced in patients treated with carbamazepine or phenytoin. *Epilepsia* 1996b;37:253-7.

Backman JT, Kyrklund C, Neuvonen M, Neuvonen PJ. Gemfibrozil greatly increases plasma concentrations of cerivastatin. *Clin Pharmacol Ther* 2002;72:685-91.

Bahadur N, Leathart JB, Mutch E, Steimel-Crespi D, Dunn SA, Gilissen R, Houdt JV, Hendrickx J, Mannens G, Bohets H, Williams FM, Armstrong M, Crespi CL, Daly AK. CYP2C8 polymorphisms in Caucasians and their relationship with paclitaxel 6alpha-hydroxylase activity in human liver microsomes. *Biochem Pharmacol* 2002;64:1579-89.

Baldwin SJ, Bloomer JC, Smith GJ, Ayrton AD, Clarke SE, Chenery RJ. Ketoconazole and sulphaphenazole as the respective selective inhibitors of P4503A and 2C9. *Xenobiotica* 1995;25:261-70.

Baldwin SJ, Clarke SE, Chenery RJ. Characterization of the cytochrome P450 enzymes involved in the in vitro metabolism of rosiglitazone. *Br J Clin Pharmacol* 1999;48:424-32.

Becquemont L, Mouajjah S, Escaffre O, Beaune P, Funck-Brentano C, Jaillon P. Cytochrome P-450 3A4 and 2C8 are involved in zopiclone metabolism. *Drug Metab Dispos* 1999;27:1068-73.

Beedham C. The role of non-P450 enzymes in drug oxidation. *Pharm World Sci* 1997;19:255-63.

Bertilsson L, Carrillo JA, Dahl ML, Llerena A, Alm C, Bondesson U, Lindström L, Rodriguez de la Rubia I, Ramos S, Benitez J. Clozapine disposition covaries with CYP1A2 activity determined by a caffeine test. *Br J Clin Pharmacol* 1994;38:471-3.

Bidstrup TB, Bjørnsdottir I, Sidelmann UG, Thomsen MS, Hansen KT. CYP2C8 and CYP3A4 are the principal enzymes involved in the human in vitro biotransformation of the insulin secretagogue repaglinide. *Br J Clin Pharmacol* 2003;56:305-14.

Bidstrup TB, Stilling N, Damkier P, Scharling B, Thomsen MS, Brøsen K. Rifampicin seems to act as both an inducer and an inhibitor of the metabolism of repaglinide. *Eur J Clin Pharmacol* 2004;60:109-14.

Bidstrup TB, Damkier P, Olsen AK, Ekblom M, Karlsson A, Brøsen K. The impact of CYP2C8 polymorphism and grapefruit juice on the pharmacokinetics of repaglinide. *Br J Clin Pharmacol* 2006;61:49-57.

Boberg M, Angerbauer R, Fey P, Kanhai WK, Karl W, Kern A, Ploschke J, Radtke M. Metabolism of cerivastatin by human liver microsomes in vitro. Characterization of primary metabolic pathways and of cytochrome P450 isozymes involved. *Drug Metab Dispos* 1997;25:321-31.

Bort R, Macé K, Boobis A, Gómez-Lechón MJ, Pfeifer A, Castell J. Hepatic metabolism of diclofenac: role of human CYP in the minor oxidative pathways. *Biochem Pharmacol* 1999;58:787-96.

Brøsen K, Skjelbo E, Rasmussen BB, Poulsen HE, Loft S. Fluvoxamine is a potent inhibitor of cytochrome P4501A2. *Biochem Pharmacol* 1993;45:1211-4.

Busby WF, Jr., Ackermann JM, Crespi CL. Effect of methanol, ethanol, dimethyl sulfoxide, and acetonitrile on in vitro activities of cDNA-expressed human cytochromes P-450. *Drug Metab Dispos* 1999;27:246-9.

Carlson JN, Haskew R, Wacker J, Maisonneuve IM, Glick SD, Jerussi TP. Sedative and anxiolytic effects of zopiclone's enantiomers and metabolite. *Eur J Pharmacol* 2001;415:181-9.

Chan LM, Lowes S, Hirst BH. The ABCs of drug transport in intestine and liver: efflux proteins limiting drug absorption and bioavailability. *Eur J Pharm Sci* 2004;21:25-51.

Chauret N, Gauthier A, Nicoll-Griffith DA. Effect of common organic solvents on in vitro cytochrome P450-mediated metabolic activities in human liver microsomes. *Drug Metab Dispos* 1998;26:1-4.

Cribb AE, Peyrou M, Muruganandan S, Schneider L. The endoplasmic reticulum in xenobiotic toxicity. *Drug Metab Rev* 2005;37:405-42.

Culy CR, Jarvis B. Repaglinide: a review of its therapeutic use in type 2 diabetes mellitus. *Drugs* 2001;61:1625-60.

Dagenais C, Graff CL, Pollack GM. Variable modulation of opioid brain uptake by P-glycoprotein in mice. *Biochem Pharmacol* 2004;67:269-76.

Dai D, Zeldin DC, Blaisdell JA, Chanas B, Coulter SJ, Ghanayem BI, Goldstein JA. Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. *Pharmacogenetics* 2001;11:597-607.

Daly AK. Pharmacogenetics of the major polymorphic metabolizing enzymes. *Fundam Clin Pharmacol* 2003;17:27-41.

Daly AK. Significance of the minor cytochrome P450 3A isoforms. *Clin Pharmacokinet* 2006;45:13-31.

Davies NM. Clinical pharmacokinetics of ibuprofen. The first 30 years. *Clin Pharmacokinet* 1998;34:101-54.

De Beule K, Van Gestel J. Pharmacology of itraconazole. *Drugs* 2001;61 Suppl 1:27-37.

Deng LJ, Wang F, Li HD. Effect of gemfibrozil on the pharmacokinetics of pioglitazone. *Eur J Clin Pharmacol* 2005;61:831-6.

Desta Z, Zhao X, Shin JG, Flockhart DA. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. *Clin Pharmacokinet* 2002;41:913-58.

Dickins M. Induction of cytochromes P450. *Curr Top Med Chem* 2004;4:1745-66.

Doser K, Meyer B, Nitsche V, Binkert-Graber P. Bioequivalence evaluation of two different oral formulations of loperamide (Diarex Lactab vs Imodium capsules). *Int J Clin Pharmacol Ther* 1995;33:431-6.

Dresser GK, Spence JD, Bailey DG. Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. *Clin Pharmacokinet* 2000;38:41-57.

Dresser MJ, Leabman MK, Giacomini KM. Transporters involved in the elimination of drugs in the kidney: organic anion transporters and organic cation transporters. *J Pharm Sci* 2001;90:397-421.

Dürr D, Stieger B, Kullak-Ublick GA, Rentsch KM, Steinert HC, Meier PJ, Fattinger K. St John's Wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. *Clin Pharmacol Ther* 2000;68:598-604.

Eckland DA, Danhof M. Clinical pharmacokinetics of pioglitazone. *Exp Clin Endocrinol Diabetes* 2000;108(Suppl 2):234-242.

Enayetallah AE, French RA, Thibodeau MS, Grant DF. Distribution of soluble epoxide hydrolase and of cytochrome P450 2C8, 2C9, and 2J2 in human tissues. *J Histochem Cytochem* 2004;52:447-54.

Evans AM. Enantioselective pharmacodynamics and pharmacokinetics of chiral non-steroidal anti-inflammatory drugs. *Eur J Clin Pharmacol* 1992;42:237-56.

Fernandez C, Martin C, Gimenez F, Farinotti R. Clinical pharmacokinetics of zopiclone. *Clin Pharmacokinet* 1995;29:431-41.

Fischer V, Einolf HJ, Cohen D. Efflux transporters and their clinical relevance. *Mini Rev Med Chem* 2005;5:183-95.

Fisher MB, Paine MF, Strelevitz TJ, Wrighton SA. The role of hepatic and extrahepatic UDP-glucuronosyltransferases in human drug metabolism. *Drug Metab Rev* 2001;33:273-97.

Fuhr U, Anders EM, Mahr G, Sörgel F, Staib AH. Inhibitory potency of quinolone antibacterial agents against cytochrome P450IA2 activity in vivo and in vitro. *Antimicrob Agents Chemother* 1992;36:942-8.

Fuhr U. Induction of drug metabolising enzymes: pharmacokinetic and toxicological consequences in humans. *Clin Pharmacokinet* 2000;38:493-504.

Furuta T, Shirai N, Sugimoto M, Ohashi K, Ishizaki T. Pharmacogenomics of proton pump inhibitors. *Pharmacogenomics* 2004;5:181-202.

Gamage N, Barnett A, Hempel N, Duggleby RG, Windmill KF, Martin JL, McManus ME. Human sulfotransferases and their role in chemical metabolism. *Toxicol Sci* 2006;90:5-22.

García-Martín E, Martínez C, Tabarés B, Frías J, Agúndez JA. Interindividual variability in ibuprofen pharmacokinetics is related to interaction of cytochrome P450 2C8 and 2C9 amino acid polymorphisms. *Clin Pharmacol Ther* 2004;76:119-27.

Geick A, Eichelbaum M, Burk O. Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J Biol Chem* 2001;276:14581-7.

Gerbal-Chaloin S, Pascussi JM, Pichard-Garcia L, Daujat M, Waechter F, Fabre JM, Carrère N, Maurel P. Induction of CYP2C genes in human hepatocytes in primary culture. *Drug Metab Dispos* 2001;29:242-51.

Ghanbari F, Rowland-Yeo K, Bloomer JC, Clarke SE, Lennard MS, Tucker GT, Rostami-Hodjegan A. A critical evaluation of the experimental design of studies of mechanism based enzyme inhibition, with implications for in vitro-in vivo extrapolation. *Curr Drug Metab* 2006;7:315-34.

Gibson GG, Skett P. *Introduction to drug metabolism*. Cheltenham: Nelson Thornes; 2001. p. 1-34.

Goa KL, Heel RC. Zopiclone. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy as an hypnotic. *Drugs* 1986;32:48-65.

Gonzalez FJ, Tukey RH. Drug metabolism. In: Brunton LL, Lazo JS, Parker KL. *Goodman and Gillman's the pharmacological basis of therapeutics*. New York: McGraw-Hill; 2006. p. 71-91

Granfors MT, Backman JT, Laitila J, Neuvonen PJ. Tizanidine is mainly metabolized by cytochrome p450 1A2 in vitro. *Br J Clin Pharmacol* 2004a;57:349-53.

Granfors MT, Backman JT, Neuvonen M, Ahonen J, Neuvonen PJ. Fluvoxamine drastically increases concentrations and effects of tizanidine: a potentially hazardous interaction. *Clin Pharmacol Ther* 2004b;75:331-41.

Grant SM, Clissold SP. Itraconazole. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in superficial and systemic mycoses. *Drugs* 1989;37:310-44.

Guay DR. Repaglinide, a novel, short-acting hypoglycemic agent for type 2 diabetes mellitus. *Pharmacotherapy* 1998;18:1195-204.

Ha HR, Chen J, Leuenberger PM, Freiburghaus AU, Follath F. In vitro inhibition of midazolam and quinidine metabolism by flavonoids. *Eur J Clin Pharmacol* 1995;48:367-71.

Hamman MA, Thompson GA, Hall SD. Regioselective and stereoselective metabolism of ibuprofen by human cytochrome P450 2C. *Biochem Pharmacol* 1997;54:33-41.

Hanefeld M. Pharmacokinetics and clinical efficacy of pioglitazone. *Int J Clin Pract Suppl* 2001;19-25.

Hatorp V, Huang WC, Strange P. Repaglinide pharmacokinetics in healthy young adult and elderly subjects. *Clin Ther* 1999;21:702-10.

Hatorp V. Clinical pharmacokinetics and pharmacodynamics of repaglinide. *Clin Pharmacokinet* 2002;41:471-83.

Hatorp V, Hansen KT, Thomsen MS. Influence of drugs interacting with CYP3A4 on the pharmacokinetics, pharmacodynamics, and safety of the prandial glucose regulator repaglinide. *J Clin Pharmacol* 2003;43:649-60.

Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. *Annu Rev Pharmacol Toxicol* 2005;45:51-88.

Henningsson A, Marsh S, Loos WJ, Karlsson MO, Garsa A, Mross K, Mielke S, Viganò L, Locatelli A, Verweij J, Sparreboom A, McLeod HL. Association of CYP2C8, CYP3A4, CYP3A5, and ABCB1 polymorphisms with the pharmacokinetics of paclitaxel. *Clin Cancer Res* 2005;11:8097-104.

Henry D, Lim LL, Garcia Rodriguez LA, Perez Gutthann S, Carson JL, Griffin M, Savage R, Logan R, Moride Y, Hawkey C, Hill S, Fries JT. Variability in risk of gastrointestinal complications with individual non-steroidal anti-inflammatory drugs: results of a collaborative meta-analysis. *BMJ* 1996;312:1563-6.

Hinton LK, Galetin A, Houston JB. Multiple inhibition mechanisms and prediction of drug-drug interactions: status of metabolism and transporter models as exemplified by gemfibrozil-drug interactions. *Pharm Res* 2008;25:1063-74.

Ho RH, Kim RB. Transporters and drug therapy: implications for drug disposition and disease. *Clin Pharmacol Ther* 2005;78:260-77.

Hollenberg PF. Characteristics and common properties of inhibitors, inducers, and activators of CYP enzymes. *Drug Metab Rev* 2002;34:17-35.

Hruska MW, Amico JA, Langaee TY, Ferrell RE, Fitzgerald SM, Frye RF. The effect of trimethoprim on CYP2C8 mediated rosiglitazone metabolism in human liver microsomes and healthy subjects. *Br J Clin Pharmacol* 2005;59:70-9.

Huang SM, Temple R, Throckmorton DC, Lesko LJ. Drug interaction studies: study design, data analysis, and implications for dosing and labeling. *Clin Pharmacol Ther* 2007;81:298-304.

Hynninen VV, Olkkola KT, Leino K, Lundgren S, Neuvonen PJ, Rane A, Valtonen M, Vyyryläinen H, Laine K. Effects of the antifungals voriconazole and fluconazole on the pharmacokinetics of s-(+)- and R-(-)-Ibuprofen. *Antimicrob Agents Chemother* 2006;50:1967-72.

Imai T, Taketani M, Shii M, Hosokawa M, Chiba K. Substrate specificity of carboxylesterase isozymes and their contribution to hydrolase activity in human liver and small intestine. *Drug Metab Dispos* 2006;34:1734-41.

Imodium label information. Available from http://www.fda.gov/medwatch/SAFETY/2005/Oct_PI/Imodium_PI.pdf. Accessed Nov 24 2007.

Ingelman-Sundberg M, Daly AK, Oscarson M, Nebert DW. Human cytochrome P450 (CYP) genes: recommendations for the nomenclature of alleles. *Pharmacogenetics* 2000;10:91-3.

Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J* 2005;5:6-13.

Isoherranen N, Kunze KL, Allen KE, Nelson WL, Thummel KE. Role of itraconazole metabolites in CYP3A4 inhibition. *Drug Metab Dispos* 2004;32:1121-31.

Jaakkola T, Backman JT, Neuvonen M, Neuvonen PJ. Effects of gemfibrozil, itraconazole, and their combination on the pharmacokinetics of pioglitazone. *Clin Pharmacol Ther* 2005;77:404-14.

Jaakkola T, Backman JT, Neuvonen M, Laitila J, Neuvonen PJ. Effect of rifampicin on the pharmacokinetics of pioglitazone. *Br J Clin Pharmacol* 2006a;61:70-8.

Jaakkola T, Backman JT, Neuvonen M, Niemi M, Neuvonen PJ. Montelukast and zafirlukast do not affect the pharmacokinetics of the CYP2C8 substrate pioglitazone. *Eur J Clin Pharmacol* 2006b;62:503-9.

Jaakkola T, Laitila J, Neuvonen PJ, Backman JT. Pioglitazone is metabolised by CYP2C8 and CYP3A4 in vitro: potential for interactions with CYP2C8 inhibitors. *Basic Clin Pharmacol Toxicol* 2006c;99:44-51.

Jalava KM, Olkkola KT, Neuvonen PJ. Effect of itraconazole on the pharmacokinetics and pharmacodynamics of zopiclone. *Eur J Clin Pharmacol* 1996;51:331-4.

Jalava KM, Partanen J, Neuvonen PJ. Itraconazole decreases renal clearance of digoxin. *Ther Drug Monit* 1997;19:609-13.

Josephy PD, Guengerich FP, Miners JO. "Phase I and Phase II" drug metabolism: terminology that we should phase out? *Drug Metab Rev* 2005;37:575-80.

Kajosaari LI, Laitila J, Neuvonen PJ, Backman JT. Metabolism of repaglinide by CYP2C8 and CYP3A4 in vitro: effect of fibrates and rifampicin. *Basic Clin Pharmacol Toxicol* 2005a;97:249-56.

Kajosaari LI, Niemi M, Neuvonen M, Laitila J, Neuvonen PJ, Backman JT. Cyclosporine markedly raises the plasma concentrations of repaglinide. *Clin Pharmacol Ther* 2005b;78:388-99.

Kajosaari LI, Jaakkola T, Neuvonen PJ, Backman JT. Pioglitazone, an in vitro inhibitor of CYP2C8 and CYP3A4, does not increase the plasma concentrations of the CYP2C8 and CYP3A4 substrate repaglinide. *Eur J Clin Pharmacol* 2006a;62:217-23.

Kajosaari LI, Niemi M, Backman JT, Neuvonen PJ. Telithromycin, but not montelukast, increases the plasma concentrations and effects of the cytochrome P450 3A4 and 2C8 substrate repaglinide. *Clin Pharmacol Ther* 2006b;79:231-42.

Kalliokoski A, Neuvonen M, Neuvonen PJ, Niemi M. No significant effect of SLCO1B1 polymorphism on the pharmacokinetics of rosiglitazone and pioglitazone. *Br J Clin Pharmacol* 2007;65:78-86.

Kamali F, Huang ML. Increased systemic availability of loperamide after oral administration of loperamide and loperamide oxide with cotrimoxazole. *Br J Clin Pharmacol* 1996;41:125-8.

Karjalainen MJ, Neuvonen PJ, Backman JT. Rofecoxib is a potent, metabolism-dependent inhibitor of CYP1A2: implications for in vitro prediction of drug interactions. *Drug Metab Dispos* 2006;34:2091-6.

Kerr BM, Thummel KE, Wurden CJ, Klein SM, Kroetz DL, Gonzalez FJ, Levy RH. Human liver carbamazepine metabolism. Role of CYP3A4 and CYP2C8 in 10,11-epoxide formation. *Biochem Pharmacol* 1994;47:1969-79.

Killinger JM, Weintraub HS, Fuller BL. Human pharmacokinetics and comparative bioavailability of loperamide hydrochloride. *J Clin Pharmacol* 1979;19:211-8.

Kim KA, Chung J, Jung DH, Park JY. Identification of cytochrome P450 isoforms involved in the metabolism of loperamide in human liver microsomes. *Eur J Clin Pharmacol* 2004;60:575-81.

Kim KA, Park PW, Kim HK, Ha JM, Park JY. Effect of quercetin on the pharmacokinetics of rosiglitazone, a CYP2C8 substrate, in healthy subjects. *J Clin Pharmacol* 2005;45:941-6.

Kim KA, Park PW, Kim KR, Park JY. Effect of multiple doses of montelukast on the pharmacokinetics of rosiglitazone, a CYP2C8 substrate, in humans. *Br J Clin Pharmacol* 2007;63:339-45.

Kim RB. Drugs as P-glycoprotein substrates, inhibitors, and inducers. *Drug Metab Rev* 2002;34:47-54.

Kimonen T, Juvonen RO, Alhava E, Pasanen M. The inhibition of CYP enzymes in mouse and human liver by pilocarpine. *Br J Pharmacol* 1995;114:832-6.

Kirchheiner J, Meineke I, Freytag G, Meisel C, Roots I, Brockmöller J. Enantiospecific effects of cytochrome P450 2C9 amino acid variants on ibuprofen pharmacokinetics and on the inhibition of cyclooxygenases 1 and 2. *Clin Pharmacol Ther* 2002;72:62-75.

Kirchheiner J, Thomas S, Bauer S, Tomalik-Scharte D, Hering U, Doroshyenko O, Jetter A, Stehle S, Tsahuridu M, Meineke I, Brockmöller J, Fuhr U. Pharmacokinetics and pharmacodynamics of rosiglitazone in relation to CYP2C8 genotype. *Clin Pharmacol Ther* 2006;80:657-67.

Klein K, Saussele T, Toscano C, Blievernicht J, Eichelbaum M, Schwab M, Zanger UM. Comprehensive analysis of drug metabolizing cytochromes P450 in human liver. In: *MDO book of abstracts*. Budapest: 2006. p. 127.

Klose TS, Blaisdell JA, Goldstein JA. Gene structure of CYP2C8 and extrahepatic distribution of the human CYP2Cs. *J Biochem Mol Toxicol* 1999;13:289-95.

Krishna DR, Klotz U. Extrahepatic metabolism of drugs in humans. *Clin Pharmacokinet* 1994;26:144-60.

Kumar S, Samuel K, Subramanian R, Braun MP, Stearns RA, Chiu SH, Evans DC, Baillie TA. Extrapolation of diclofenac clearance from in vitro microsomal metabolism data: role of acyl glucuronidation and sequential oxidative metabolism of the acyl glucuronide. *J Pharmacol Exp Ther* 2002;303:969-78.

Kyrklund C, Backman JT, Neuvonen M, Neuvonen PJ. Gemfibrozil increases plasma pravastatin concentrations and reduces pravastatin renal clearance. *Clin Pharmacol Ther* 2003;73:538-44.

König J, Seithel A, Gradhand U, Fromm MF. Pharmacogenomics of human OATP transporters. *Naunyn Schmiedebergs Arch Pharmacol* 2006;372:432-43.

Lamba JK, Lin YS, Thummel K, Daly A, Watkins PB, Strom S, Zhang J, Schuetz EG. Common allelic variants of cytochrome P4503A4 and their prevalence in different populations. *Pharmacogenetics* 2002;12:121-32.

Lau YY, Huang Y, Frassetto L, Benet LZ. Effect of OATP1B transporter inhibition on the pharmacokinetics of atorvastatin in healthy volunteers. *Clin Pharmacol Ther* 2007;81:194-204.

Lee CR, North KE, Bray MS, Couper DJ, Heiss G, Zeldin DC. CYP2J2 and CYP2C8 polymorphisms and coronary heart disease risk: the Atherosclerosis Risk in Communities (ARIC) study. *Pharmacogenet Genomics* 2007;17:349-58.

Lee EJ, Williams K, Day R, Graham G, Champion D. Stereoselective disposition of ibuprofen enantiomers in man. *Br J Clin Pharmacol* 1985;19:669-74.

Lewis DF. 57 varieties: the human cytochromes P450. *Pharmacogenomics* 2004;5:305-18.

Li XQ, Björkman A, Andersson TB, Ridderström M, Masimirembwa CM. Amodiaquine clearance and its metabolism to N-desethylamodiaquine is mediated by CYP2C8: a new high affinity and turnover enzyme-specific probe substrate. *J Pharmacol Exp Ther* 2002;300:399-407.

Lilja JJ, Backman JT, Neuvonen PJ. Effect of gemfibrozil on the pharmacokinetics and pharmacodynamics of racemic warfarin in healthy subjects. *Br J Clin Pharmacol* 2005;59:433-9.

Lin JH, Lu AY. Inhibition and induction of cytochrome P450 and the clinical implications. *Clin Pharmacokinet* 1998;35:361-90.

Lin JH, Yamazaki M. Role of P-glycoprotein in pharmacokinetics: clinical implications. *Clin Pharmacokinet* 2003;42:59-98.

Läpple F, von Richter O, Fromm MF, Richter T, Thon KP, Wisser H, Griese EU, Eichelbaum M, Kivistö KT. Differential expression and function of CYP2C isoforms in human intestine and liver. *Pharmacogenetics* 2003;13:565-75.

Madan A, Usuki E, Burton LA, Ogilvie BW, Parkinson A. In vitro approaches for studying the inhibition of drug-metabolizing enzymes and identifying the drug-metabolizing enzymes responsible for the metabolism of drugs. In: Rodrigues AD. *Drug-drug interactions*. New York: Marcel Dekker; 2002. p. 217-294

Malaiyandi V, Sellers EM, Tyndale RF. Implications of CYP2A6 genetic variation for smoking behaviors and nicotine dependence. *Clin Pharmacol Ther* 2005;77:145-58.

Mano Y, Usui T, Kamimura H. The UDP-glucuronosyltransferase 2B7 isozyme is responsible for gemfibrozil glucuronidation in the human liver. *Drug Metab Dispos* 2007;35:2040-4.

Martínez C, García-Martín E, Blanco G, Gamito FJ, Ladero JM, Agúndez JA. The effect of the cytochrome P450 CYP2C8 polymorphism on the disposition of (R)-ibuprofen enantiomer in healthy subjects. *Br J Clin Pharmacol* 2005;59:62-9.

Masters PA, O'Bryan TA, Zurlo J, Miller DQ, Joshi N. Trimethoprim-sulfamethoxazole revisited. *Arch Intern Med* 2003;163:402-10.

Meyer UA. Overview of enzymes of drug metabolism. *J Pharmacokinet Biopharm* 1996;24:449-59.

Miller DB, Spence JD. Clinical pharmacokinetics of fibric acid derivatives (fibrates). *Clin Pharmacokinet* 1998;34:155-62.

Miners JO, Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. *Br J Clin Pharmacol* 1998;45:525-38.

Nadin L, Murray M. Participation of CYP2C8 in retinoic acid 4-hydroxylation in human hepatic microsomes. *Biochem Pharmacol* 1999;58:1201-8.

Nakagomi-Hagihara R, Nakai D, Tokui T. Inhibition of human organic anion transporter 3 mediated pravastatin transport by gemfibrozil and the metabolites in humans. *Xenobiotica* 2007a;37:416-26.

Nakagomi-Hagihara R, Nakai D, Tokui T, Abe T, Ikeda T. Gemfibrozil and its glucuronide inhibit the hepatic uptake of pravastatin mediated by OATP1B1. *Xenobiotica* 2007b;37:474-86.

Nebert DW, Russell DW. Clinical importance of the cytochromes P450. *Lancet* 2002;360:1155-62.

Neuvonen PJ, Kantola T, Kivistö KT. Simvastatin but not pravastatin is very susceptible to interaction with the CYP3A4 inhibitor itraconazole. *Clin Pharmacol Ther* 1998;63:332-41.

Neuvonen PJ, Niemi M, Backman JT. Drug interactions with lipid-lowering drugs: mechanisms and clinical relevance. *Clin Pharmacol Ther* 2006;80:565-81.

Niemi M, Backman JT, Neuvonen M, Neuvonen PJ, Kivistö KT. Rifampin decreases the plasma concentrations and effects of repaglinide. *Clin Pharmacol Ther* 2000;68:495-500.

Niemi M, Neuvonen PJ, Kivistö KT. The cytochrome P4503A4 inhibitor clarithromycin increases the plasma concentrations and effects of repaglinide. *Clin Pharmacol Ther* 2001a;70:58-65.

Niemi M, Neuvonen PJ, Kivistö KT. Effect of gemfibrozil on the pharmacokinetics and pharmacodynamics of glimepiride. *Clin Pharmacol Ther* 2001b;70:439-45.

Niemi M, Backman JT, Granfors M, Laitila J, Neuvonen M, Neuvonen PJ. Gemfibrozil considerably increases the plasma concentrations of rosiglitazone. *Diabetologia* 2003a;46:1319-23.

Niemi M, Backman JT, Neuvonen M, Neuvonen PJ. Effects of gemfibrozil, itraconazole, and their combination on the pharmacokinetics and pharmacodynamics of repaglinide: potentially hazardous interaction between gemfibrozil and repaglinide. *Diabetologia* 2003b;46:347-51.

Niemi M, Leathart JB, Neuvonen M, Backman JT, Daly AK, Neuvonen PJ. Polymorphism in CYP2C8 is associated with reduced plasma concentrations of repaglinide. *Clin Pharmacol Ther* 2003c;74:380-7.

Niemi M, Backman JT, Neuvonen PJ. Effects of trimethoprim and rifampin on the pharmacokinetics of the cytochrome P450 2C8 substrate rosiglitazone. *Clin Pharmacol Ther* 2004a;76:239-49.

Niemi M, Kajosaari LI, Neuvonen M, Backman JT, Neuvonen PJ. The CYP2C8 inhibitor trimethoprim increases the plasma concentrations of repaglinide in healthy subjects. *Br J Clin Pharmacol* 2004b;57:441-7.

Niemi M, Backman JT, Juntti-Patinen L, Neuvonen M, Neuvonen PJ. Coadministration of gemfibrozil and itraconazole has only a minor effect on the pharmacokinetics of the CYP2C9 and CYP3A4 substrate nateglinide. *Br J Clin Pharmacol* 2005a;60:208-17.

Niemi M, Backman JT, Kajosaari LI, Leathart JB, Neuvonen M, Daly AK, Eichelbaum M, Kivisto KT, Neuvonen PJ. Polymorphic organic anion transporting polypeptide 1B1 is a major determinant of repaglinide pharmacokinetics. *Clin Pharmacol Ther* 2005b;77:468-78.

Niemi M, Pasanen MK, Neuvonen PJ. SLCO1B1 polymorphism and sex affect the pharmacokinetics of pravastatin but not fluvastatin. *Clin Pharmacol Ther* 2006;80:356-66.

Niemi M. Role of OATP transporters in the disposition of drugs. *Pharmacogenomics* 2007;8:787-802.

Noble S, Langtry HD, Lamb HM. Zopiclone. An update of its pharmacology, clinical efficacy and tolerability in the treatment of insomnia. *Drugs* 1998;55:277-302.

NovoNorm product information. Available from http://www.emea.europa.eu/humandocs/PDFs/EPAR/Novonorm/H-187-PI-en.pdf. Accessed Jan 26, 2008

Nozawa T, Sugiura S, Nakajima M, Goto A, Yokoi T, Nezu J, Tsuji A, Tamai I. Involvement of organic anion transporting polypeptides in the transport of troglitazone sulfate: implications for understanding troglitazone hepatotoxicity. *Drug Metab Dispos* 2004;32:291-4.

Obach RS, Zhang QY, Dunbar D, Kaminsky LS. Metabolic characterization of the major human small intestinal cytochrome p450s. *Drug Metab Dispos* 2001;29:347-52.

Ogilvie BW, Zhang D, Li W, Rodrigues AD, Gipson AE, Holsapple J, Toren P, Parkinson A. Glucuronidation converts gemfibrozil to a potent, metabolism-dependent inhibitor of CYP2C8: implications for drug-drug interactions. *Drug Metab Dispos* 2006;34:191-7.

Ohyama K, Nakajima M, Nakamura S, Shimada N, Yamazaki H, Yokoi T. A significant role of human cytochrome P450 2C8 in amiodarone N-deethylation: an approach to predict the contribution with relative activity factor. *Drug Metab Dispos* 2000;28:1303-10.

Olkkola KT, Backman JT, Neuvonen PJ. Midazolam should be avoided in patients receiving the systemic antimycotics ketoconazole or itraconazole. *Clin Pharmacol Ther* 1994;55:481-5.

Omura T, Sato R. A new cytochrome in liver microsomes. *J Biol Chem* 1962;237:1375-6.

Ong CE, Coulter S, Birkett DJ, Bhasker CR, Miners JO. The xenobiotic inhibitor profile of cytochrome P4502C8. *Br J Clin Pharmacol* 2000;50:573-80.

Parikh S, Ouedraogo JB, Goldstein JA, Rosenthal PJ, Kroetz DL. Amodiaquine metabolism is impaired by common polymorphisms in CYP2C8: implications for malaria treatment in Africa. *Clin Pharmacol Ther* 2007;82:197-203.

Parkinson A. Biotransformation of xenobiotics. In: Klaassen CD. *Casarett & Doull's Toxicology - The Basic Science of Poisons*. New York: McGraw-Hill; 2001. p. 133-224

Pasanen MK, Neuvonen M, Neuvonen PJ, Niemi M. SLCO1B1 polymorphism markedly affects the pharmacokinetics of simvastatin acid. *Pharmacogenet Genomics* 2006;16:873-9.

Pasanen MK, Fredrikson H, Neuvonen PJ, Niemi M. Different effects of SLCO1B1 polymorphism on the pharmacokinetics of atorvastatin and rosuvastatin. *Clin Pharmacol Ther* 2007;82:726-33.

Pelkonen O, Maenpaa J, Taavitsainen P, Rautio A, Raunio H. Inhibition and induction of human cytochrome P450 (CYP) enzymes. *Xenobiotica* 1998;28:1203-53.

Pelkonen O, Rautio A, Raunio H, Pasanen M. CYP2A6: a human coumarin 7-hydroxylase. *Toxicology* 2000;144:139-47.

Periti P, Mazzei T, Mini E, Novelli A. Pharmacokinetic drug interactions of macrolides. *Clin Pharmacokinet* 1992;23:106-31.

Picard N, Cresteil T, Djebli N, Marquet P. In vitro metabolism study of buprenorphine: evidence for new metabolic pathways. *Drug Metab Dispos* 2005;33:689-95.

Projean D, Baune B, Farinotti R, Flinois JP, Beaune P, Taburet AM, Ducharme J. In vitro metabolism of chloroquine: identification of CYP2C8, CYP3A4, and CYP2D6 as the main isoforms catalyzing N-desethylchloroquine formation. *Drug Metab Dispos* 2003a;31:748-54.

Projean D, Morin PE, Tu TM, Ducharme J. Identification of CYP3A4 and CYP2C8 as the major cytochrome P450 s responsible for morphine N-demethylation in human liver microsomes. *Xenobiotica* 2003b;33:841-54.

Prueksaritanont T, Ma B, Yu N. The human hepatic metabolism of simvastatin hydroxy acid is mediated primarily by CYP3A, and not CYP2D6. *Br J Clin Pharmacol* 2003;56:120-4.

Prueksaritanont T, Richards KM, Qiu Y, Strong-Basalyga K, Miller A, Li C, Eisenhandler R, Carlini EJ. Comparative effects of fibrates on drug metabolizing enzymes in human hepatocytes. *Pharm Res* 2005;22:71-8.

Rahman A, Korzekwa KR, Grogan J, Gonzalez FJ, Harris JW. Selective biotransformation of taxol to 6 alpha-hydroxytaxol by human cytochrome P450 2C8. *Cancer Res* 1994;54:5543-6.

Ribera E, Pou L, Fernandez-Sola A, Campos F, Lopez RM, Ocaña I, Ruiz I, Pahissa A. Rifampin reduces concentrations of trimethoprim and sulfamethoxazole in serum in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother* 2001;45:3238-41.

Richter T, Mürdter TE, Heinkele G, Pleiss J, Tatzel S, Schwab M, Eichelbaum M, Zanger UM. Potent mechanism-based inhibition of human CYP2B6 by clopidogrel and ticlopidine. *J Pharmacol Exp Ther* 2004;308:189-97.

Rifkind AB, Lee C, Chang TK, Waxman DJ. Arachidonic acid metabolism by human cytochrome P450s 2C8, 2C9, 2E1, and 1A2: regioselective oxygenation and evidence for a role for CYP2C enzymes in arachidonic acid epoxygenation in human liver microsomes. *Arch Biochem Biophys* 1995;320:380-9.

Rodrigues AD. Integrated cytochrome P450 reaction phenotyping: attempting to bridge the gap between cDNA-expressed cytochromes P450 and native human liver microsomes. *Biochem Pharmacol* 1999;57:465-80.

Rodríguez-Antona C, Niemi M, Backman JT, Kajosaari LI, Neuvonen PJ, Robledo M, Ingelman-Sundberg M. Characterization of novel CYP2C8 haplotypes and their contribution to paclitaxel and repaglinide metabolism. *Pharmacogenomics J* 2007; Epub: doi:10.1038/sj.tpj.6500482

Rowland-Yeo K, Rostami-Hodjegan A, Tucker GT. Abundance of cytochromes P450 in human liver: a meta-analysis. *Br J Clin Pharmacol* 2003;57:687-8.

Sabordo L, Sallustio BC, Evans AM, Nation RL. Hepatic disposition of the acyl glucuronide1-O-gemfibrozil-beta-D-glucuronide: effects of dibromosulfophthalein on membrane transport and aglycone formation. *J Pharmacol Exp Ther* 1999;288:414-20.

Sahi J, Black CB, Hamilton GA, Zheng X, Jolley S, Rose KA, Gilbert D, LeCluyse EL, Sinz MW. Comparative effects of thiazolidinediones on in vitro P450 enzyme induction and inhibition. *Drug Metab Dispos* 2003;31:439-46.

Sallustio BC, Fairchild BA, Shanahan K, Evans AM, Nation RL. Disposition of gemfibrozil and gemfibrozil acyl glucuronide in the rat isolated perfused liver. *Drug Metab Dispos* 1996;24:984-9.

Schneck DW, Birmingham BK, Zalikowski JA, Mitchell PD, Wang Y, Martin PD, Lasseter KC, Brown CD, Windass AS, Raza A. The effect of gemfibrozil on the pharmacokinetics of rosuvastatin. *Clin Pharmacol Ther* 2004;75:455-63.

Schoch GA, Yano JK, Wester MR, Griffin KJ, Stout CD, Johnson EF. Structure of human microsomal cytochrome P450 2C8. Evidence for a peripheral fatty acid binding site. *J Biol Chem* 2004;279:9497-503.

Shen DD, Kunze KL, Thummel KE. Enzyme-catalyzed processes of first-pass hepatic and intestinal drug extraction. *Adv Drug Deliv Rev* 1997;27:99-127.

Sheweita SA. Drug-metabolizing enzymes: mechanisms and functions. *Curr Drug Metab* 2000;1:107-32.

Shitara Y, Hirano M, Sato H, Sugiyama Y. Gemfibrozil and its glucuronide inhibit the organic anion transporting polypeptide 2 (OATP2/OATP1B1:SLC21A6)-mediated hepatic uptake and CYP2C8-mediated metabolism of cerivastatin: analysis of the mechanism of the clinically relevant drug-drug interaction between cerivastatin and gemfibrozil. *J Pharmacol Exp Ther* 2004;311:228-36.

Shitara Y, Horie T, Sugiyama Y. Transporters as a determinant of drug clearance and tissue distribution. *Eur J Pharm Sci* 2006;27:425-46.

Skarke C, Jarrar M, Schmidt H, Kauert G, Langer M, Geisslinger G, Lötsch J. Effects of ABCB1 (multidrug resistance transporter) gene mutations on disposition and central nervous effects of loperamide in healthy volunteers. *Pharmacogenetics* 2003;13:651-60.

Smilack JD. Trimethoprim-sulfamethoxazole. *Mayo Clin Proc* 1999;74:730-4.

Soyama A, Saito Y, Hanioka N, Murayama N, Nakajima O, Katori N, Ishida S, Sai K, Ozawa S, Sawada JI. Non-synonymous single nucleotide alterations found in the CYP2C8 gene result in reduced in vitro paclitaxel metabolism. *Biol Pharm Bull* 2001;24:1427-30.

Soyama A, Hanioka N, Saito Y, Murayama N, Ando M, Ozawa S, Sawada J. Amiodarone N-deethylation by CYP2C8 and its variants, CYP2C8*3 and CYP2C8 P404A. *Pharmacol Toxicol* 2002;91:174-8.

Staffa JA, Chang J, Green L. Cerivastatin and reports of fatal rhabdomyolysis. *N Engl J Med* 2002;346:539-40.

Stone BM. Pencil and paper tests--sensitivity to psychotropic drugs. *Br J Clin Pharmacol* 1984;18 Suppl 1:15S-20S.

Tan SC, Patel BK, Jackson SH, Swift CG, Hutt AJ. Stereoselectivity of ibuprofen metabolism and pharmacokinetics following the administration of the racemate to healthy volunteers. *Xenobiotica* 2002;32:683-97.

Tanaka E, Terada M, Misawa S. Cytochrome P450 2E1: its clinical and toxicological role. *J Clin Pharm Ther* 2000;25:165-75.

Tayrouz Y, Ganssmann B, Ding R, Klingmann A, Aderjan R, Burhenne J, Haefeli WE, Mikus G. Ritonavir increases loperamide plasma concentrations without evidence for P-glycoprotein involvement. *Clin Pharmacol Ther* 2001;70:405-14.

Templeton IE, Thummel KE, Kharasch ED, Kunze KL, Hoffer C, Nelson WL, Isoherranen N. Contribution of itraconazole metabolites to inhibition of CYP3A4 in vivo. *Clin Pharmacol Ther* 2008;83:77-85.

Theuretzbacher U, Ihle F, Derendorf H. Pharmacokinetic/pharmacodynamic profile of voriconazole. *Clin Pharmacokinet* 2006;45:649-63.

Todd PA, Ward A. Gemfibrozil. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in dyslipidaemia. *Drugs* 1988;36:314-39.

Tompkins LM, Wallace AD. Mechanisms of cytochrome P450 induction. *J Biochem Mol Toxicol* 2007;21:176-81.

Tornio A, Pasanen MK, Laitila J, Neuvonen PJ, Backman JT. Comparison of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) as inhibitors of cytochrome P450 2C8. *Basic Clin Pharmacol Toxicol* 2005;97:104-8.

Totah RA, Rettie AE. Cytochrome P450 2C8: substrates, inhibitors, pharmacogenetics, and clinical relevance. *Clin Pharmacol Ther* 2005;77:341-52.

Tracy TS, Wirthwein DP, Hall SD. Metabolic inversion of (R)-ibuprofen. Formation of ibuprofenyl-coenzyme A. *Drug Metab Dispos* 1993;21:114-20.

Turpeinen M, Tolonen A, Uusitalo J, Jalonen J, Pelkonen O, Laine K. Effect of clopidogrel and ticlopidine on cytochrome P450 2B6 activity as measured by bupropion hydroxylation. *Clin Pharmacol Ther* 2005;77:553-9.

Turpeinen M, Raunio H, Pelkonen O. The functional role of CYP2B6 in human drug metabolism: substrates and inhibitors in vitro, in vivo and in silico. *Curr Drug Metab* 2006;7:705-14.

Wadelius M, Pirmohamed M. Pharmacogenetics of warfarin: current status and future challenges. *Pharmacogenomics J* 2007;7:99-111.

Walsky RL, Gaman EA, Obach RS. Examination of 209 drugs for inhibition of cytochrome P450 2C8. *J Clin Pharmacol* 2005a;45:68-78.

Walsky RL, Obach RS, Gaman EA, Gleeson JP, Proctor WR. Selective inhibition of human cytochrome P4502C8 by montelukast. *Drug Metab Dispos* 2005b;33:413-8.

van Heiningen PN, Hatorp V, Kramer Nielsen K, Hansen KT, van Lier JJ, De Merbel NC, Oosterhuis B, Jonkman JH. Absorption, metabolism and excretion of a single oral dose of (14)C-repaglinide during repaglinide multiple dosing. *Eur J Clin Pharmacol* 1999;55:521-5.

Wang EJ, Lew K, Casciano CN, Clement RP, Johnson WW. Interaction of common azole antifungals with P glycoprotein. *Antimicrob Agents Chemother* 2002a;46:160-5.

Wang H, Negishi M. Transcriptional regulation of cytochrome p450 2B genes by nuclear receptors. *Curr Drug Metab* 2003;4:515-25.

Wang JS, Neuvonen M, Wen X, Backman JT, Neuvonen PJ. Gemfibrozil inhibits CYP2C8-mediated cerivastatin metabolism in human liver microsomes. *Drug Metab Dispos* 2002b;30:1352-6.

Wang JS, DeVane CL. Involvement of CYP3A4, CYP2C8, and CYP2D6 in the metabolism of (R)- and (S)-methadone in vitro. *Drug Metab Dispos* 2003;31:742-7.

Varhe A, Olkkola KT, Neuvonen PJ. Oral triazolam is potentially hazardous to patients receiving systemic antimycotics ketoconazole or itraconazole. *Clin Pharmacol Ther* 1994;56:601-7.

Waugh J, Keating GM, Plosker GL, Easthope S, Robinson DM. Pioglitazone: a review of its use in type 2 diabetes mellitus. *Drugs* 2006;66:85-109.

Weinshilboum RM, Otterness DM, Szumlanski CL. Methylation pharmacogenetics: catechol O-methyltransferase, thiopurine methyltransferase, and histamine N-methyltransferase. *Annu Rev Pharmacol Toxicol* 1999;39:19-52.

Wen X, Wang JS, Backman JT, Kivistö KT, Neuvonen PJ. Gemfibrozil is a potent inhibitor of human cytochrome P450 2C9. *Drug Metab Dispos* 2001;29:1359-61.

Wen X, Wang JS, Backman JT, Laitila J, Neuvonen PJ. Trimethoprim and sulfamethoxazole are selective inhibitors of CYP2C8 and CYP2C9, respectively. *Drug Metab Dispos* 2002;30:631-5.

Venkatakrishnan K, Von Moltke LL, Greenblatt DJ. Human drug metabolism and the cytochromes P450: application and relevance of in vitro models. *J Clin Pharmacol* 2001;41:1149-79.

Venkatakrishnan K, Obach RS. Drug-drug interactions via mechanism-based cytochrome P450 inactivation: points to consider for risk assessment from in vitro data and clinical pharmacologic evaluation. *Curr Drug Metab* 2007;8:449-62.

Werck-Reichhart D, Feyereisen R. Cytochromes P450: a success story. *Genome Biol* 2000;1:Reviews3003.1-3003.9.

Westwood IM, Kawamura A, Fullam E, Russell AJ, Davies SG, Sim E. Structure and mechanism of arylamine N-acetyltransferases. *Curr Top Med Chem* 2006;6:1641-54.

Wilkinson GR. Drug metabolism and variability among patients in drug response. *N Engl J Med* 2005;352:2211-21.

Villikka K, Kivistö KT, Lamberg TS, Kantola T, Neuvonen PJ. Concentrations and effects of zopiclone are greatly reduced by rifampicin. *Br J Clin Pharmacol* 1997;43:471-4.

Willson TM, Kliewer SA. PXR, CAR and drug metabolism. *Nat Rev Drug Discov* 2002;1:259-66.

Wrighton SA, Brian WR, Sari MA, Iwasaki M, Guengerich FP, Raucy JL, Molowa DT, Vandenbranden M. Studies on the expression and metabolic capabilities of human liver cytochrome P450IIIA5 (HLp3). *Mol Pharmacol* 1990;38:207-13.

Wrighton SA, Stevens JC. The human hepatic cytochromes P450 involved in drug metabolism. *Crit Rev Toxicol* 1992;22:1-21.

Wrighton SA, Schuetz EG, Thummel KE, Shen DD, Korzekwa KR, Watkins PB. The human CYP3A subfamily: practical considerations. *Drug Metab Rev* 2000;32:339-61.

Yamazaki H, Shibata A, Suzuki M, Nakajima M, Shimada N, Guengerich FP, Yokoi T. Oxidation of troglitazone to a quinone-type metabolite catalyzed by cytochrome P-450 2C8 and P-450 3A4 in human liver microsomes. *Drug Metab Dispos* 1999;27:1260-6.

Yamazaki M, Li B, Louie SW, Pudvah NT, Stocco R, Wong W, Abramovitz M, Demartis A, Laufer R, Hochman JH, Prueksaritanont T, Lin JH. Effects of fibrates on human organic anion-transporting polypeptide 1B1-, multidrug resistance protein 2- and P-glycoprotein-mediated transport. *Xenobiotica* 2005;35:737-53.

Yano JK, Wester MR, Schoch GA, Griffin KJ, Stout CD, Johnson EF. The structure of human microsomal cytochrome P450 3A4 determined by X-ray crystallography to 2.05-A resolution. *J Biol Chem* 2004;279:38091-4.

Yasar U, Lundgren S, Eliasson E, Bennet A, Wiman B, de Faire U, Rane A. Linkage between the CYP2C8 and CYP2C9 genetic polymorphisms. *Biochem Biophys Res Commun* 2002;299:25-8.

Yasar U, Bennet AM, Eliasson E, Lundgren S, Wiman B, De Faire U, Rane A. Allelic variants of cytochromes P450 2C modify the risk for acute myocardial infarction. *Pharmacogenetics* 2003;13:715-20.

You G. The role of organic ion transporters in drug disposition: an update. *Curr Drug Metab* 2004;5:55-62.

Youdim MB, Bakhle YS. Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. *Br J Pharmacol* 2006;147 Suppl 1:S287-96.

Zanger UM, Fischer J, Raimundo S, Stüven T, Evert BO, Schwab M, Eichelbaum M. Comprehensive analysis of the genetic factors determining expression and function of hepatic CYP2D6. *Pharmacogenetics* 2001;11:573-85.

Zanger UM, Raimundo S, Eichelbaum M. Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemistry. *Naunyn Schmiedebergs Arch Pharmacol* 2004;369:23-37.

Zanger UM, Klein K, Saussele T, Blievernicht J, M HH, Schwab M. Polymorphic CYP2B6: molecular mechanisms and emerging clinical significance. *Pharmacogenomics* 2007;8:743-59.

Zhang QY, Dunbar D, Ostrowska A, Zeisloft S, Yang J, Kaminsky LS. Characterization of human small intestinal cytochromes P-450. *Drug Metab Dispos* 1999;27:804-9.

ORIGINAL PUBLICATIONS