TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

SARJA - SER. D OSA - TOM. 922

MEDICA - ODONTOLOGICA

EPIDEMIOLOGY OF CYTOCHROME P450-MEDIATED DRUG-DRUG INTERACTIONS

by

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UNIVERSITY OF TURKU
Turku 2010

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ISBN 978-951-29-4411-8 (PRINT)
ISBN 978-951-29-4412-5 (PDF)
ISSN 0355-9483
Painosalama Oy – Turku, Finland 2010

"Scio me nihil scire"
"I know that I know nothing"

Socrates

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ABSTRACT

Drug-drug interactions (DDIs) comprise an important cause of adverse drug reactions leading to excess hospitalizations. Drug metabolism is catalyzed by 75% by cytochrome P450 (CYP) enzymes and thus they are often involved in pharmacokinetic DDIs. In general, DDIs are studied in randomized controlled clinical trials in selected study populations. The overall aim of the present studies was to perform observational pharmacoepidemiological surveys on CYP-mediated DDIs in diseases important at the population level.

The prevalence of co-administrations of four prodrugs (losartan, codeine, tramadol, and clopidogrel), three sulphonylureas (glibenclamide, glimepiride, and glipizide), or two statins (lovastatin and simvastatin) with well established agents altering CYP activity, as well as of statins with fibrates, was studied in Finland utilizing data from a university hospital medication database (inpatients) and the National Prescription Register of the Social Insurance Institution of Finland, Kela (outpatients). Clinical consequences of potential DDIs were estimated by reviewing laboratory data, and information from hospital care and cause-of-death registers.

Concomitant use of study substrates with interacting medication was detected in up to one fifth of patients in both hospital and community settings. Potential CYP3A4 interactions in statin users did not manifest in clear adverse laboratory values but pharmacodynamic DDIs between statins and fibrates predisposed patients to muscular toxicity. Sulphonylurea DDIs with CYP2C9 inhibitors increased the risk of hypoglycaemia. CYP3A4 inhibitor use with clopidogrel was not associated with significant changes in mortality but non-fatal thrombosis and haemorrhage complications were seen less often in this group. Concomitant administration of atorvastatin with clopidogrel moderately attenuated the antithrombotic effect by clopidogrel. The overall mortality was increased in CYP3A4 inducer and clopidogrel co-users. Atorvastatin used concomitantly with prodrug clopidogrel seems to be beneficial in terms of total and LDL cholesterol concentrations, and overall mortality compared with clopidogrel use without interacting medication.

In conclusion, CYP-mediated DDIs are a common and often unrecognized consequence of irrational drug prescribing.

Keywords: cytochrome P450, drug metabolism, drug-drug interactions, pharmacoepidemiology

Tuire Tirkkonen

SYTOKROMI P450 -VÄLITTEISTEN LÄÄKEAINEYHTEISVAIKUTUSTEN EPIDEMIOLOGIAA

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TIIVISTELMÄ

Lääkeaineyhteisvaikutukset (lääkeinteraktiot) ovat merkittäviä lääkehaittojen aiheuttajia, jotka vaativat huomattavan usein sairaalahoitoa rasittaen turhaan terveydenhuollon resursseja. Sytokromi P450 (CYP) -entsyymit katalysoivat 75 %:a lääkeaineiden aineenvaihduntareaktioista, jolloin myös farmakokineettiset lääkeinteraktiot ovat usein mekanismiltaan CYP-välitteisiä. Lääkeinteraktioita tutkitaan yleensä terveillä vapaaehtoisilla satunnaistetuissa, kontrolloiduissa kliinisissä kokeissa. Nyt esillä olevissa havannoivissa töissä oli tarkoituksena tutkia kansanterveydellisesti merkittäviin sairauksiin liittyviä CYP-välitteisiä lääkeinteraktioita farmakoepidemiologisin menetelmin.

Yhteiskäytön vallitsevuutta tutkittiin neljän aihiolääkeaineen (losartaani, kodeiini, tramadoli ja klopidogreeli), kolmen sulfonyyliurean (glibenklamidi, glimepiridi ja glipitsidi) tai kahden statiinin (lovastatiini ja simvastatiini) sekä tunnettujen CYP-aktiivisuutta muuttavien lääkeaineiden, ja lisäksi statiinien ja fibraattien välillä käyttämällä Turun yliopistollisen keskussairaalan (TYKS) lääkitystietokantaa ja Kelan tilastoa korvatuista resepteistä. Potentiaalisten interaktioiden kliinisiä merkityksiä arvioitiin TYKSin laboratoriotietokannan sekä hoitoilmoitus- ja kuolinsyyrekistereiden avulla.

Valittujen CYP-substraattien ja interaktoita aiheuttavien lääkkeiden yhteiskäyttöä havaittiin jopa viidenneksellä sairaala- ja avohoitopotilaista. Potentiaaliset CYP3A4-interaktiot eivät aiheuttaneet selviä haittoja ilmaisevia muutoksia statiinilla hoidettujen potilaiden laboratorioarvoissa, mutta fibraattien ja statiinien farmakodynaamiset lääkeinteraktiot altistivat potilaat lihasvaurioille. Sulfonyyliureoiden ja CYP2C9-inhibiittoreiden samanaikainen käyttö lisäsi hypoglykemiariskiä. Kuolleisuudessa ei ollut merkitsevää eroa CYP3A4-inhibiittoreita käyttävillä klopidogreelipotilailla kontrolleihin verrattuna, mutta ei-kuolemaanjohtavia veritulppa- ja vuotokomplikaatioita havaittiin harvemmin. Atorvastatiinin käyttö heikensi hieman klopidogreelin verenhyytymistä estäviä vaikutuksia. Kokonaiskuolleisuus oli kohonnut CYP3A4-induktoreita ja klopidogreelia samanaikaisesti käyttävien ryhmässä. Atorvastatiinin ja aihiolääke klopidogreelin yhteiskäyttö vaikutti edullisesti kokonais- ja LDL-kolesterolipitoisuuksiin sekä kokonaiskuolleisuuteen verrattuna klopidogreelihoitoon ilman interaktioita aiheuttavaa lääkitystä.

Yhteenvetona voidaan todeta, että CYP-välitteiset lääkeyhteisvaikutukset ovat yleinen ja usein tunnistamaton irrationaalisen lääkkeenmääräämisen seuraus.

Avainsanat: sytokromi P450, lääkeainemetabolia, lääkeaineyhteisvaikutukset, farmakoepidemiologia

TABLE OF CONTENTS

ABBREVIATIONS

ADME	absorption, distribution, metabolism, and excretion
ADR	adverse drug reaction
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ATC	Anatomical Therapeutic Chemical
AUC	area under the plasma concentration-time curve
C_{max}	maximum concentration
CI	confidence interval
CYP	cytochrome P450
DDI	drug-drug interaction
EMA (EMEA)	European Medicines Agency
FDA	Food and Drug Administration
HDL	high-density lipoprotein
HIV	human immunodeficiency virus
HR	hazard ratio
ICD-10	the tenth revision of the International Classification of Diseases
LDL	low-density lipoprotein
n	number
NOMESCO	Nordic Medico-Statistical Committee
NSAID	non-steroidal anti-inflammatory drug
NCSP	NOMESCO Classification of Surgical Procedures
OR	odds ratio
P	probability
P-gp	P-glycoprotein
RCT	randomized controlled clinical trial
$T_{1/2el}$	elimination half-life
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by Roman numerals I–IV:

I Tirkkonen T, Laine K. Drug interactions with the potential to prevent prodrug activation as a common source of irrational prescribing in hospital inpatients. *Clin Pharmacol Ther.* 2004; 76 (6): 639-47.

II Tirkkonen T, Ryynänen A, Vahlberg T, Irjala K, Klaukka T, Huupponen R, Laine K. Frequency and clinical relevance of drug interactions with lovastatin and simvastatin: an observational database study. *Drug Safety.* 2008; 31 (3): 231-240.

III Tirkkonen T, Heikkilä P, Huupponen R, Laine K. Potential CYP2C9-mediated drug-drug interactions in hospitalised type 2 diabetes mellitus patients on sulphonylureas glibenclamide, glimepiride, or glipizide. *J Intern Med.* 2010 Aug 4. [Epub ahead of print]

IV Tirkkonen T, Heikkilä P, Vahlberg T, Huupponen R, Laine K. Epidemiology of CYP3A4-mediated clopidogrel drug-drug interactions and their clinical consequences. Submitted.

In addition, some unpublished data are presented in this thesis.

1 INTRODUCTION

A drug-drug interaction (DDI) occurs when co-administration of two or more drugs alters the pharmacokinetics or -dynamics of one or both of the interacting drugs. Pharmacokinetic DDIs involve changes in either absorption, distribution, metabolism, or excretion phases in drug kinetics. Pharmacodynamic interactions do not involve changes in drug concentrations but are due to change in response to a given drug concentration. Metabolism plays an importat role in elimination by transforming drug molecules into an excretable form and by changing their biological activity. Cytochrome P450 (CYP) enzymes constitute a predominant family of metabolizing enzymes in the human body and are also involved in most of metabolic DDIs.

Adverse drug reactions (ADRs) are a remarkable problem in health care. It has been estimated that 26% of ADRs leading to hospitalizations are due to DDIs. Drug-drug interactions cause not only adverse drug reactions and toxicity but also lack of efficacy. Pharmacokinetic DDIs, especially CYP-mediated DDIs, are widely studied starting already during the drug development process. However, it is important to emphasize also the pharmacodynamic side in DDI studies.

DDI studies are usually performed in healthy volunteers and selected groups of patients by using randomized controlled studies while a pharmacoepidemiological approach has been applied mainly to detect drug-related adverse events at the population level (pharmacovigilance) but not much to study DDIs. Finland represents an adequate field for pharmacoepidemiological studies due to its valid and comprehensive patient registers with a rather homogenous population.

The group of CYP substrates includes drugs from different drug therapy fields. The inhibitors and inducers of CYP enzymes include different types of drugs, also from the therapeutic point of view. This makes DDI control challenging especially at the CYP isoenzyme level.

DDIs of drugs used by large populations or by vulnerable patient groups, such as elderly people, are of special importance. However, more research is needed. The chosen substrates of the present studies represent agents that are often used by these groups; drugs used in the treatment of hypertension, pain, dyslipidemia, type 2 diabetes mellitus, and increased blood coagulability. The correct use of drugs is essential for proper effectiveness and safety in these situations.

2 REVIEW OF THE LITERATURE

2.1 Drug metabolism

Drugs as other xenobiotics are foreign chemicals to the human body. Pharmacokinetics consists of absorption, distribution, metabolism, and excretion (ADME), of which metabolism and excretion contribute to drug elimination. Xenobiotic metabolism is also known as biotransformation. (Rang *et al.* 2007)

Generally drug molecules are lipophilic to maintain effective absorption and distribution. Most drugs are, however, excreted in the urine. Renal excretion favours hydrophilic compounds and therefore biotransformation is needed to metabolize drugs to more polar products. Water-soluble compounds are excreted also into the bile. (Rang *et al.* 2007)

The main organ in drug metabolism is the liver but also the gut, lungs, and skin are important. Apart from the liver, it is common for these organs that they are the main routes for xenobiotics to enter the body. When oral administration is concerned the amount of the drug in systemic circulation is usually less than what is absorbed from the gastrointestinal tract. This phenomenon is due to first-pass metabolism or presystemic extraction. (Rang *et al.* 2007)

Drug metabolism can be divided into two main steps: phase I and phase II reactions. Phase I reactions are catabolic functionalization reactions based on hydrolysis, reduction, and oxidation. Phase II reactions involve conjugation and are anabolic processes. They include glucuronidation, sulfonation, methylation, acetylation, and amino acid and glutathione conjugation. Biotransformation is catalyzed by various enzyme systems, examples of which are shown in Table 2.1. One substrate may use several metabolic pathways. Phase I reactions introduce a functional group into the substrate molecule and phase II reactions attach a substituent to this reactive site of the derivative. If the drug is a mixture of stereoisomers the chirality also affects the metabolic behaviour. The activity of metabolites is usually less than that of the parent substrate but formation of active and toxic metabolites is possible. (Brophy *et al.* 2006, Parkinson and Ogilvie 2008)

Drug metabolism involves remarkable inter- and intraindividual variations. The main causes for the alteration in drug metabolism are genetic polymorphisms in the genes coding catalyzing enzymes, concomitant use of other drugs or exposure to other xenobiotics (including drugs) that inhibit or induce metabolic enzymes, age, and physiological status and disease state. (Ingelman-Sundberg *et al.* 1999)

Drug metabolism is related to both efficacy and safety of drugs. It is important to determine by which enzymes a drug is metabolized to predict the effects of drug-drug interactions or interindividual variations. (Gonzalez and Tukey 2006)

Table 2.1 Examples of the catalyzing enzymes in different phase I and phase II metabolic reactions

Reaction	Enzyme
Phase I	
hydrolysis	carboxylesterase
reduction	carbonyl reductase
oxidation	**cytochrome P450**
Phase II	
glucuronidation	UDP-glucuronosyltransferase (UGT)
sulfonation	sulfotransferase (SULT)
methylation	methyltransferase (MT)
acetylation	*N*-acetyltransferase (NAT)
amino acid conjugation	amino acid specific NATs
glutathione conjugation	glutathione-*S*-transferase (GST)

2.1.1 Cytochrome P450 (CYP) enzymes

Cytochrome P450 enzymes (CYPs) are a superfamily of enzymes that contain a non-covalently bound haem in the polypeptide chain. CYPs are located in the endoplasmic reticulum consisting of phospholipid bilayers in the cytoplasm. When hydrophobic drug molecules enter the cell, they become embedded in the lipid bilayer where they then come into direct contact with the CYP enzymes. Haeme is the O_2 binding moiety, the active site, in the CYP-mediated catalytic cycle to carry out the oxidation of substrates by either *N*-dealkylation, *O*-dealkylation, aromatic hydroxylation, *N*-oxidation, *S*-oxidation, deamination, or, dehalogenation. (Gonzalez and Tukey 2006) The catalytic CYP cycle consists of seven steps: 1) binding of the substrate to the ferric form of the enzyme, 2) reduction of the haem group from the ferric to the ferrous state by an electron provided by NADPH via CYP reductase, 3) binding of molecular oxygen, 4) transfer of a second electron from CYP reductase and/or cytochrome b5, 5) cleavage of the O–O bond, 6) substrate oxygenation, 7) product release (Lin and Lu 1998). Cytochrome P450s were named in 1961 based on the finding that when the haem iron is reduced and bound to carbon monoxide the pigment (P) has a spectral peak at 450 nm (Omura and Sato 1962).

The human CYP enzyme family comprises 57 genes (Nebert and Russell 2002). Cytochrome P450 enzymes are present in most of the tissues that involve drug metabolism and dietary xenobiotics as well as synthesis of endogenous hormones. (Gonzalez and Tukey 2006) (Table 2.2) Further on, this thesis will concentrate on drug metabolism related CYPs.

CYPs are the most important enzymes involved in drug metabolism; they account for about 75% of all enzymatic biotransformation. Thus CYPs play also a major role in the phase I of the human metabolism. (Guengerich 2008) An evaluation of the mechanism for the metabolic clearance of 315 different drugs revealed that 56% of them were primarily cleared via CYP metabolism (Ingelman-Sundberg *et al.* 1999).

Table 2.2 Human *CYP* families (modified from Nebert and Russell 2002)

Family	n of subfamilies	n of genes	Substrates/Functions
CYP1	2	3	**xenobiotics**, arachidonic acid, eicosanoids
CYP2	13	16	**xenobiotics**, arachidonic acid, eicosanoids
CYP3	1	4	**xenobiotics**, arachidonic acid, eicosanoids
CYP4	5	12	fatty acids, arachidonic acid, eicosanoids
CYP5	1	1	thromboxane A_2 synthase
CYP7	2	2	cholesterol, bile acid synthesis
CYP8	2	2	prostacyclin synthase, bile acid synthesis
CYP11	2	3	steroidogenesis
CYP17	1	1	steroid 17β-hydroxylase, 17/20-lyase
CYP19	1	1	aromatase, estrogen synthesis
CYP20	1	1	unknown
CYP21	1	1	steroid 21-hydroxylase
CYP24	1	1	vitamin D_3 24-hydroxylase
CYP26	3	3	retinoic acid hydroxylation
CYP27	3	3	bile acid biosynthesis, vitamin D_3 hydroxylation
CYP39	1	1	24-hydroxycholesterol 7α-hydroxylase
CYP46	1	1	cholesterol 24-hydroxylase
CYP51	1	1	lanosterol 14α-desmethylase
Total	**42**	**57**	

Of the 57 CYP proteins encoded by the human genome (Table 2.2) only five are responsible for the oxidative metabolism of 95% of all drugs (Figure 2.1). These five subtypes are CYP3A4, CYP2D6, CYP2C9, CYP2C19, and CYP1A1/2. (Guengerich 2008, Johnson 2008) All isoenzymes in the same family have at least 40% and those in the same subfamily at least 55% amino acid similarity. An individual enzyme is identified by a number following the number and letter indicating the family and subfamily, respectively. (Nelson *et al.* 1996)

The proportions on the CYP enzymes in human liver are presented in Figure 2.2 as reported with respect to age-, sex-, and race-related changes by Shimada *et al.* in 1994. The immunochemical *in vitro* study was performed by using human liver microsomes of Caucasian and Japanese subjects. Although the total CYP concentration was higher in Caucasians than in Japanese, the relative levels did not differ except that CYP2A6 and CYP2B6 levels were higher in Caucasians. (Shimada *et al.* 1994)

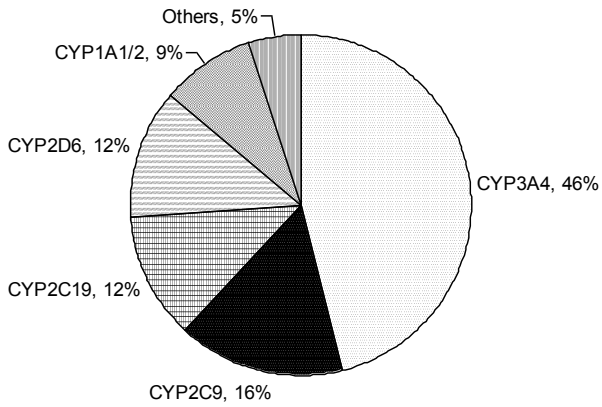


Figure 2.1 Propotions of the CYP enzymes responsible of the oxidative drug metabolism (Johnson 2008)

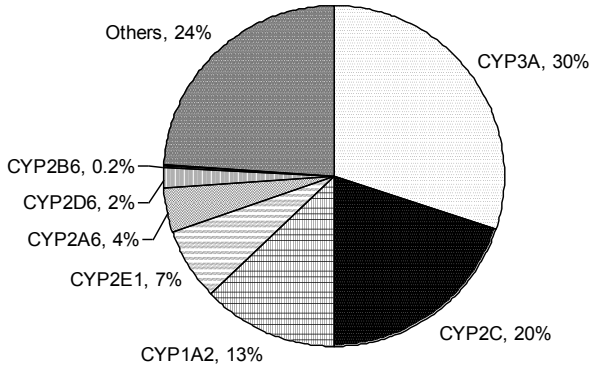


Figure 2.2 Proportions of CYP isoenzyme concentrations in human liver microsomes (Shimada *et al.* 1994)

2.1.1.1 CYP3A4

CYP3A4 isoenzyme is abundantly expressed in liver and small intestine where it contributes substantially to the first-pass metabolism of numerous drugs. It metabolizes more drugs than any other biotransforming enzyme. Among the substrates of CYP3A4 there are members of several important drug classes: antiarrhythmic agents, anxiolytics, HIV protease inhibitors, lipid-lowering agents, and strong opioids. The substrates vary widely in size and structure. The active site of CYP3A4 is wide and it is capable of binding large substrates, two small molecules simultaneously, or individual substrates to discrete regions. CYP3A4 is both inhibitable and inducible. The number of the agents inhibiting or inducing CYP3A4 is small compared with the

amount of the substrates. The group of CYP3A4 inhibitors includes azole antifungals, macrolide antibiotics, and HIV protease inhibitors. The most important drugs among the inducers are antitubercular agent rifampicin and the antiepileptics carbamazepine, phenobarbital, and phenytoin. (Parkinson and Ogilvie 2008)

2.1.1.2 CYP2C9

CYP2C9 is a genetically polymorphic CYP isoenzyme with two main variant alleles: *CYP2C9*2* and *CYP2C9*3*. These single-nucleotide polymorphisms (SNPs) decrease the catabolic activity of CYP2C9; *CYP2C9*3* is associated with marked decrease and *CYP2C9*2* with moderate decrease in the enzyme activity. In Northern Europe, the allele frequencies of these SNPs are 7.4% and 11.5%, respectively (Sistonen *et al.* 2009). Individuals with the *CYP2C9*3* allele are considered poor metabolizers (PMs) of CYP2C9. Homozygous *CYP2C9*3/*3* is present in 0.3% of Caucasians (Goldstein 2001). Substrates of CYP2C9 tend to represent acid- or sulfonamide-containing compounds. The main groups of the substrates are antidiabetic agents and non-steroidal anti-inflammatory drugs (NSAIDs). *S*-warfarin is one particular substrate of which the major metabolic pathway is CYP2C9-mediated. Its therapeutic index is narrow and thus careful dosing is essential, because warfarin-treated patients are vulnerable for treatment failure and adverse effects due to concentration alterations. In addition to interindividual changes in drug concentrations due to genetic factors, the alterations may result from inhibition or induction of CYP2C9. The list of CYP2C9 inhibitors includes some azole antifungals, amiodarone (an antiarrhythmic agent), and fluvoxamine (an antidepressive agent). Rifampicin (an antitubercular agent) is a potent inducer of CYP2C9. (Parkinson and Ogilvie 2008)

2.1.1.3 CYP2D6

CYP2D6 represents only 2% of the haepatic CYPs but it accounts for 12% of oxidative drug metabolism (Figures 2.1 and 2.2.). CYP2D6 substrates include antiarrhythmic agents, antidepressants, neuroleptics, and weak opioids. The substrates of CYP2D6 contain a basic nitrogen that interacts with an anionic residue in the binding site of the enzyme. Strong inhibitors, like quinidine, can interact favourably with the anionic site but are not oxidized by the ezyme. In contrast to other CYPs, CYP2D6 is considered to be non-inducible. Based on the polymorphisms of CYP2D6 individuals can be categorized into four genotypes: poor metabolizers (PMs), intermediate metabolizers (IMs), extensive metabolizers (EMs), and ultra-rapid metabolizers (UMs) (Table 2.3). Five to 7% of Caucasians are PMs, the prevalence of UMs range from 1─2% to 5─10% in Northern European and Southern European Caucasians, respectively. (Parkinson and Ogilvie 2008)

Table 2.3 Relationship between genotype and phenotype for a polymorphically expressed CYP2D6 (modified from Parkinson and Ogilvie 2008)

Alleles	Phenotype
(*wt*/*wt*)n [a]	UM
wt/*wt*	EM
wt/**x*	EM
wt/**y*	EM
x*/x*	EM or IM
x*/y*	IM
y*/y*	PM

abbreviations: *wt*, fully active wild type; **x*, partly active; **y*, inactive
[a] n ≥ 2

2.1.2 Prodrugs

Some drugs become pharmacologically active only after biotransformation. If the parent compound of a drug lacks activity, the drug is called a prodrug. Drugs with no or little pharmacological activity metabolizing to therapeutically active metabolites are designed for improving oral bioavailability by facilitating absorbtion or decreasing presystemic metabolism, lengthening the duration of action by slow metabolic release, or by improving the chemical stability of the active agent allowing tissue-selective delivery leading to its *in situ* activation. (Testa 2009)

2.2 Drug-drug interactions

When the effects of a drug are markedly altered as a result of coadministration of another drug it is a case of a drug-drug interaction (DDI). DDIs may be pharmacokinetic or pharmacodynamic, or combinations of these two interaction types. Pharmacokinetic interactions concern the ADME phases and result in increased or decreased delivery of drugs to the sites of action. In pharmacodynamic interactions the effects change without alterations in drug concentrations. As in drug metabolism, there are interindividual differences (see chapter 2.1) that make some patients more vulnerable to DDIs than others. (Oates 2006)

2.2.1 Pharmacokinetic drug-drug interactions

Most of the drugs are given orally and they are absorbed to the systemic circulation through the mucous membranes in the gastrointestinal tract. Absorption interactions may affect on the rate or the extent of absorption. The mechanism can be based either on changes in gastrointestinal pH or motility, complex formation, changes in transporter protein activity, (see examples in Table 2.4) or result from the combinations of these. The absorption can be impaired also due to malabsorption state, the

bioavailability of phenoxymethylpenicillin, for example, is reduced by neomycin-induced malabsorbtion syndrome. (Stockley 2002a)

After absorption the drug molecules are dissolved in plasma water or bound to plasma proteins, particularly to albumin. Equilibrium is established between the two forms and only the unbound molecules are pharmacologically active. The molecules occupying protein binding sites may be displaced by another drug which leads to increased concentration of the active form. (Stockley 2002a) However, changes in protein binding caused by drug-drug interactions will usually not influence the clinical exposure due to increased metabolism of the free drug (Benet and Hoener 2002).

The passive drug disposition from blood to specific tissues depends on pH and lipid solubility of the drug and is highest in well-perfused organs like liver, kidney, and brain. The distribution may also result from active transportation which is the case especially in the central nervous system where the blood-brain barrier (BBB) restrains the transition through the vascular endothelium. (Buxton 2006) The transporter proteins in BBB are either influx transporters like OATP (organic anion-transporting polypeptide) and MCT (monocarboxylate transporter) or efflux transporters like P-gp (P-glycoprotein), BCRP (breast-cancer-resistance protein), OAT (organic anion transporter), and MRP (multidrugresistance-associated protein) (Urquhart and Kim 2009). With positron emission tomography (PET) in healthy volunteers it has been shown that CNS (central nervous system) exposure to P-gp substrate verapamil is significantly increased in the presence of P-gp inhibitor cyclosporine (Sasongko *et al.* 2005). (Table 2.4)

The drugs that alter blood flow in the liver may have a marked effect on the extent of the first-pass metabolism and the bioavailability of other drugs (Stockley 2002a). (Table 2.4)

Metabolic DDIs may occur during the phase I or phase II biotransformation reactions. They are mainly based on inhibition or induction of the metabolic enzymes (Table 2.1). Drug metabolism may end up to four different consequences: 1) an active parent substrate forms an inactive metabolite, 2) an active substrate forms an active metabolite, 3) an inactive substrate forms an active compound, 4) a parent compound transforms into toxic metabolite. CYPs are the main enzymes in metabolism and thus the main target enzymes for metabolic drug interactions. The mechanisms of enzyme inhibition and induction as well as DDIs involving CYP enzymes are discussed in detail in chapters 2.2.4 and 2.3.6. (Table 2.4)

Table 2.4 Mechanisms of interactions in the different ADME phases and clinically significant examples of the interacting drugs

ADME phase	Interaction mechanism	Interacting drug	Target drug and the effect	References
Absorption				
	gastrointestinal pH ↑	cimetidine	ketoconazole absorption ↓	(Blum *et al.* 1991a)
	chelation	iron	tetracycline serum C ↓	(Neuvonen *et al.* 1970)
	adsorption	activated charcoal	glipizide absorption ↓	(Kivisto and Neuvonen 1990)
	complexation	colestyramine	glipizide absorption ↓	(Kivisto and Neuvonen 1990)
	gastrointestinal motility ↑	metoclopramide	digoxin serum C ↓	(Manninen *et al.* 1973)
	gastrointestinal motility ↓	propantheline	digoxin serum C ↑	(Manninen *et al.* 1973)
	P-gp induction in gut	rifampicin	digoxin plasma C ↓	(Greiner *et al.* 1999)
	P-gp inhibition in gut	cyclosporine	paclitaxel bioavailability ↑	(Meerum Terwogt *et al.* 1999)
Distribution				
	P-gp inhibition in BBB	cyclosporine	verapamil AUC_{brain}/AUC_{blood} ↑	(Sasongko *et al.* 2005)
Metabolism				
	haepatic blood flow ↓	cimetidine	propranolol bioavailability ↑	(Feely *et al.* 1981)
	CYP inhibition	itraconazole	lovastatin C_{max} and AUC ↑	(Kivisto *et al.* 1998)
	CYP inhibition	fluvoxamine	prodrug proguanil C ↑, metabolite C ↓	(Jeppesen *et al.* 1997)
	CYP inhibition	disulfiram	paracetamol NAPQI formation ↓	(Hazai *et al.* 2002)
	CYP induction	rifampicin	midazolam plasma C↓	(Backman *et al.* 1996a)
	CYP induction	rifampicin	AUC of losartan and E-3174 ↓	(Williamson *et al.* 1998)
	UGT inhibition	valproate	lamotrigine AUC and $T_{1/2el}$ ↑, CL ↓	(Yuen *et al.* 1992)
	UGT induction	rifampicin	morphine analgesic effect ↓	(Fromm *et al.* 1997)
	COMT inhibition	entacapone	levodopa AUC and $T_{1/2el}$ ↑	(Myllyla *et al.* 1993)
Excretion				
	urinary pH ↑	$Al(OH)_3/Mg(OH)_2$ antacid	acetylsalicylic acid serum C ↓	(Levy *et al.* 1975)
	tubular excretion ↓	phenylbutazone	tolbutamide serum C ↑	(Ober 1974)
	prostaglandin suppression	indomethacin	lithium renal CL ↓	(Reimann *et al.* 1983)
	P-gp inhibition in kidney	clarithromycin	digoxin C and toxicity ↑	(Yu 1999)

abbreviations: pH, power of hydrogen; C, concentration; P-gp, P-glycoprotein; BBB, blood-brain barrier; AUC, area under the plasma concentration-time curve; CYP, cytochrome P450; C_{max}, maximum concentration; NAPQI, N-acetyl-p-benzoquinone-imine; UGT, UDP-glucuronosyltransferase; $T_{1/2el}$, elimination half-life; CL, clearance; COMT, catechol-*O*-methyl transferase; $Al(OH)_3$, aluminium hydroxide; $Mg(OH)_2$, magnesium hydroxide

Most of the drugs are excreted in urine or bile as water soluble metabolites. The mechanisms of the excretion phase interactions are based on changes in urinary pH, active tubular excretion, renal blood flow, re-metabolism by the gut flora (clinically irrelevant), and activity of transporter proteins in the gut and kidney. (Stockley 2002a) (Table 2.4)

Transporter proteins, like P-glycoprotein (P-gp; also known as multidrug resistance transporter 1, MDR1) coded by *ABCB1* gene (Gottesman 2002), play an important role in DDIs from the pharmacokinetic point of view because they affect the drug ADME in all four phases. Inhibition or induction of the transporters may enhance or impair: 1) the absorption in gut, 2) the distribution through the blood barriers like blood-brain barrier (BBB), blood-placental barrier (BPB), and blood-testis barrier (BTB), 3) the enzymatic metabolism rate by altering the drug concentrations, 4) the excretion in urine or bile (Table 2.4). Noteworthy alterations affect the efficacy and safety of drugs with narrow therapeutic index such as digoxin. On the other hand, the DDIs can be used to manipulate transporter (P-gp) activity, thus improving the cell uptake of drugs in cancer cells and through the BBB (Varadi *et al.* 2002, Newman *et al.* 2002) (Table 2.4). P-gp and CYP3A have overlapping substrate specificity and tissue distribution suggesting synergy in the regulation of drug exposure (Wacher *et al.* 1995, Yu 1999, Zhang and Benet 2001).

2.2.2 Pharmacodynamic drug-drug interactions

In pharmacodynamic interactions the drug effect is changed in the presence of another drug at the site of action (receptors or ion channels, for example) without a change in drug concentration. However, the reaction is often indirect and involves interference with physiological mechanism. Disturbances in electrolyte balance during the use of potassium-depleting diuretics (e.g. furosemide) increase the sensitivity of myocardium to digitalis glycosides (e.g. digoxin) causing digitalis toxicity. (Stockley 2002a)

In addition to adverse effects, pharmacodynamic interactions have beneficial effects, and they can be employed to gain therapeutic advantages. Additive or synergistic effects are used in achieving fewer drug-specific adverse effects by using submaximal doses of the drugs in concern. Combinations are common in the treatment of, for example hypertension, infections, and pain. For optimal drug therapy there are even manufactured combination products, such as losartan + diuretic (for hypertension), rifampicin + isoniazide + pyrazinamide (for tuberculosis), and codeine + NSAID (for pain). (Oates 2006) One reason for manufacturing combination products is the improvement of compliance (Erdine 2010).

Solely toxic effects of pharmacodynamic interactions include additive prolongation of QT interval and serotonin syndrome. They both represent life-threatening ADRs. Two or more drugs prolonging QT interval increase the risk of *torsades de pointes*. Drugs

increasing serotonin activity may in concomitant use lead to over-stimulation of serotonin (5-HT) receptors in the central nervous system. This may occur even when one serotonergic drug is replaced with another. Serotonin syndrome is an iatrogenic condition that is difficult to diagnose due to variability of clinical manifestations and lack of awareness of the syndrome (Sun-Edelstein *et al.* 2008). (Stockley 2002a)

2.2.3 Drug-drug interactions inflicting adverse drug reactions

Serious and fatal ADRs are frequent and represent an important clinical issue. Fatal ADRs have been reported to be between the fourth and sixth leading cause of death in the US. When studying either patients experiencing an ADR in hospital (ADRIn) or patients admitted to hospital due to an ADR (ADRAd) the incidences of serious ADRs were 2.1 and 4.7%, and the incidences of fatal ADRs 0.19 and 0.13% (of ADRIn and ADRAd, respectively). When combining ADRIns and ADRAds the overall incidence of serious ADRs was 6.7% (95% CI 5.2─8.2) of hospital patients and the overall incidence of fatal ADRs 0.32% (95% CI 0.23─0.41). (Lazarou *et al.* 1998)

It has been estimated that 26% of ADRs (McDonnell and Jacobs 2002) and 8% of all adverse drug events (Kelly 2001) leading to hospitalizations are caused by DDIs. Many of the DDI-involved hospitalizations could have been avoided with closer patient monitoring or the use of alternative medications (Juurlink *et al.* 2003). Polypharmacy and the use of drugs with narrow therapeutic index increase the risk for ADRs. The frequency of at least one interaction is predicted to be 50% for those who receive at least four drugs and even 90% for the patients receiving eight drugs or more (Weideman *et al.* 1998). In a high-risk population of emergency department a potential adverse DDI was found even in 47% of the patients receiving three or more drugs (two or more in patients ≥ 50 years of age) (Goldberg *et al.* 1996). In primary health care patients at risk (receiving two or more drugs) the incidence rate of potential DDI was 12% for all and 22% for elderly (≥ 65 years of age) (Linnarsson 1993).

2.2.4 Drug-drug interactions involving CYP enzymes

Due to the major role of oxidative metabolism in drug elimination the alterations in CYP enzyme activity represent the main reason for DDIs. Many drugs can compete for the same enzyme which increases the significance of CYP inhibition. Generally the ADRs resulting from changes in drug concentrations are emphasized if the drug is metabolized by a single CYP pathway and has a narrow therapeutic index. Inhibitory interactions lead usually to more dramatic consequences to the patient but induction decreases efficacy and side effects. In case of prodrugs inhibition can reduce clinical efficacy. When the DDI concerns a polymorphic CYP enzyme, the EMs are more susceptible to enzyme inhibition and induction than PMs. DDIs can also be stereoselective (see Table 2.4). (Lin and Lu 1998, Pelkonen 2002)

Different human CYPs and their implication to DDIs as substrate, inhibitor, or inducer are listed in Table 2.5. The figures are based either on *in vivo* or *in vitro* studies in various models including tissue slices, microsomes, cell cultures, and purified and recombinant enzymes. (Rendic 2002)

Table 2.5 Proportions of the human isoenzymes involved in CYP-mediated drug-drug interactions in all and separately as substrate, inhibitor, and inducer (modified from Rendic 2002)

CYP	All (%)	Substrate (%)	Inhibitor (%)	Inducer (%)
1A1	3	3	3	6
1A2	10	10	12	3
1B1	1	1	1	1
2A6	3	3	2	2
2B6	4	4	3	13
2C	25	25	27	21
2E1	4	3	4	7
2D6	16	15	22	2 *
3A4	34	36	26	45
Total	100	100	100	100

* The common understanding is that CYP2D6 is non-inducible (Parkinson and Ogilvie 2008) but according to Rendic, haloperidol as well as organic solvents isopropranol and dimethyl sulfoxide stimulate CYP2D6 activity (Rendic 2002 referring to Kudo and Odomi 1998, Shin *et al.* 2001).

2.2.4.1 CYP enzyme inhibition

CYP enzyme inhibition can be divided roughly into reversible and irreversible processes or into three categories: reversible inhibition, quasi-irreversible inhibition, and irreversible inhibition (Figure 2.3). Among these, reversible inhibition is the most common mechanism responsible for the DDIs. (Lin and Lu 1998)

Reversible inhibition can be further divided into competitive, non-competitive, uncompetitive, and mixed-type inhibition. In competitive inhibition the binding of the inhibitor prevents the binding of the substrate to the active site of the enzyme. In non-competitive inhibition the inhibitor binds not to the active but to another site of the enzyme. The presence of the inhibitor has no effect on binding of substrate but its metabolism is still hindered. In uncompetitive inhibition the inhibitor does not bind to the free enzyme but to the enzyme-substrate complex. Also in this case the substrate cannot be metabolized by the enzyme. Mixed-type inhibition displays elements of both competitive and non-competitive inhibition. (Lin and Lu 1998, Pelkonen *et al.* 2008) (Figure 2.3)

Reversible enzyme inhibition is transient and the normal function of the enzyme is able to continue after the inhibitor has been eliminated from the body. Reversible inhibition

involves probably the first step of the CYP catalytic cycle (see chapter 2.1.1). The inhibitors causing reversible inhibition act rapidly. They bind to the enzyme with weak bonds which are formed and broken down easily. Many of the CYP inhibitors causing reversible inhibition are nitrogen-containing drugs. They bind to the prosthetic haem iron or to the lipophilic region of the enzyme. Inhibitors that bind to both regions simultaneously are more potent. (Lin and Lu 1998, Pelkonen *et al.* 2008)

Both irreversible and quasi-irreversible inhibitions require the formation of active metabolites. They evolve during at least one CYP catalytic process cycle. The loss of enzyme activity persists even after the elimination of the inhibitor from the body and *de novo* biosynthesis of new enzymes is required to restore the CYP activity. (Lin and Lu 1998)

The CYP inhibitors causing irreversible inhibition contain such functional groups that can be oxidized by the CYP enzyme to reactive intermediates. The intermediates inactivate the enzyme prior to the release from the active site. The inhibitors causing irreversible inhibition are divided into mechanism-based inactivators and suicide substrates (Figure 2.3). Mechanism-based inhibitors contain terminal double or triple bond and can be oxidized by CYP to radical intermediates that alkylate the prosthetic haem group and inactivate the enzyme. Suicide inhibition inactivates the enzyme completely by covalent binding to apoprotein. (Lin and Lu 1998)

In quasi-irreversible inhibition the metabolite forms a stable complex with the CYP prosthetic haem. The complex is called the metabolic intermediate (MI) complex. It sequesters the enzyme into a functionally inactive state. (Lin and Lu 1998)

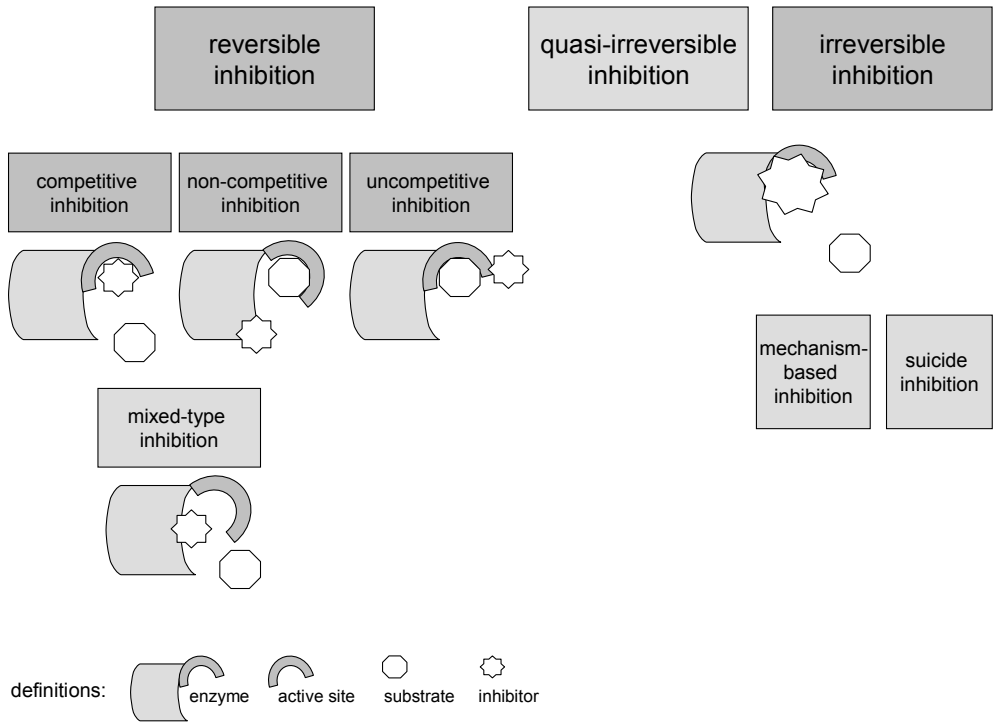


Figure 2.3 Substrate and inhibitor binding to enzyme in different types of CYP inhibition (Lin and Lu 1998, Pelkonen *et al.* 2008)

2.2.4.2 CYP enzyme induction

From a biological point of view induction protects the cells from toxic xenobiotics by increasing the metabolic activity. In drug therapy there are two concerns related to CYP induction: 1) induction may reduce pharmacological effects by increasing drug metabolism, 2) induction may result in increased toxicity due to the increased production of the toxic metabolites. (Lin and Lu 1998)

In most cases CYP enzyme induction by xenobiotics is mediated by a group of ligand-activated transcription factors and ensues from increased gene transcription. However, some non-transcriptional mechanisms are also known. (Lin and Lu 1998, Pelkonen *et al.* 2008) As a consequence of CYP induction both the amount of enzyme and endoplasmic reticulum in hepatocytes increase. CYP induction interactions are delayed at the beginning and at the end of the concomitant use, and may then arise even after withdrawal of the inducer. It is possible to adapt the interaction by raising the dosage of the affected drug. This requires, however, careful monitoring, and includes the risk of overdose when withdrawing the inducing drug. (Stockley 2002a)

2.2.4.3 Methods in CYP-mediated drug-drug interaction research

CYP enzymes are widely studied due to their remarkable potential to cause clinically significant DDIs. Testing the CYP profile of a new drug (as a substrate, inhibitor, or inducer for CYPs) is a prerequisite for the marketing authorization (EMEA 1997, FDA 1997, FDA 1999). Screening of drug candidates for their DDI potential is performed *in vitro* and *in vivo* (human studies) and is encouraged to be started in the early stages of the drug development process (Figure 2.4). Examples of probe substrates for studying the CYP inhibition or induction effects of the new drug are listed in Table 2.6. Probe drugs used one at a time or as a cocktail are presented also in two Nordic articles (Pelkonen *et al.* 1998, Christensen *et al.* 2003).

Table 2.6 Examples of marker substrates used in studying interaction potential of new drugs *in vitro* and *in vivo* [modified from EMA draft guideline currently under revision (EMA 2010) and FDA directions to drug development process (FDA 2006a)]

	Markers	
CYP	***in vitro***	***in vivo***
1A2	phenacetin * **	theophylline * **
		caffeine * **
2A6	coumarin **	
	nicotine **	
2B6	efavirenz * **	efavirenz * **
	bupropion * **	*S*-bupropion *
2C8	paclitaxel *	amodiaquine *
	amodiaquine *	repaglinide **
	taxol **	rosiglitazone **
2C9	*S*-warfarin * **	*S*-warfarin * **
	diclofenac * **	tolbutamide * **
	tolbutamide **	
2C19	*S*-mephenytoin * **	omeprazole * **
		esomeprazole **
		lansoprazole **
		pantoprazole **
2D6	bufuralol * **	metoprolol *
	dextromethorphan **	desipramine * **
		dextromethorphan **
		atomoxetine **
2E1	chlorzoxazone **	chlorzoxazone **
3A4	midazolam * **	midazolam * **
	testosterone * **	buspirone **
	nifedipine *	felodipine **
	triazolam *	lovastatin **
	dexamethasone *	eletriptan **
		sildenafil **
		simvastatin **
		triazolam **

* accodring to EMA
** according to FDA

Human liver microsomes from several donors are the most important tool in studying CYP activity *in vitro*. CYP antibodies, cloned *CYP* cDNAs (complementary deoxyribonucleic acid) and recombinant proteins as well as isolated hepatocytes and radiolabelled drugs may be used to confirm the results from the microsome studies. (FDA 1997) *In vitro* interaction studies should be performed before phase I clinical studies (EMA 2010). The early data of pharmacokinetics is important because the DDIs *in vivo* will not depend only on the potency but also on the dose and the concentration of the compound in the active site (Rodrigues and Lin 2001). *In vitro* studies can assess the presence or absence of enzyme inhibition but have a limited capability to identify induction (FDA 1999). According the new EU guideline (EMA 2010) the extent of enzyme induction should be investigated in hepatocytes from ≥ 3 donors for CYP3A, CYP2B6, and CYP1A2. In addition to this, the activity of mRNA (messenger ribonucleic acid) as well as the function of nuclear receptors PXR, CAR, and AH can be measured. The later preclinical stages of drug development and the data available from animal studies can attempt be used to predict DDIs in humans. The US Food and Drug Administration (FDA) has defined animal studies important in toxicology but they are not regulated (FDA 1997). (Figure 2.4)

In general, if no interactions are detected in appropriately performed *in vitro* studies there is no need for further surveys (EMEA 1997, FDA 1997). If inhibition has been seen *in vitro* the pharmacokinetics of the probe drug is studied alone and at a steady state of the inhibiting drug (EMA 2010).

According to the European Medicines Agency (EMA, earlier EMEA) draft guideline on investigation of drug interactions metabolic DDIs should be studied *in vivo* if the metabolic pathways are responsible for more than 25% of the total clearance or if metabolites are estimated to have more than 50% of the pharmacological activity (EMA 2010). Pharmacokinetic *in vivo* interaction studies in humans begin in the phase I of the drug development process and are performed more elaborately during the phase II and phase III studies (Figure 2.4). They are usually carried out in healthy volunteers. Subjects drawn from the general patient population offer certain advantages, including the opportunity to investigate pharmacodynamic endpoints not presented in healthy volunteers. Subjects are genotyped or phenotyped if any of the enzymes mediating the metabolism are polymorphically distributed, notably CYP2D6 and CYP2C19. (EMEA 1997, FDA 1999)

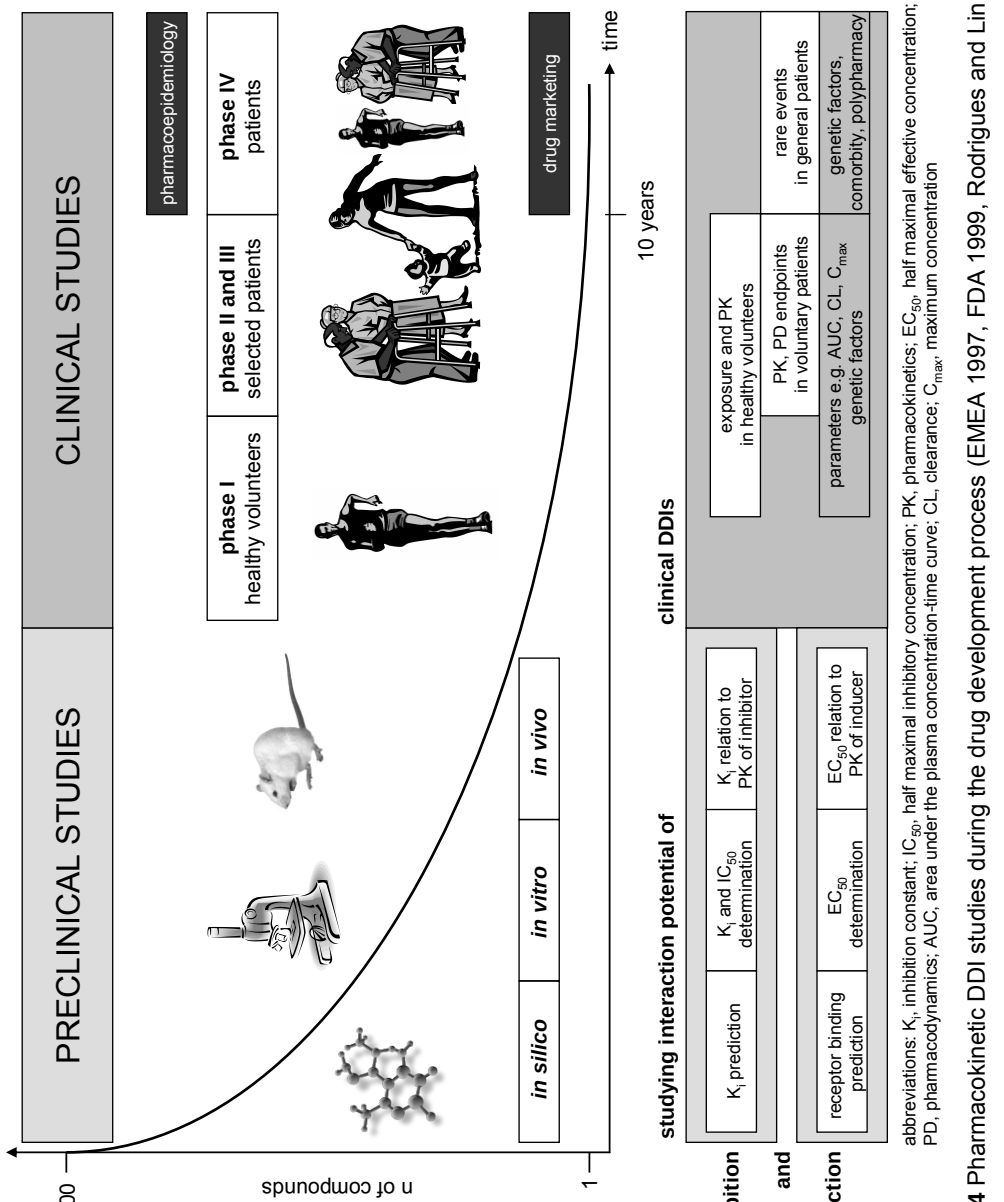
The study design in *in vivo* metabolic DDI studies is usually a randomized crossover study type (EMEA 1997, FDA 1999). Studies can be run as unblinded unless pharmacodynamic endpoints are part of the assessment. The time at observing endpoints depends on whether inhibition or induction is studied. When the drugs are given chronically a one-sequence crossover design is possible. When the drugs or their metabolites exhibit long elimination half-life ($T_{1/2el}$) also parallel design may be used. (FDA 1999) This is, however, not recommended due to wide inter-individual

variability (EMA 2010). The recommended measures and parameters are exposure measures such as area under the plasma concentration-time curve (AUC), maximum concentration (C_{max}) and time to maximum concentration (T_{max}), and pharmacokinetic parameters like clearance (CL), volume of distribution (V_d) and $T_{1/2el}$. A specific objective is to determine whether the interaction is sufficiently potent to necessitate dosage adjustments and additional therapeutic monitoring. To provide adequate dosage recommendation also steady state studies and parameters such as trough concentration (C_{min}) are valuable. (EMEA 1997, FDA 1999)

Population studies (phase IV) in a sufficient number of patients are valuable addition to phase II and III trials to get acquainted with unsuspected interactions or to confirm absence of suspected interactions (Figure 2.4). A relatively new method, population pharmacokinetics, can also detect unsuspected DDIs. (EMEA 1997, FDA 1999)

Generally extensive efforts have been made to characterize the human CYP system, and with recent advances in molecular biology and *in silico* methods the high-throughput screening (HTS) assays can now be performed earlier in the drug-development process to identify CYP profiles (Rodrigues and Lin 2001). *Ex vivo* testing, in perfused placenta, for example, is also a method to test CYP activity and interaction potential but is not in routine use (Deshmukh *et al.* 2003).

On average only 0.1 ‰ of the candidate molecules reach the drug market as full-fledged products (Figure 2.4). The reasons why the drug candidates fail to reach the market are shown in Figure 2.5. Nowadays, human pharmacokinetics is only a marginal cause of dropouts with 8% proportion (Guengerich and MacDonald 2007). More than two decades ago inappropriate pharmacokinetics was the major problem and the reason for 39.4% of the development discontinuations (Prentis *et al.* 1988). The extrapolation of *in vivo* systems has become more accurate with the help of developed *in vitro* methods and *in silico* techniques, such as crystallizing and modelling the structures of human CYP enzymes (Guengerich 2008).



abbreviations: K_i, inhibition constant; IC_{50}, half maximal inhibitory concentration; PK, pharmacokinetics; EC_{50}, half maximal effective concentration; PD, pharmacodynamics; AUC, area under the plasma concentration-time curve; CL, clearance; C_{max}, maximum concentration

Figure 2.4 Pharmacokinetic DDI studies during the drug development process (EMEA 1997, FDA 1999, Rodrigues and Lin 2001)

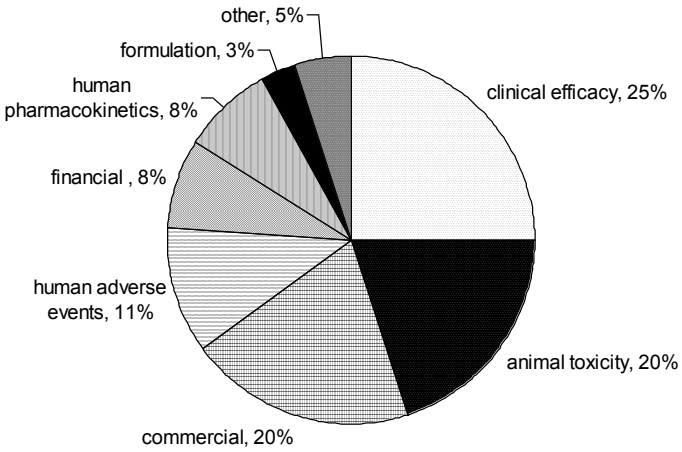


Figure 2.5 Proportions of the reasons why the drug candidates have not reached the end of the drug development process and have not been launched to the market (modified from Guengerich and MacDonald 2007)

2.3 CYP-mediated metabolism and interaction profile of the investigated drugs

2.3.1 Losartan

Losartan is an angiotensin II (ATII) type 1 (AT_1) receptor blocker used as an antihypertensive agent (See 2001). Losartan is a prodrug; its active metabolite is responsible for the decrease in blood pressure (Munafo *et al.* 1992). The parent compound is transformed to carboxylic acid metabolite EXP3174 (E-3174) by CYP2C9 (Yasar *et al.* 2001) (Figure 2.6). Concomitant use of CYP2C9 inhibitors, fluconazole and bucolone, has been shown to prevent the formation of the active metabolite in healthy volunteers (Kaukonen *et al.* 1998, Kobayashi *et al.* 2008) Also in patients with *CYP2C9*3* variant allele (see chapter 2.1.1.2) the metabolism of single dose losartan to EXP3174 as well as its hypotensive effect are significantly reduced (Sekino *et al.* 2003). Losartan is manufactured as an unmixed product but also in combination with hydrochlorothiazide diuretic (see chapter 2.2.2).

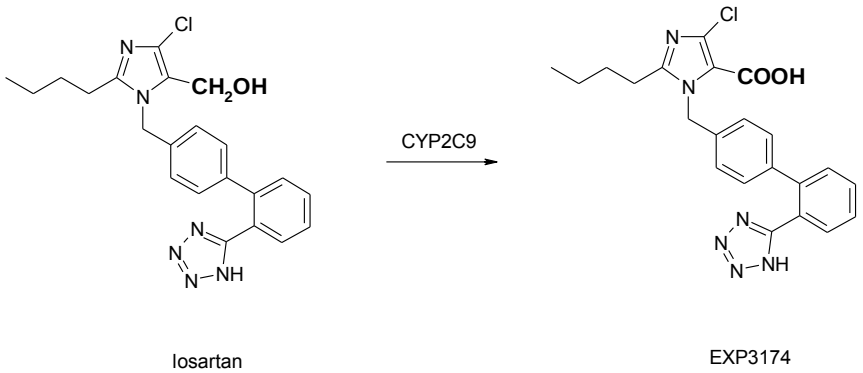


Figure 2.6 Chemical structures of losartan and its active metabolite EXP3174 (active sites in bold)

2.3.2 Codeine and tramadol

Codeine and tramadol are weak opioid analgesics. They are both prodrugs. Codeine (methylmorphine) is converted into morphine by CYP2D6 (Dayer *et al.* 1988) (Figure 2.7 a). The *O*-demethylation is essential for analgesia. The use of CYP2D6 inhibitor quinidine has been shown to reduce the analgesic effect and codeine abuse (Desmeules *et al.* 1991, Sindrup *et al.* 1992, Sindrup *et al.* 1996, Caraco *et al.* 1996, Caraco *et al.* 1999, Kathiramalainathan *et al.* 2000). Also the threshold of experimental pain is increased in EMs but not PMs of CYP2D6 (see chapter 2.1.1.3) lacking the activation process (Sindrup *et al.* 1990, Poulsen *et al.* 1996b). Codeine itself has an exceptionally low affinity to opioid receptors. Tramadol is a codeine analog with weak μ-opioid receptor affinity. It is used as a racemic mixture. The (+)-enantiomer binds to μ receptor and increases serotonin activity, the (–)-enantiomer stimulates α_2-adrenergic receptors and inhibits noradrenalin reuptake. The analgesic effect of tramadol is partly due to its ability to increase noradrenalin and serotonin activity. However, most important is the metabolism to active *O*-desmethyltramadol by CYP2D6 (Poulsen *et al.* 1996a) (Figure 2.7 b). Concomitant use of CYP2D6 inhibitor paroxetine decreases the analgesic effect of tramadol (Laugesen *et al.* 2005). Weak opioids are often used in combination with NSAIDs (see chapter 2.2.2) and are available also as combination products, with ibuprofen or paracetamol, for example. Codeine has also antitussive effects. (Gutstein and Akil 2006)

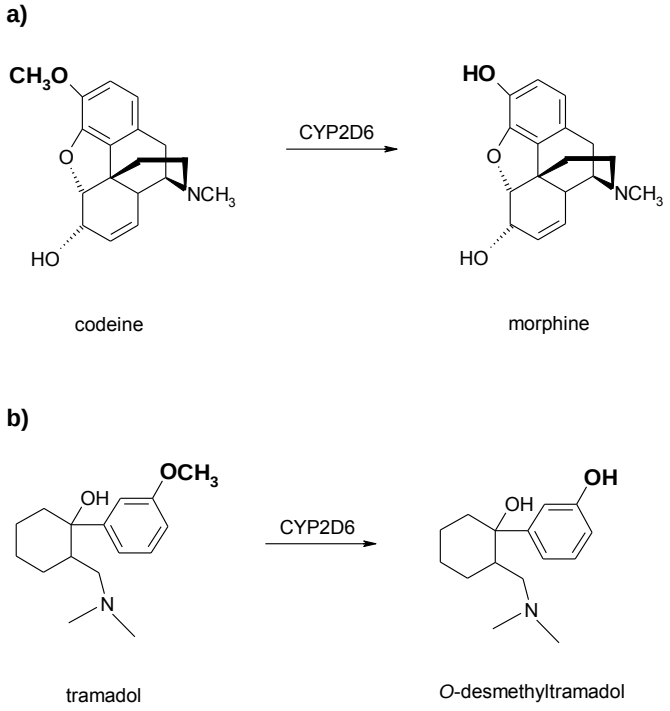


Figure 2.7 Chemical structures of codeine (a) and tramadol (b), and their active metabolites (active sites in bold)

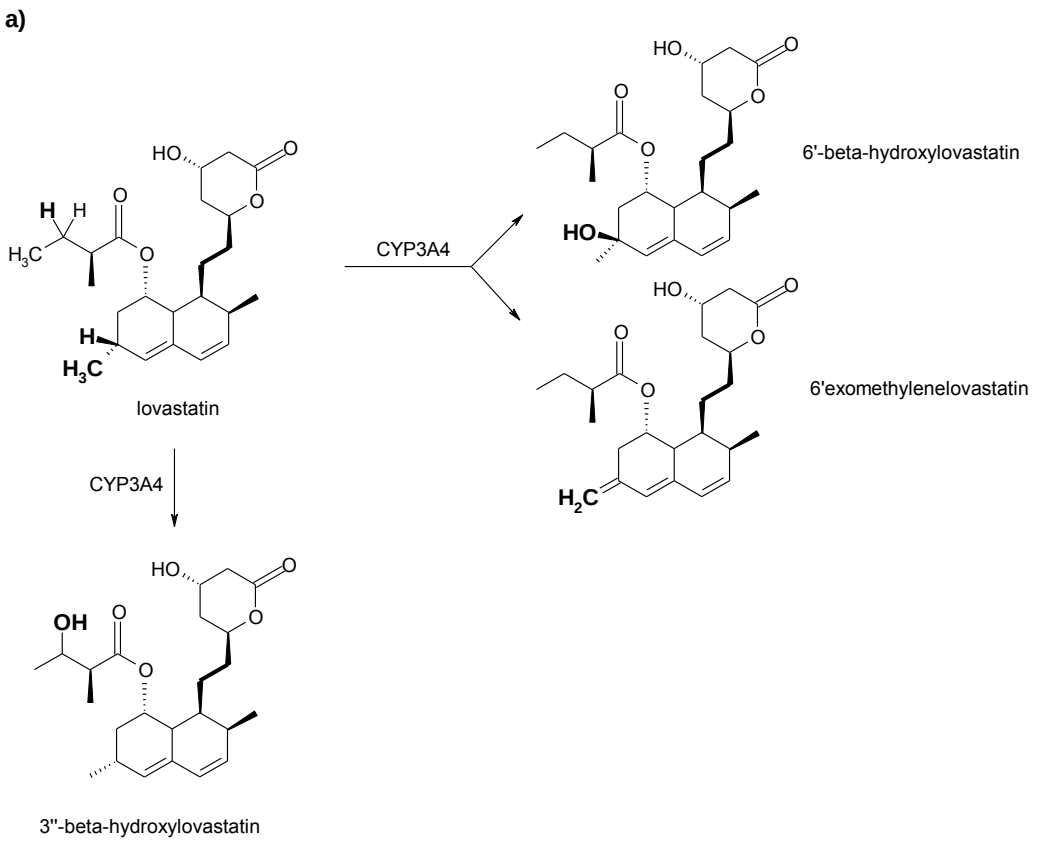
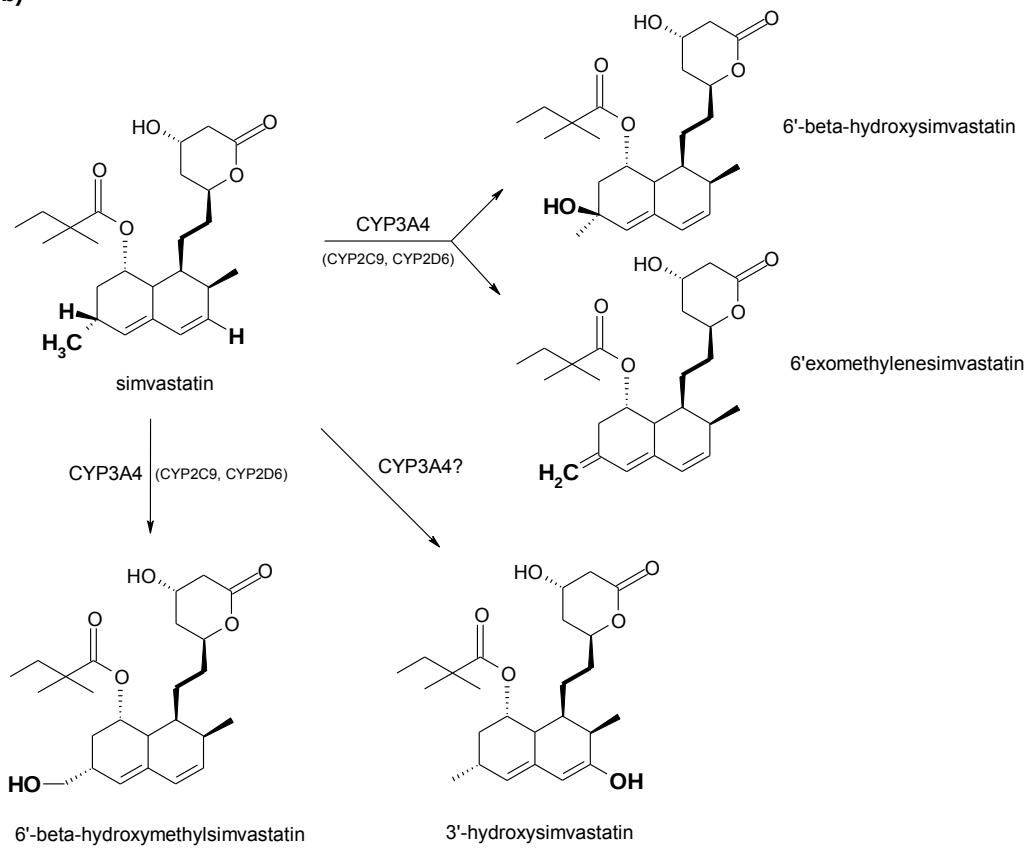


Figure 2.8 Chemical structures of lovastatin (a) and simvastatin (b), and their CYP metabolites (active sites in bold)

2.3.3 Lovastatin and simvastatin

Statins are HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitors used in treatment of dyslipidemia. They are effective both in the primary and secondary prevention of aterosclerotic heart disease. They lower LDL (low-density lipoprotein) cholesterol concentrations in plasma by increasing the LDL clearance and upregulation of LDL receptors. They also decrease VLDL (very-low-density lipoprotein) cholesterol and triglyceride concentrations and increase HDL (high-density lipoprotein) cholesterol levels slightly. Combination products with ezetimibe, for example, are available. Lovastatin and simvastatin as lactone prodrug forms are less active than the respective β-hydroxy acid metabolites (Figure 2.8). Both parent drugs undergo extensive first-pass metabolism. CYP3A4 is mainly responsible for the biotransformation. Lovastatin is oxidized to three known primary metabolites: 6'β-hydroxylovastatin, 6'-exomethylene metabolite, and 3''β-hydroxylovastatin. All these metabolites are pharmacologically active. It is probable that the first two derive from a

b)



single metabolic intermediate (Figure 2.8 a). Simvastatin is metabolized to at least four primary metabolites: 6'β-hydroxysimvastatin, 6'-exomethylene metabolite (which may derive from a common metabolic precursor), 6'β-hydroxymethyl metabolite, and 3'-hydroxysimvastatin (Figure 2.8 b). CYP3A4 inhibitors (e.g. clarithromycin, erythromycin, telithromycin, itraconazole, ketoconazole, diltiazem, and verapamil) have been shown to increase the exposure to lovastatin and simvastatin. By increasing the statin concentration these kinds of DDIs increase the incidence of skeletal muscle toxicity, an ADR concerning the entire class of otherwise well-tolerated statins, and the risk of potentially fatal rhabdomyolysis. CYP3A4 inducers rifampicin and carbamazepine, on the other hand, have been shown to reduce simvastatin concentrations (Kyrklund *et al.* 2000, Ucar *et al.* 2004). The synergistic effects of fibrates and statins give therapeutic advantages in severe dyslipidemia but their concomitant use increases the risk of myopathy. (Williams and Feely 2002, Neuvonen *et al.* 2006, Caron *et al.* 2007)

2.3.4 Glibenclamide, glimepiride, and glipizide

Glibenclamide (also known as glyburide), glimepiride, and glipizide are second-generation sulphonylureas. These antidiabetic agents bind to the SUR1 receptors in pancreatic β-cells and close the potassium-dependent ATP channels when potassium intake decreases and the cell membrane depolarizes. The calcium intake then initiates the insulin excretion from β-cells. (Kirchheiner *et al.* 2005) Sulphonylureas bind also to SUR2 receptor subtypes SUR2A in cardiac tissue and SUR2B in smooth muscle which may have relevance in mechanisms of cardiac morbidity and peripheral vascular resistance in type 2 diabetes mellitus (Ashcroft and Gribble 2000).

Glibenclamide is an antidiabetic drug that is extensively metabolized by CYP2C9 in the liver. The main metabolites are 3- and 4-hydroxyglibenclamide (Figure 2.9 a). They both have antihyperglycaemic activity and contribute to the glucose lowering effect of glibenclamide. Glibenclamide is excreted into urine (50%) and into faeces (50%). Long $T_{1/2el}$ of the parent compound and the metabolites leads to long-lasting hypoglycaemic events and increases the risk of hypoglycaemic episodes. (Kirchheiner *et al.* 2005) In studies in healthy subjects the clearance of glibenclamide is less than half and insulin secretion significantly higher in PMs (*CYP2C9*3/*3* homozygous, see chapter 2.1.1.2) compared with wild type subjects (Kirchheiner *et al.* 2002). Also heterozygous carriers of *CYP2C9*3* allele have greater glibenclamide and glimepiride AUCs in plasma compared with wild-type subjects (Niemi *et al.* 2002, Yin *et al.* 2005).

From glimepiride CYP2C9 forms a hydroxyl metabolite (Figure 2.9 b) that has approximately one third of the activity of the parent compound. This metabolite is oxidized further to carboxylic acid. (Kirchheiner *et al.* 2005) In healthy volunteers it has been shown that concomitant use of CYP2C9 inhibitors fluconazole and fluvoxamine prolongs $T_{1/2el}$ and increase C_{max} of glimepiride (Niemi *et al.* 2001a). In the same study fluconazole but not fluvoxamine increased also the AUC of glimepiride compared with placebo. Gemfibrozil increases the AUC of glimepiride modestly (Niemi *et al.* 2001d). On the other hand, rifampicin has been shown to decrease the AUC and $T_{1/2el}$ in healhy volunteers (Niemi *et al.* 2000).

Glipizide is structurally very similar to glibenclamide (see Figures 2.9 a and c) differing only in the aryl ring portion. The role of CYP2C9 is also similar; glipizide is transformed into 3- and 4-hydroxymetabolites (Figure 2.9 c). (Kirchheiner *et al.* 2005) A CYP2C9 inducer, rifampicin, decreases the plasma AUC and C_{max} and shortens the $T_{1/2el}$ of both glibenclamide and glipizide in healthy volunteers (Niemi *et al.* 2001b).

All the studies on sulphonylurea kinetcs mentioned above (Niemi *et al.* 2000, Niemi *et al.* 2001a, Niemi *et al.* 2001b, Niemi *et al.* 2001d, Kirchheiner *et al.* 2002, Niemi *et al.* 2002, Yin *et al.* 2005) were performed in settings with single dose sulphonylurea exposures. However, in continous exposure to high concentrations of sulphonylureas, the relationship between the drug concentration and the hypoglycaemic effet appears to

be bell-shaped. This is probably due to the downregulation of β-cell sensitivity. (Melander *et al.* 1998)

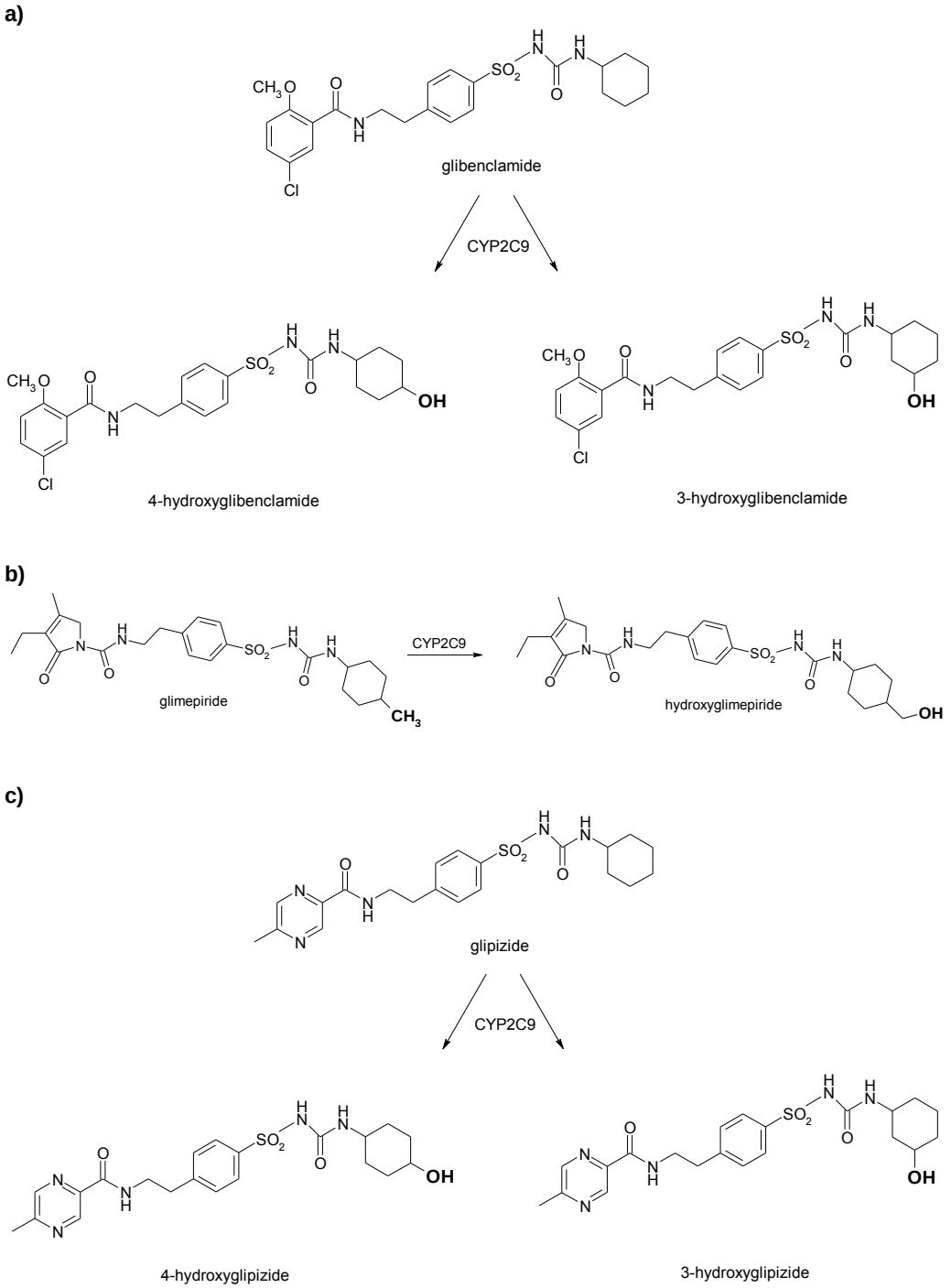


Figure 2.9 Chemical structures of glibenclamide (a), glimepiride (b) and glipizide (c), and their hydroxylated metabolites (active sites in bold)

2.3.5 Clopidogrel

Clopidogrel is an ADP-receptor antagonist used to inhibit platelet aggregation. The approved indications are to reduce the rate of stroke, myocardial infarction (MI), and death in patients with recent MI or stroke, established peripheral arterial disease, or acute coronary syndrome. The fixed dose is 75 mg per day possibly with initial loading dose of 300 mg. (Majerus and Tollefsen 2006) Clopidogrel is a thienopyridine prodrug activated by CYP enzymes. However, only 15% of clopidogrel metabolism is CYP-related, for 85% of clopidogrel is hydrolyzed by esterases to an inactive carboxylic acid derivative. The activation of clopidogrel consists of two steps. CYPs are responsible for the oxidation of the thiophene ring to 2-oxo-clopidogrel and for the further oxidation resulting in opening of the thiophene ring and formation of carboxyl and thiol groups (Figure 2.10). (Clarke and Waskell 2003, Nguyen *et al.* 2005) The thiol group binds with ADP-receptor $P2Y_{12}$ when the normal activation of glycoprotein GPIIb/IIIa in fibrinogen clotting is prevented (Savi *et al.* 2001). Recently it has been defined that the formation of 2-oxo-clopidogrel is mediated by CYP2C19, CYP1A2, and CYP2B6 (by 44.9, 35.8, and 19.4%, respectively) whereas the active metabolite, R-130964, is formed by CYP3A4, CYP2B6, CYP2C19, and CYP2C9 (with contribution of 39.8, 32.9, 20.6, and 6.8%, respectively) (Kazui *et al.* 2010) (Figure 2.10).

Ketoconazole, a well known CYP3A4 inhibitor, has been shown to decrease the AUC and C_{max} of the active R-130964 metabolite of clopidogrel (Farid *et al.* 2007). *In vitro* clopidogrel metabolism is inhibited by more than 90% by atorvastatin also metabolized primarily by CYP3A4 (Clarke and Waskell 2003). In a platelet activation study in coronary artery implantation patients measuring platelet aggregation inhibition, atorvastatin but not pravastatin (a statin not undergoing CYP metabolism) attenuated clopidogrel activation (Lau *et al.* 2003). In the same study erythromycin and troleandomycin (both CYP3A4 inhibitors) impaired the platelet activation inhibition of clopidogrel whereas rifampicin (a CYP3A4 inducer) enhanced it. These effects of rifampicin on clopidogrel efficacy have also been seen in another study in healthy volunteers (Lau *et al.* 2004). The first published CYP2C19-mediated DDI associated with diminished clopidogrel activation was due to concomitant use of omeprazol (Gilard *et al.* 2006). Thereafter, patients carrying mutant *CYP2C19*2* have been associated with significantly diminished platelet aggregation and increased risk of stent thrombosis and cardiovascular ischemic event following coronary stent placement (Sibbing *et al.* 2009, Shuldiner *et al.* 2009). *CYP2C19*17* carriers are associated with significantly increased bleeding risk (Sibbing *et al.* 2010).

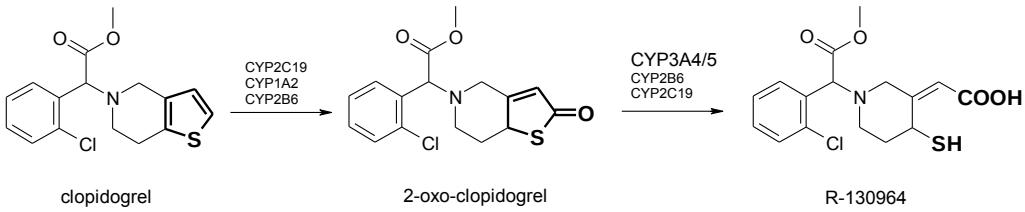


Figure 2.10 Chemical structures of clopidogrel and its CYP metabolites (active sites in bold)

2.3.6 Inhibitors and inducers of CYP3A4, CYP2C9, and CYP2D6 isoenzymes

The CYP inhibitors and inducers included in the studies are listed in Table 2.7 with short introductions to referred literature.

Table 2.7 Cytochrome P450 2C9, 2D6, and 3A4 inhibitors and inducers included in the studies with literature refecences

Interacting drug	Documentation level	Target drug and the effect	References
CYP2C9 inhibitors			
amiodarone	6 healthy volunteers	warfarin plasma C ↑	(O'Reilly *et al.* 1987)
	7 healthy volunteers	phenytoin AUC ↑	(Nolan *et al.* 1990)
fluconazole	20 healthy volunteers	phenytoin AUC and C_{min} ↑	(Blum *et al.* 1991b)
	16 healthy volunteers	losartan AUC and C_{max} ↑, E-3174 AUC and C_{max} ↓	(Kazierad *et al.* 1997)
	11 healthy volunteers	losartan: E-3174 AUC ↓	(Kaukonen *et al.* 1998)
	12 healthy volunteers	fluvastatin AUC, $T_{1/2el}$, and C_{max} ↑	(Kantola *et al.* 2000)
fluvoxamine	14 healthy volunteers	tolbutamide CL ↓	(Madsen *et al.* 2001)
gemfibrozil	10 healthy volunteers	glimepiride AUC and $T_{1/2el}$ ↑	(Niemi *et al.* 2001d)
metronidazole	32 patients	warfarin: INR ↑	(Laine *et al.* 2000)
miconazole	6 healthy volunteers and human liver microsomes	warfarin CL ↓, AUC and $T_{1/2el}$ ↑, PT ↑; warfarin hydroxylation ↓	(O'Reilly *et al.* 1992)
phenytoin	16 healthy volunteers	losartan: E-3174 AUC ↓	(Fischer *et al.* 2002)
sulfamethoxazole	7 healthy volunteers	tolbutamide CL ↓ and $T_{1/2el}$ ↑	(Wing and Miners 1985)
tamoxifen	13 patients	losartan MR ↑	(Boruban *et al.* 2006)
trimethoprim	7 healthy volunteers	tolbutamide CL ↓ and $T_{1/2el}$ ↑	(Wing and Miners 1985)
valproate	11 patients	losartan MR ↑ (significant after 4 weeks)	(Gunes *et al.* 2007)
zafirlukast	16 healthy volunteers	*S*-warfarin AUC and $T_{1/2el}$ ↑, PT ↑	(www.astrazeneca-us.com/pi/accolate.pdf)
CYP2D6 inhibitors			
celecoxib	12 healthy volunteers	metoprolol AUC ↑	(Werner *et al.* 2003)
chloroquine	20 healthy volunteers	debrisoquine MR ↑	(Simooya *et al.* 1998)
chlorpromazine	43 patients	haloperidol and reduced haloperidol plasma C ↑	(Suzuki *et al.* 2001)
clomipramine	151 patients	sparteine MR ↑	(DUAG 1999)
dextropropoxyphene	14 healthy volunteers	debrisoquine MR ↑	(Sanz and Bertilsson 1990)
flecainide	8 healthy volunteers and human liver microsomes	dextromethorphan MR ↑; bufuralol hydroxylation ↓	(Haefeli *et al.* 1990)
fluoxetine	13 patients	dextromethorphan MR ↑	(Vandel *et al.* 1995)
	13 patients	tolterodine CL ↓	(Brynne *et al.* 1999)
	31 + 12 healthy volunteers	dextromethorphan MR ↑	(Alfaro *et al.* 1999, Alfaro *et al.* 2000)
	26 healthy volunteers	dextromethorphan MR ↑	(Amchin *et al.* 2001)
hydroxychloroquine	7 healthy volunteers	metoprolol AUC and plasma C ↑	(Somer *et al.* 2000)
levomepromazine	7 patients	deprisoquine MR ↑	(Kallio *et al.* 1990)
	10 patients	codeine MR ↑	(Vevelstad *et al.* 2009)

moclobemide	4 healthy volunteers	dextromethorphan MR ↑	(Hartter *et al.* 1998)
paroxetine	9 patients	clozapine and norclozapine plasma C ↑	(Spina *et al.* 2000)
	9 healthy volunteers	sparteine MR ↑	(Brosen *et al.* 1993)
	9 healthy volunteers	desipramine CL ↓	(Brosen *et al.* 1993)
	9 healthy volunteers	perphenazine AUC and C_{max} ↑, and CNS side effects ↑	(Ozdemir *et al.* 1997)
	31 + 12 healthy volunteers	dextromethorphan MR ↑	(Alfaro *et al.* 1999, Alfaro *et al.* 2000)
	5 healthy volunteers	nortriptylin CL ↓, C_{max} and C_{min} ↑, 10-hydroxynortriptyline AUC ↓	(Laine *et al.* 2001)
	16 healthy volunteers	tramadol AUC ↑, M1 AUC ↓	(Laugesen *et al.* 2005)
propafenone	12 healthy volunteers	propranolol C_{max}, T_{max}, $T_{1/2el}$, and C_{ss} ↑	(Kowey *et al.* 1989)
	8 healthy volunteers	mexiletine oral CL ↓	(Labbe *et al.* 2000)
quinidine	5 patients	flecainide CL ↓	(Birgersdotter *et al.* 1992)
	22 patients	dextromethorphan MR ↑	(Zhang *et al.* 1992)
	8 healthy volunteers	debrisoquine MR ↑	(Speirs *et al.* 1986)
	7 + 16 + 10 + 17 healthy volunteers	codeine: morphine plasma C undetectable and PD ↓	(Desmeules *et al.* 1991, Sindrup *et al.* 1992, Caraco *et al.* 1996, Caraco *et al.* 1999)
	17 healthy volunteers	codeine MR in plasma and cerebrospinal fluid ↓	(Sindrup *et al.* 1996)
terbinafine	9 healthy volunteers	dextromethorphan MR ↑	(Abdel-Rahman *et al.* 1999)
	12 healthy volunteers	desipramine AUC and C_{max} ↑	(Madani *et al.* 2002)
	12 healthy volunteers	paroxetine AUC, C_{max}, and $T_{1/2el}$ ↑	(Yasui-Furukori *et al.* 2007)
	12 healthy volunteers	venlafaxine AUC ↑	(Yasui-Furukori *et al.* 2007)
thioridazine	13 patients	mianserin and desmethylmianserin plasma C ↑	(Yasui *et al.* 1997)
	8 healthy volunteers	debrisoquine MR ↑	(Spina *et al.* 1991a)
CYP3A4 inhibitors			
clarithromycin	9 healthy volunteers	repaglinide and insulin AUC, C_{max}, and $T_{1/2el}$ ↑	(Niemi *et al.* 2001c)
	15 healthy volunteers	simvastatin and simvastatin acid AUC and C_{max} ↑	(Jacobson 2004)
cyclosporine	10 patients	simvastatin AUC and C_{max} ↑	(Arnadottir *et al.* 1993)
	14 patients	simvastatin acid plasma C ↑	(Campana *et al.* 1995)
	44 patients	lovastatin AUC ↑	(Olbricht *et al.* 1997)
diltiazem	30 patients	midazolam and alfentanil AUC and $T_{1/2el}$ ↑	(Ahonen *et al.* 1996)
	11 patients	simvastatin AUC and C_{max} ↑, LDL-Chol ↓	(Watanabe *et al.* 2004)
	10 + 7 healthy volunteers	triazolam AUC, C_{max}, $T_{1/2el}$, and PD ↑	(Varhe *et al.* 1996a, Kosuge *et al.* 1997)
	9 healthy volunteers	buspirone AUC and C_{max} ↑	(Lamberg *et al.* 1998)

Interacting drug	Documentation level	Target drug and the effect	References
	10 healthy volunteers	lovastatin AUC and C_{max} ↑	(Azie *et al.* 1998)
	10 healthy volunteers	simvastatin AUC, C_{max}, and $T_{1/2el}$ ↑, simvastatin C_{max} ↑	(Mousa *et al.* 2000)
erythromycin	12 healthy volunteers	simvastatin and simvastatin acid AUC and C_{max} ↑	(Kantola *et al.* 1998)
	12 healthy volunteers	midazolam AUC ↑	(Okudaira *et al.* 2007)
fluconazole	12 healthy volunteers	triazolam AUC, C_{max}, and $T_{1/2el}$ ↑	(Varhe *et al.* 1996b)
fluoxetine	11 healthy volunteers	alprazolam CL ↓, AUC and $T_{1/2el}$ ↑	(Haddad *et al.* 2007)
imatinib	20 patients	simvastatin AUC, C_{max}, and $T_{1/2el}$ ↑	(O'Brien *et al.* 2003)
itraconazole	12 + 10 healthy volunteers	lovastatin and lovastatin acid AUC and C_{max} ↑	(Neuvonen and Jalava 1996, Kivisto *et al.* 1998)
	10 healthy volunteers	simvastatin and simvastatin acid AUC, C_{max}, and $T_{1/2el}$ ↑	(Neuvonen *et al.* 1998)
	10 healthy volunteers	alprazolam CL ↓, AUC and $T_{1/2el}$ ↑	(Yasui *et al.* 1998)
	18 + 15 healthy volunteers	atorvastatin AUC, C_{max}, and $T_{1/2el}$ ↑	(Mazzu *et al.* 2000, Jacobson 2004)
ketoconazole	12 healthy volunteers	quetiapine CL ↓ and C_{max} ↑	(Grimm *et al.* 2006)
telithromycin	12 healthy volunteers	repaglinide AUC and C_{max} ↑	(Kajosaari *et al.* 2006)
verapamil	9 healthy volunteers	buspirone AUC and C_{max} ↑	(Lamberg *et al.* 1998)
	12 + 12 healthy volunteers	simvastatin and simvastatin acid AUC and C_{max} ↑	(Kantola *et al.* 1998, Jacobson 2004)
	12 healthy volunteers	fexofenadine oral CL ↓	(Lemma *et al.* 2006)
CYP3A4 inducers			
carbamazepine	6 patients and 7 healthy controls	midazolam AUC, C_{max}, and $T_{1/2el}$ ↓	(Backman *et al.* 1996b)
	9 patients * and 6 controls	vincristine CL ↑, AUC and $T_{1/2el}$ ↓	(Villikka *et al.* 1999)
	12 healthy volunteers	simvastatin and simvastatin acid AUC, C_{max}, and $T_{1/2el}$ ↓	(Ucar *et al.* 2004)
	12 healthy volunteers	quetiapine CL ↑ and C_{max} ↓	(Grimm *et al.* 2006)
dexamethasone	12 healthy volunteers and human liver microsomes	dextromethorphan MR ↓; dextromethorphan hydroxylation ↑	(McCune *et al.* 2000)
phenobarbital	6 patients	carbamazepine-10,11-epoxide CL ↑ and $T_{1/2\ el}$ ↓	(Spina *et al.* 1991b)
phenytoin	6 patients and 7 healthy controls	midazolam AUC, C_{max}, and $T_{1/2el}$ ↓	(Backman *et al.* 1996b)
	9 patients * and 6 controls	vincristine CL ↑, AUC and $T_{1/2el}$ ↓	(Villikka *et al.* 1999)
rifampicin	10 healthy volunteers	simvastatin and simvastatin acid AUC and C_{max} ↓	(Kyrklund *et al.* 2000)
	12 healthy volunteers	everolimus CL ↑, C_{max} and $T_{1/2el}$ ↓	(Kovarik *et al.* 2002)

abbreviations: C, concentration; AUC, area under the plasma concentration-time curve; C_{min}, trough concentration; C_{max}, maximum concentration; $T_{1/2el}$, elimination half-life; CL, clearance; INR, international normalized ratio; PT, prothrombin time; MR, metabolic ratio; CNS, central nervous system; T_{max}, time to maximum concentration C_{ss}, steady state concentration; PD, pharmacodynamics; LDL-Chol, low-density lipoprotein cholesterol

* 8 receiving carbamazepine, 1 phenytoin

2.4 Pharmacoepidemiological studies

Pharmacoepidemiology is defined as a study of the use and the effects of drugs in a large number of people (Strom 2005). The main focus is often on the risks of uncommon, unexpected, and latent adverse reactions (Garbe and Suissa 2007). On the other hand, both beneficial and adverse drug effects can be investigated (Garbe and Suissa 2007), which enables a better assessment of the risk-benefit balance for drug use (Strom 2005). In the drug development trajectory pharmacoepidemiology is presented in phase IV (Figure 2.4). The contribution of phase IV studies to the drug development process is a relatively new field, although US Food and Drug Administration (FDA) has required post-marketing research to one third of the approved drugs since the 1970s (Strom 2005). During the 14 years between 1993 and 2006, FDA withdrew 20 drugs from the US market for safety reasons with an average of 1.5 drug withdrawals per year (range 0–4) (Issa *et al.* 2007).

In addition to industry and regulatory point of view, pharmacoepidemiology is an important tool in academic studies on clinical pharmacological problems. They are usually run as observational designs with different case-control or cohort settings (Strom 2005, Garbe and Suissa 2007).

Drug utilization studies are an important tool in improving rational drug use. The definition "prescribing, dispensing, administering, and ingesting of drugs" implies the several steps involved in drug utilization. The World Health Organization (WHO) gives even a broader definition as "marketing, distribution, prescription and use of drugs in a society, with special emphasis on the resulting medical, social and economic consequences". (Garbe and Suissa 2007)

2.4.1 Advantages and limitations of pharmacoepidemiology

The 20 drugs FDA withdrew from the US market during 1993–2006 had been in use approximately four and a half years (range 6–519 months) (Issa *et al.* 2007). Due to the limited sample size (approximately up to 3000) the pre-marketing phase III studies are unlikely to detect uncommon adverse effects (Strom 2005). Phase III studies proving the efficacy and safety of the new drug are mostly conducted as randomized controlled clinical trials (RCTs). The sample sizes needed to be exposed to sufficient statistical power of a rare ADR are prohibitively large for premarketing studies. This means that rare ADRs will usually be detected only when the drug has been used in large patient populations after drug marketing. (Garbe and Suissa 2007) In RCTs the follow-up time may also be too short to detect long-term effects (Schneeweiss 2007). The short duration renders the detection of ADRs developing after a long induction period or cumulative drug intake impossible (Garbe and Suissa 2007).

RCTs differ from routine clinical care in several ways: selected study populations defined by strict inclusion and exclusion criteria are not representative of subsequent users for the drugs because the most vulnerable patient groups (the elderly, patients with comorbid conditions, pregnant women, and children) are under-represented; the surveillance of the patients is more intensive in clinics chosen for research centres due to better facilities and more frequent patient monitoring for the ADRs; treatment regimens allow no individual treatment variation in contrast to the adjustments made constantly in routine care; placebo controlled efficacy data from the trials conducted to compare an active substance against no treatment are rarely relevant in routine practice where one or more alternative therapies are available for most conditions. (Garbe and Suissa 2007, Schneeweiss 2007)

In addition to issues of resources, proper RCTs cannot always be implemented because of ethical issues. The character of the disease may affect the RCT process. If the disease is presupposed to cause prominent sufferings, the drug may be launched by fast track procedure. This is the case with HIV and cancer drugs, for example. When the drug therapy is absolutely essential for the patient the use of placebo in RCTs is not ethically accepted but active therapy must be offered for each study groups. (FDA 2006b) The fast track process may also be used in launching vaccination products under the threat of pandemia (EMEA 2008).

Pharmacoepidemiology can be used for comparing active treatment groups and studying new indications and user groups. Register-based studies offer a possibility to research retrospective data of high quality. A sophisticated study plan improves the chances for quick response with low cost. There are no recall- or interview-biases. However, patients' compliance as well as the indications of drug therapy and the use of over-the-counter (OTC) drugs are unknown (Garbe and Suissa 2007). Pharmacoepidemiological studies carry also some specific methodological challenges like immortal time bias, confounding by indication, and depletion of susceptibles (Garbe and Suissa 2007). To illustrate and understand confounding factors it is recommended to use causal diagrams or directed acyclic graphs (Schneeweiss 2007, Greenland *et al.* 1999). The use of propensity scores is proposed as a new method of adjusting for covariate imbalances (Garbe and Suissa 2007).

In register studies one special characteristic of the data is that the information has not been gathered for research purposes. The features in data impede register-based research compared with other quantitative research especially when combining data from different register sources. (retki.stakes.fi/EN/index.htm)

2.4.2 Data sources in pharmacoepidemiological research

The first approach in pharmacoepidemiological studies was based on spontaneous reports of drug-related morbidity or mortality. Later controlled studies have been

performed to examine whether the outcomes occur more often in an exposed than in unexposed population. (Strom 2005)

After the thalidomide disaster in the early 1960s WHO set up its International Drug Monitoring Program. An independent centre in Uppsala, Sweden is responsible for the collection of data about ADRs from 95 countries (in 2009) around the world, especially from the WHO member states, and the generation of signals of drugs which might possibly have problematic side effects. Uppsala Monitoring Centre receives more than half a million individual case safety reports annually and holds more than 4.7 million active reports in its database. (www.who-umc.org) In Finland physicians, dentists, and pharmacists are asked to report any noticed or suspected adverse drug reactions to the national register from which the gathered information is forwarded to WHO (www.fimea.fi).

Spontaneous reporting schemes are effective in the recognition of ADRs occurring shortly after initiation of the drug therapy but less successful in identifying reactions with long induction periods. Spontaneous reporting systems suffer from underreporting and, on the other hand, some ADRs are more likely to be reported than others because of their known association with the therapy. It is noteworthy that one false case report may lead to misconception and numerous false ADR reports. This phenomenon is called media bias. (Garbe and Suissa 2007)

A great number of pharmacoepidemiological studies have been conducted as field studies, but thereafter existing data sources, including multipurpose cohort studies or large health databases, have been used increasingly. Record linkage study databases can be divided into two categories: administrative databases and physician-based databases. Pharmacy-based prescription databases may be included in both categories depending on the local practice. (Garbe and Suissa 2007)

As an example of an administrative database the Saskatchewan Health Database based on a health insurance program include the patient records of more than one million inhabitants living in Saskatchewan province, Canada. All the residents of the province have been enrolled in the publicly funded health system which makes the population representative and fairly stable (compared with the situation in the US, for example, where the insurance policy is over-representing social welfare recipients). The systematic data collection has been conducted since 1962 and computerized since 1976. The data include population registry, cancer registry, hospitalization information, medical services data, outpatient prescription drug information, and vital statistics. More than 100 pharmacoepidemiological studies have been completed using the Saskatchewan Health Database as the data source. (www.health.gov.sk.ca, Garbe and Suissa 2007)

The General Practice Research Database (GPRD), as an example of a physician-based database, is a database of anonymized longitudinal medical records from primary care.

Since 1987 electronic data has been collected from 488 primary care practices throughout the United Kingdom covering about 5.5% of the population. Containing comprehensive observational data of 39 million person years from clinical practice, it is a valuable tool for academic research. There are over 550 research papers published in peer-reviewed journals. GPRD includes also lifestyle information parameters (body mass index, height, weight, and smoking and alcohol consumption) which are important confounders not usually recorded in health databases. (www.gprd.com)

2.4.2.1 Finnish registers for pharmacoepidemiological use

In Finland we have long traditions of maintaining registers. The general register of vital statistics including births, deaths, and marriages was initiated as early as 1749. The first nationwide computerized register was Cancer Register established in 1952. Registration of new cases of tuberculosis and sexually transmitted diseases was initiated in the 1950s. In the 1960s computerized registers of congenital malformations, occupational diseases, adverse drug reactions, causes of death, as well as the first version of the Hospital Discharge Register were introduced. (Gissler and Haukka 2004) Current nationwide health registers are presented in Table 2.8.

The system of identification numbers was launched in the 1960s along with the general health insurance. All Finnish citizens and permanent residents of Finland have a unique personal identification number, also known as identity number or social security number. This provides good opportunities for compilation of health and social welfare data. The legislation from 1987 (revised in 1999 to meet EU requirements) includes strict data protection laws prohibiting the collection of sensitive health and social information but an exemption provides for data collection for statistical and scientific purposes aiming to improve health and welfare. The institution maintaining the register has the right to grant an authorization for a researcher, but when hospital records are linked to register data the permission must be applied for from the Ministry for Social Affairs and Health. Statement from an ethics committee is not obligatory in register studies. (Gissler and Haukka 2004)

Finland, as well as the other Nordic countries, provides excellent possibilities for high-quality register-based research. However, there are still some obstacles hindering effective use of register data. The Finnish Information Centre for Register Research (ReTki) was introduced in the beginning of the 21st century aiming to promote the use of national registers for research purposes, particularly in social and health sciences. (retki.stakes.fi/EN/index.htm)

Table 2.8 Finnish nationwide health registers

Register holder	Register	Reference
National Institute for Health and Welfare	Finnish Care Register (initially Hospital Discharge Register) Finnish Cancer Registry Register of congenital malformations Finnish Myocardial Infarction Register * Finnish Stroke Register * National Cardiovascular Disease Register *	www.thl.fi/en_US/web/en/Home
Statistics Finland	Causes of Death	www.stat.fi/index_en.html
Kela, the Social Insurance Institution of Finland	Prescription Register	kela.fi/in/internet/english.nsf
Finnish Medicines Agency Fimea	Finnish ADR register	www.fimea.fi
Finnish Institute of Occupational Health	Register of Occupational Diseases	www.ttl.fi/en/Pages/default.aspx

abbreviation: ADR, adverse drug reaction

* combination register for reseach use offered by register holder

3 AIMS OF THE STUDY

The overall aim was to study the prevalence and clinical consequences of cytochrome P450-mediated drug-drug interactions with a pharmacoepdemiological approach. The specific aims were:

1. To investigate the prevalence of DDIs between drugs inhibiting the activity of CYP2C9 or CYP2D6 and the prodrugs losartan, codeine, and tramadol

2. To study the prevalence and clinical consequences of CYP2C9 inhibitor use together with the insulin secretagogues glibenclamide, glimepiride, and glipizide in hospitalized type 2 diabetes mellitus patients

3. To investigate the prevalence of concomitant use of CYP3A4 inhibitors and inducers with simvastatin, lovastatin and clopidogrel, and its clinical consequences both in hospital and open care settings

4. To study the prevalence and clinical consequences of interactions between statins and fibrates, and to characterize the prevalence and clinical effects of the use of atorvastatin by patients on clopidogrel

4 MATERIALS AND METHODS

4.1 Data sources

4.1.1 Turku University Hospital patient registers

At Turku University Hospital the complete information on the medication of all patients has been prospectively recorded by nurses into an electronic Unix-based database since the beginning of 1996. The laboratory data has been in electronic form since 1994. The laboratory database contains information not only for hospitalized patients but includes also information on tests performed in several health centres within the service area of the hospital. The Finnish Multilab software forms an aggregate laboratory database. The laboratory tests can be offered and the results followed by this standardized cache operating system. Also the traditional patient files are still archived.

4.1.2 National Prescription Register

The National Prescription Register of Kela, the Social Insurance Institution of Finland exists since 1994 and includes all reimbursed medication purchases. The register has been created for administrative purposes but also researchers can be granted permission to use the databank. The information is collected monthly from all pharmacies and includes about 25 million prescriptions every year. Information on the indication of the medication is scarce but further information may be available indirectly if the patient is entitled to special reimbursement. Medicines used in hospitals, over-the-counter (OCT) drugs, and relatively inexpensive packages are examples of drug treatments that are not registered in the database. (Klaukka 2001)

4.1.3 Finnish Care Register

The Finnish Care Register (HILMO), initially known as Hospital Discharge Register, includes data of hospital discharges and treatment periods in hospitals. It is the most often used register in health care research in Finland. The HILMO register has been in use since the 1960s and gathers now data on about 1.2 million hospital discharges annually. Validation studies have shown that HILMO contains 95% of all hospital discharges and, when compared against corresponding medical records, the most relevant information, like diagnoses and surgical procedures, were recorded correctly in 95% of the discharges. (www.stakes.fi/verkkojulkaisut/papers/DP1-2006.pdf) The

current register keeper is the National Institute for Health and Welfare (www.thl.fi/en_US/web/en/Home).

4.1.4 Causes of Death register

The Causes of Death register is produced by Statistics Finland. The register consists of statistics on causes of death and on trends in mortality. Statistics Finland also maintains an archive of death certificates, which have been available since 1936. Since 1969 the data has been available as a longitudinal file and since 1996 the ICD-10 classification system (see chapter 4.1.7) has been in use. The statistics on causes of deaths are produced annually. (www.stat.fi/til/ksyyt/index_en.html)

4.1.5 The Finnish ADR register

The Finnish Medicines Agency Fimea, earlier National Agency for Medicines, is responsible for continuous drug safety monitoring. In addition to that, Fimea maintains the adverse drug reaction register. The Finnish ADR register is a compilation of individual ADR reports and has been available since the 1960s. The data is extracted from reports from holders of marketing authorizations and from spontaneous reports from health care professionals. Spontaneous reporting system is considered important since especially rare ADRs can be detected only after wider use when divergent patient populations are treated. Fimea collects information on suspected ADRs, especially when they are serious, unexpected, or the suspected drug is new and has been on the market less than two years. It is noteworthy that the products may also lack efficacy or have adverse effects when used in combination with other medicines. (www.fimea.fi)

4.1.6 ATC codes

The Anatomical Therapeutic Chemical (ATC) classification system created and updated by WHO Collaborating Centre for Drug Statistics Methodology in Norway divides substances into different groups according to the organ or system on which they act and their therapeutic, pharmacological and chemical properties. The classification has five different levels (see Table 4.1). The first level contains 14 main groups. The second level describes pharmacological/therapeutic subgroups, the third and fourth levels are chemical/pharmacological/therapeutic subgroups, and the fifth level is the chemical substance. (www.whocc.no)

Table 4.1 Structure of ATC codes with glibenclamide ATC as an example (www.whocc.no)

Level	Symbol	Example	Definition
1	letter	A	alimentary tract and metabolism
2	two numbers	A10	drugs used in diabetes
3	letter	A10B	blood glucose lowering drugs, excl. insulin
4	letter	A10BB	sulfonamides, urea derivatives
5	two numbers	A10BB01	glibenclamide

4.1.7 ICD-10 codes

International Classification of Diseases (ICD) is used to classify diseases and other medical disorders recorded on many types of health and vital records including death certificates and health records like morbidity statistics derived from hospital case records and discharge data. ICD is an internationally recognized classification system and a statistical tool for between-country comparisons for clinical, epidemiological, and quality purposes. When WHO was founded in 1948 it took over the responsibility for the ICD when the sixth revision was introduced but the classification originates from the International List of Causes of Death initiated in the 1850s. The current tenth revision of ICD, ICD-10, by the World Health Assembly was adopted in the Nordic countries between 1994 and 1999. (www.who.int/en/, www.helsedirektoratet.no/nordclass_english/)

4.1.8 NCSP codes

The Nordic Medico-Statistical Committee (NOMESCO) published the first edition of the NOMESCO Classification of Surgical Procedures (NCSP) in 1996. Finland introduced the national version NCSP-F in 1997. (www.helsedirektoratet.no/nordclass_english/)

4.2 Study subjects and methods

The drug-drug interactions of cytochrome P450 enzyme substrates losartan, codeine, tramadol, lovastatin, simvastatin, glibenclamide, glimepiride, glipizide, and clopidogrel were studied with interacting drugs listed in Table 4.2. The interacting drugs were identified by performing literature searches in the MEDLINE database (www.ncbi.nlm.nih.gov/pubmed/). Also the book *Stockley's Drug Interactions* (Stockley 2002b) and an interaction card on CYP-mediated DDIs compiled for physicians' checklist [klifa.utu.fi/interaktiokortti.pdf (in Finnish)] were referred in Studies I and IV, respectively.

In addition to inhibitors and inducers of relevant CYP isoenzymes, concomitant use of fibrates with lovastatin and simvastatin was studied. The use of fibrates increases the risk of muscular toxicity of statins (Williams and Feely 2002). Cases of life-threatening, even fatal, rhabdomyolysis have been published (Pierce *et al.* 1990, van Puijenbroek *et al.* 1996, Federman *et al.* 2001, Kursat *et al.* 2005, Unal *et al.* 2008).

A CYP3A4 substrate atorvastatin has been shown to inhibit clopidogrel metabolism by more than 90% *in vitro* (Clarke and Waskell 2003, Jacobsen *et al.* 2000) and attenuate clopidogrel activation as measured by platelet aggregation inhibition *ex vivo* (Lau *et al.* 2003, Neubauer *et al.* 2003). However, later *in vivo* (Wienbergen *et al.* 2003, Saw *et al.* 2003, Mukherjee *et al.* 2005, Saw *et al.* 2007, Lotfi *et al.* 2008, Geisler *et al.* 2008) and *ex vivo* studies (Muller *et al.* 2003, Mitsios *et al.* 2004, Serebruany *et al.* 2004, Gorchakova *et al.* 2004) show no difference in outcomes between the patients using CYP-metabolized statins or non-CYP statins with clopidogrel. Only one clinical study indicates that the use of atorvastatin or other substances potentially inhibiting CYP3A4 activity has been associated with increased risk of cardiovascular outcome after percutaneous coronary intervention in clopidogrel-treated patients when compared with clopidogrel alone (Brophy *et al.* 2006). Because the role of atorvastatin as CYP3A4 inhibitor is controversial, it was excluded from the inhibitor group but analyzed as a separate study group in the clopidogrel study (Study IV). (Table 4.2)

The medication data was reviewed and thereby the study patients, both in Turku University Hospital (Studies I–IV) and nationwide in Finland (Studies II and IV), identified by searching medication registers using the ATC codes (see chapter 4.1.6) The ATC codes of the drugs included in the final analyses are listed in Table 4.2. Only pharmaceutical dosage forms leading to systemic exposure were included. The alterations of the ATC codes during the study years were taken into account, for example the ATC codes of statins have been changed from group B04AB to C10AA (www.whocc.no).

Table 4.2 The ATC codes of the study drugs found in the searches and included in the analyses with the related study numbers. The lists involve the ATC changes during the study years.

Study drugs	ATCs			Study number
substrates				
losartan	C09CA01	C09DA01		I
codeine *	M01AE51	N02AA59	N02AA79	I
tramadol	N02AX02	N02AX52		I
lovastatin	B04AB02	C10AA02		II
simvastatin	B04AB01	C10AA01		II
glibenclamide	A10BB01			III
glimepiride	A10BB12			III
glipizide	A10BB07			III
clopidogrel	B01AC04			IV
CYP2C9 inhibitors				
amiodarone **	C01BD01			I, III
fluconazole **	J02AC01			I, III

Study drugs	ATCs				Study number
fluvoxamine **	N06AB08				I, III
gemfibrozil	C10AB04				I, III
metronidazole **	A02BD02	A02BD03	J01XD01	P01AB01	I, III
miconazole **	A01AB09 †	A07AC01	J02AB01		I, III
phenytoin	N03AB02	N03AB52			I
sulfamethoxazole ** ‡	J01EC01	J01EE01			I, III
tamoxifen	L02BA01				I, III
trimethoprim	J01EA01	J01EE01	J01EE02		III
valproate	N03AG01				III
zafirlukast	R03DC01				I, III
CYP2D6 inhibitors					
celecoxib	M01AH01				I
chloroquine	P01BA01				I
chlorpromazine	N05AA01				I
clomipramine	N06AA04				I
dextropropoxyphene	M03BB53	N02AC04	N02AC54	N02AC74	I
flecainide	C01BC04				I
fluoxetine	N06AB03				I
hydroxychloroquine	P01BA02				I
levomepromazine	N05AA02				I
moclobemide	N06AG02				I
paroxetine	N06AB05				I
propafenone	C01BC03				I
quinidine	C01BA01	C01BA51	C01BA71		I
terbinafine	D01BA02				I
thioridazine	N05AC02				I
CYP3A4 inhibitors					
clarithromycin	A02BDXX	J01FA09			II, IV §
cyclosporine	L04AA01				II, IV
diltiazem	C08DB01				II, IV
erythromycin	J01FA01				II, IV §
fluconazole	J02AC01				IV
fluoxetine	N06AB03				IV
itraconazole	J02AC02				II, IV §
ketoconazole	J02AB02				II §, IV §
telithromycin	J01FA15				IV §
verapamil	C08DA01	C08DA51	C09BB10		II, IV
CYP3A4 inducers					
carbamazepine	N03AF01				II, IV
dexamethasone	C05AA09	H02AB02			IV §
phenobarbital	N03AA02				II #
phenytoin	N03AB02	N03AB52			II, IV
rifampicin	J04AB02	J04AM02			II #, IV §
others					
bezafibrate	B04AC02	C10AB02			II
clofibrate	B04AC01	C10AB01			II #
gemfibrozil	B04AC04	C10AB04			II
atorvastatin	C10AA05				IV

* only as analgesic
** CYP2C9 inhibitors considered clinically most relevant in Study III
† oral gel, the only topical drug form included in the studies
‡ in Finland all sulphamethoxazole products are combinations including also trimethoprim
§ found in outpatient data only
# found in inpatient data only

A single subject could have more than one interaction period during the follow-up and each of them was counted in the analysis separately; multiple periods of the same group of interacting drugs were allowed but patients receiving both CYP2C9 inhibitor and CYP2C9 inducer (Study III) or CYP3A4 inhibitor and CYP3A4 inducer (Studies II and IV) during the study periods were excluded. Patients receiving CYP3A4 inhibitor or CYP3A4 inducer with fibrate were included in the fibrate group (Study II) and patients receiving CYP3A4 inhibitor and atorvastatin were included in the CYP3A4 inhibitor group. In Study I phenytoin was regarded as CYP2C9 inhibitor (see Table 2.7) but in Study III the patients receiving phenytoin were excluded due to uncertain interaction potential of this possible competitive CYP2C9 inhibitor (Fischer *et al.* 2002). The controls did not receive interacting medication at any time during the whole study periods.

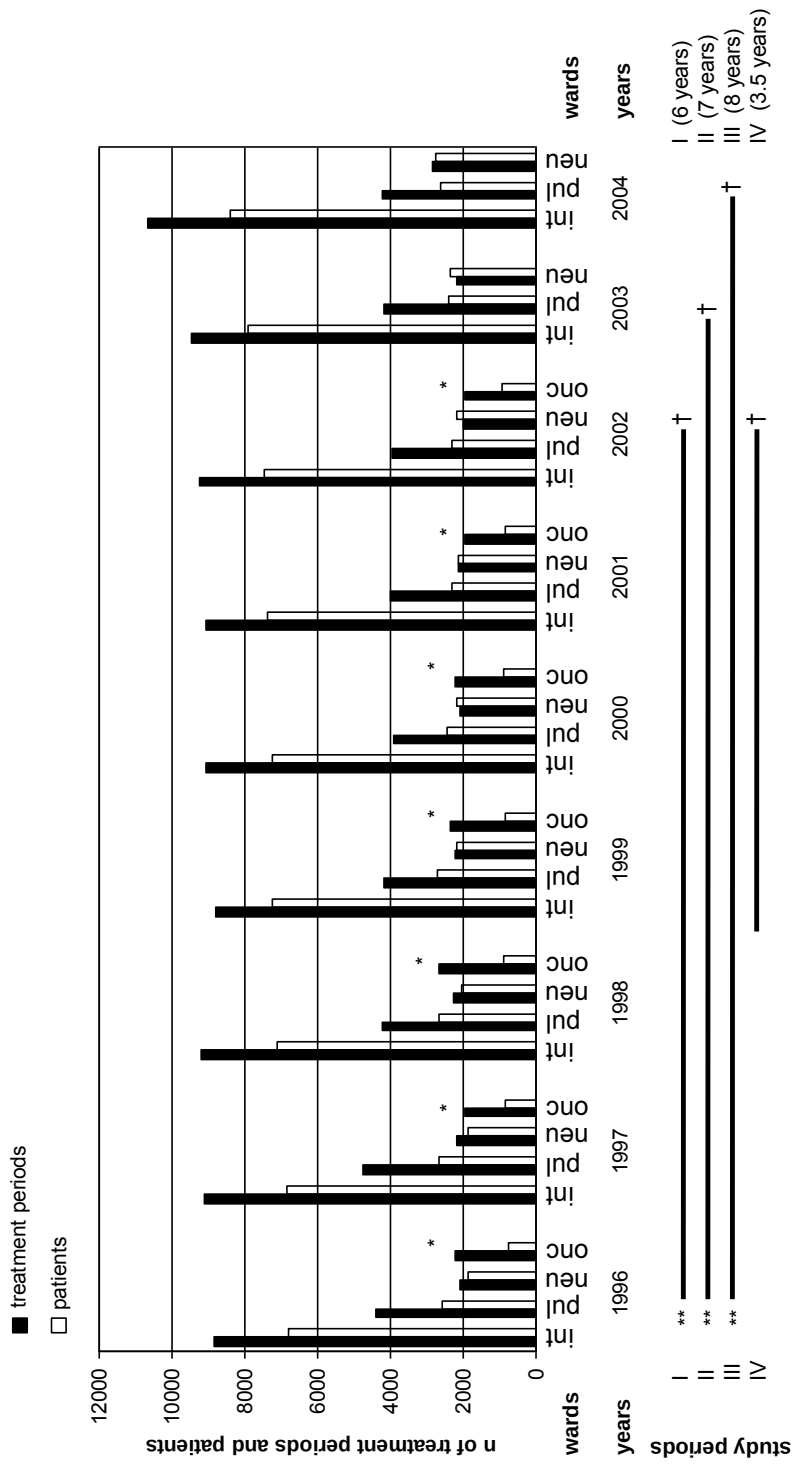
The selections of the study patients were exposure-based so that the designs would be considered as cohort studies. However, the controls were not matched to the cases but all the patients receiving the study substrates without defined interacting drugs (Table 4.2) were seen as controls.

4.2.1 Inpatients

Exposure to study substrates with or without concomitant interacting treatment (Studies I–IV) was searched retrospectively in the Turku University Hospital electronic medication database (chapter 4.1.1). In addition to patient identification, the following drug-related data was collected from the database: trade name, strength, dosage form and ATC code of the drug; dosage of the drug; starting and stopping dates of medication and the ward. The data was reviewed manually to ascertain the adequate allocation of the patients to the different study groups. Concomitant use was considered as a potential interaction and the patient was classified to the interaction group if the co-administration of chosen drug pairs overlapped at least for two days.

The study population consisted of all patients and treatment periods in the wards of internal medicine (n = 8), pulmonary diseases (n = 3), neurology (n = 2), and oncology (n = 2) in Turku University Hospital during the study periods (see Figure 4.1). These wards were considered as medication intensive and the stay of patients in these wards long enough to allow meaningful follow-up. Patients treated in the emergency room, intensive care units, or outpatient clinics were not included in the study. The oncology wards were included in Study I only because in these wards the use of analgesics is voluminous but, on the other hand, the disease status of the patients complicated.

The personnel of the wards in Turku University Hospital were not aware of the study. During the study periods there was no computerized prescription support tool warning for drug-drug interactions integrated with the hospital data processing systems.



abbreviations: int, internal medicine; pul, pulmonary diseases; neu, neurology; onc, oncology
* oncology wards included in the Study I only
** half a calendar year (starting July 1)
† half a calendar year (ending June 30)

Figure 4.1 Number of treatment periods and patients in the study wards and the timely coverage of the study periods

To assure the quality of the data a half year run-in period was allowed after the initiation of the medication database in the beginning of 1996 before the start date of the data searches, July 1, 1996 in Studies I–III (see Figure 4.1). Glimepiride was launched onto the Finnish market on July 7, 1997 [namweb.nam.fi/namweb/do/haku/process (in Finnish)]. The beginning of the study period in Study III thus included glibenclamide and glipizide only. Clopidogrel became available in Finland on July 15, 1998 and the study period of Study IV was decided to start on January 1, 1999.

In Study IV, after identifying the patients in the electronic database, the archived patient files were studied manually. This gave further information on the patients, for example indication of the clopidogrel treatment, predisposing factors (diabetes mellitus, cardiac insufficiency, and hypertension), and other concomitant medication. The concomitant medication was referred as drugs in use on discharge day. The co-medication was transformed into ATC codes and divided into six categories: the codes beginning with B (blood and blood forming organs), C (cardiovascular system), J (antiinfectives for systemic use), M (musculo-skeletal system), N (nervous system), and R (respiratory system). In addition, the drugs increasing or decreasing bleeding risk were categorized as listed in Table 4.3. In case less than five levels of the ATC classification (see chapter 4.1.6) are mentioned, all the substrates of the subgroup are included.

Table 4.3 Classification of concomitant medication other than CYP3A4 inhibitors, CYP3A4 inducers, and atorvastatin in clopidogrel treated patients in Study IV

Drugs	ATCs					
drugs increasing bleeding risk						
vitamin K antagonists	B01AA					
antithrombotic agents	B01AB	B01AC	B01AD	B01AX	B01AE	
acetylsalicylic acid	B01AC06	M01BA03	N02BA01	N02BA51	N02BA71	
glucocorticoids *	H02AB	R03BA				
NSAIDs	M01AA	M01AB	M01AC	M01AE	M01AG	M01AX **
coxibes	M01AH					
antiinflammatory/antirheumatic agents in combination with corticosteroids	M01BA					
other antiinflammatory/antirheumatic agents in combination with other drugs	M01BX					
tramadol	N02AX02	N02AX52				
SSRIs	N06AB					
drugs decreasing bleeding risk						
H_2-receptor antagonists	A02BA					
prostaglandins	A02BB					
proton pump inhibitors	A02BC					
tranexamic acid	B02AA02					
blood coagulation factors	B02BD					

abbreviations: NSAID, non-steroidal anti-inflammatory drug; SSRI, selective serotonin reuptake inhibitor
* systemic and inhaled
** excluding the ATC of glucosamine

In Studies II–IV the clinical outcomes of the potential interactions were assessed by examining the patients' routinely determined laboratory values in the laboratory database of Turku University Hospital. The analyzed laboratory parameters are listed in Table 4.4. The laboratory data of the already identified study patients was reviewed by using automatic data processing (ADP) codes of National nomenclature of laboratory tests maintained by the Association of Finnish Local and Regional Authorities (www.kunnat.net/k_etusivu.asp?path=1;161;279). The changes made to the ADP codes during the study years were taken into account.

Table 4.4 Laboratory parameters measuring clinical outcomes of the potential interactions

Laboratory parameter	Abbreviation *	Study number
total cholesterol	fP-Chol	II, IV
high-density lipoprotein cholesterol	fP-HDL-Chol	II, IV
high-density lipoprotein cholesterol / total cholesterol ratio	HDL-Chol/Chol-ratio	II, IV
low-density lipoprotein cholesterol	fP-LDL-Chol	II, IV
triglycerides	fP-Trigly	II, IV
creatine kinase	P-CK	II, IV
alanine amino transferase	P-ALAT	II, III
gamma-glutamyl transferase	P-γGT	II, III
fasting plasma glucose	fP-Gluc	III
glycosylated haemoglobin	B-GHb-A1C	III
plasma potassium	P-K	III
plasma sodium	P-Na	III
urinary glucose bodies	U-Gluc	III
urinary ketone bodies	U-Keto	III
haematocrit	B-HCT	IV
haemoglobin	B-Hb	IV
leucocyte count	fB-Leuc	IV
erythrocyte count	B-Eryt	IV
mean corpuscular haemoglobin	E-MCH	IV
mean corpuscular volume	E-MCV	IV
thrombocyte count	B-Trom	IV

* f, fasting; P, plasma; B, blood; U, urine; E, erythrocyte

In Studies III and IV the follow-up period of the laboratory value analyses was the same as the drug exposure period (interaction or control) with an exception of the CYP3A4 inducer group in Study IV. For this particular group the values measured within one day after the beginning and one week after the end of the interaction were taken into account. If the exact stopping date of CYP2C9 inhibitor exposure could not be ascertained in Study III (the patient was discharged with ongoing interacting medication, for example) the duration of the CYP2C9 inhibitor use was approximated according to standard clinical practice: trimethoprim 5 days, metronidazole 14 days, fluconazole 5 weeks, and miconazole 5 weeks. Treatment with other CYP2C9 inhibitors was assumed to be continuous and all the recorded laboratory values were included in the analyses. If both the starting and stopping dates of the sulphonylurea treatment were unclear the patient was excluded from the laboratory analyses. In Study

II the laboratory values that were measured within seven days after the beginning and seven days after the end of the exposure were included. In addition, only patients with a minimum of seven days' exposure were included. The maximum follow-up for laboratory test results was one year in Studies II–IV.

In case of several measurement values during follow-up the average of the values was used and the results are reported as mean values. In Study IV the blood haemoglobin data was collected also as the minimum values. In Study III the urinary glucose and ketone bodies were handled as positive or negative findings. If all the findings were negative the case was classified as negative, otherwise positive. For other parameters in Study III (see Table 4.4) in addition to average the minimum and maximum values were collected.

In Study II the risk of being outside the reference target range was calculated based on the target values for laboratory determinations valid in Finland in 1999 (i.e. the mid-point of the study period): fP-Chol < 5.0 mmol/l; fP-Trigly < 2.0 mmol/l; fP-HDL-Chol > 1.0 mmol/l for men, > 1.2 mmol/l for women; HDL-Chol/Chol-ratio > 0.25; fP-LDL-Chol < 3.5 mmol/l; P-CK < 285 U/l for men, < 165 U/l for women; P-γGT < 90 U/l for men, < 75 U/l for women; and P-ALAT < 60 U/l for men, < 45 U/l for women. (See definitions for abbreviations in Table 4.4.) The target range for fasting plasma glucose is 4–6 mmol/l. This was used when estimating the minimum fP-Gluc values in Study III.

4.2.2 Outpatients

Exposure to lovastatin, simvastatin, and clopidogrel with or without concomitant interacting treatment was searched retrospectively in the Prescription register of Kela, the Social Insurance Institution of Finland (see chapter 4.1.2).

In Study II all the prescriptions dispensed in the pharmacies that were reimbursed during a three-month study period (from April 1 to June 30, 2001) were included in the searches. The patients that purchased an interacting drug (see Table 4.2) within the same three-month period were considered to be exposed to concomitant use. The three-month period was chosen because the maximum reimbursable amount per purchase is the supply for three months of treatment. The second quarter year was chosen because at the end of the year patients may hoard drugs to exploit the reached annual maximum of out-of-pocket cost after which the necessary medicines are free. Due to this phenomenon the use of the first quarter year usually underestimates and the last overestimates drug consumption.

Clopidogrel became reimbursable in 2002. The outpatients of Study IV were identified by searching the prescriptions for clopidogrel and interacting drugs in years 2002–2004. The duration of clopidogrel treatment was calculated assuming a uniform 75 mg per day dosing, thus the number of the purchased tablets equaled the number of treatment days. A grace period of less than 14 days between two calculated clopidogrel treatment periods was allowed and the medication was considered to be continuous; otherwise a new

treatment period was considered to start after the gap. An interaction was assumed when the prescription of the potential interacting drug was purchased within the clopidogrel period. Patients with certain long-lasting diseases are entitled for a higher refund from prescription medicines. We collected the information on patients' status for special reimbursement for diabetes mellitus, cardiac insufficiency, and hypertension.

The clopidogrel treatment related complications, hospitalizations, and deaths due to thromboembolism and bleedings listed in Tables 4.5 and 4.6, were searched in the Finnish Care Register and Causes of Death register (chapters 4.1.3 and 4.1.4). In case the exact ICD-10 or NCSP is not mentioned all the existing codes of the group are included (e.g. I20.0 refers to codes I20.0, I20.01, I20.02, I20.03, I20.08, and I20.09). Also the information of deaths for any reason was reviewed from the Causes of Death register to measure the overall mortality and to avoid overestimation of the lengths of the follow-up periods.

Table 4.5 The ICD-10 codes used for data searches in the Finnish Care Register and Causes of Death register in Study IV (apps.who.int/ classifications/apps/icd/icd10online/)

Complication	ICD-10
thrombosis	
unstable angina	I20.0
acute myocardial infarction	I21
subsequent myocardial infarction	I22
cerebral infarction	I63
arterial embolism and thrombosis	I74
haemorrhage	
iron deficiency anaemia	D50.0
acute posthaemorrhagic anaemia	D62
haemorrhagic condition, unspecified	D69.9
conjunctival haemorrhage	H11.3
hyphaema	H21.0
choroidal haemorrhage and rupture	H31.3
retinal haemorrhage	H35.6
vitreous haemorrhage	H43.1
otorrhagia	H92.2
haemopericardium as current complication following acute myocardial infarction	I23.0
subarachnoid haemorrhage	I60
intracerebral haemorrhage	I61
other non-traumatic intracranial haemorrhage	I62
oesophageal varices with bleeding	I85.0
haemothorax	J94.2
gastric ulcer	K25
duodenal ulcer	K26
peptic ulcer, site unspecified	K27
gastrojejunal ulcer	K28
haemorrhage of anus and rectum	K62.5
haemoperitoneum	K66.1
melena	K92.1
gastrointestinal haemorrhage, unspecified	K92.2
haemorrhage from respiratory passages	R04
unspecified haematuria	R31
haemorrhage, not elsewhere classified	R58
haemorrhage and haematoma complicating a procedure, not elsewhere classified	T81.0
unintentional cut, puncture, perforation, or haemorrhage during surgical and medical care	Y60

Table 4.6 The NCSP codes used for data searches in the Finnish Care Register in Study IV (http://194.89.160.67/codeserver/distribution-action.do?action=find&type=1 &key=849)

Reoperation	NCSP
coronary arteries	
angiography of heart and/or coronary arteries	FN1AC
endovascular dilatation of coronary arteries (PTCA)	FN1AT
extensive angiography of heart and/or coronary arteries	FN1BC
extensive endovascular dilatation of coronary (PTCA) arteries	FN1BT
very extensive angiography of heart and/or coronary arteries	FN1CC
connection to coronary artery from internal mammary artery	FNA
connection to coronary artery from gastroepiploic artery	FNB
aortocoronary venous bypass	FNC
aortocoronary bypass using prosthetic graft	FND
coronary bypass using free arterial graft	FNE
peripheral arteries	
reoperation for superficial haemorrhage in surgery of heart and major thoracic vessels	FWD00
reoperation for thrombosis or embolus in surgery of heart and major thoracic vessels	FWG00
cerebral artery PTA	PA2AT
cerebral artery extensive PTA	PA2BT
cerebral artery very extensive PTA	PA2CT
carotis artery PTA	PA6AT
carotis artery PTA with stent	PA6BT
carotis artery very extensive PTA	PA6CT
extensive carotis artery PTA with stent	PA7XT
very extensive dilatation of carotid artery with stent	PA7YT
thrombectomy or embolectomy of arteries of aortic arch and branches	PAE
thrombendarterectomy of arteries of aortic arch and branches	PAF
bypass from arteries of aortic arch and branches	PAH
insertion of endovascular graft into arteries of aortic arch and branches	PAQ
upper extremity artery PTA	PB1AT
extensive upper extremity artery PTA	PB1BT
thrombectomy or embolectomy of arteries of upper extremity	PBE
thrombendarterectomy of arteries of upper extremity	PBF
bypass from arteries of upper extremity	PBH
thrombectomy or embolectomy of visceral arteries	PCE
thrombendarterectomy of visceral arteries	PCF
bypass from suprarenal abdominal aorta and visceral arteries	PCH
insertion of endovascular graft into visceral arteries	PCQ
PTA of aorta	PD1AT
extensive PTA of aorta	PD1BT
implantaion of endoprothesis to aorta in conjunction to PTA	PD1YT
pelvic artery PTA	PD3AT
pelvic artery extensive PTA	PD3BT
thrombectomy or embolectomy of infrarenal abdominal aorta and iliac arteries	PDE
thrombendarterectomy of infrarenal abdominal aorta and iliac arteries	PDF
bypass from infrarenal abdominal aorta and iliac arteries	PDH
insertion of endovascular graft into infrarenal abdominal aorta and iliac arteries	PDQ
femoral artery PTA	PE1AT
femoral artery extensive PTA	PE1BT
thrombectomy or embolectomy of femoral artery and branches	PEE
thrombendarterectomy of femoral artery and branches	PEF
bypass from femoral artery and branches	PEH
insertion of endovascular graft into femoral artery and branches	PEQ
intravascular dilatation of arteries of knee, lower leg and ankle (PTA)	PF1AT
extensive PTA of arteries of knee, lower leg and ankle	PF1BT
thrombectomy or embolectomy of popliteal artery and arteries of lower leg and foot	PFE
bypass from femoral artery to infrapopliteal arteries and from popliteal artery to arteries of lower leg and foot	PFH
insertion of endovascular graft into popliteal artery or artery of lower leg	PFQ
percutaneous plastic repair of bypass from femoral or popliteal artery to infrapopliteal arteries	PFU85
PTA of arteries on several areas	PG1BT

abbreviations: PTCA, percutaneous transluminal coronary angioplasty; PTA, percutaneous transluminal angioplasty

Rhadomyolysis cases in Finland during the seven year-time window of hospital-based research were inquired from the Finnish ADR register of Fimea (chapter 4.1.5).

4.3 Statistical analyses

In the hospitalized patients, chi square was used to test between-group differences in sex distribution (Studies I–IV) as well as in ward distribution (Studies I–III). Age differences were tested with one way analysis of variance (ANOVA) or with Mann-Whitney test (Studies II–IV and Study I, respectively). Differences in mean daily doses were tested with unpaired t-test (Study I) or with one way ANOVA (Studies II–III). In Study IV Kruskal-Wallis Test was used for testing the between-group differences in number of concomitant drugs other than affecting to CYP3A4 metabolism. In Studies II–III the results are given both for separate substrates (lovastatin and simvastatin or glibenclamide, glimepiride, and glipizide) and for pooled statin and sulphonylurea groups.

In addition to univariate analyses, laboratory values were also compared between the groups with analysis of covariance (ANCOVA) after adjustment for age, sex, and mean dose (Study II); age, sex, mean dose, and ward (Study III); age, sex, and number of drugs increasing and inhibiting bleeding risk (Study IV). In Study III the above mentioned analyses were repeated with the data for CYP2C9 inhibitors considered clinically most relevant based on the literature (amiodarone, fluconazole, fluvoxamine, metronidazole, miconazole, sulphamethoxazole). Because of positively skewed distribution, triglyceride values were log-transformed before analysis in Study II. Logistic regression analysis was used for between-group comparison of the risk being outside the target range of the laboratory values (Study II).

In the nationwide part of Study IV the patients were included in the control group until the purchase of the interaction medication to avoid immortal time bias. When once considered as an interaction case, the patient could not move back to the control group even the exposure for the interacting medication had ended. Between-group differences were tested with one way ANOVA for age and Cochran-Mantel-Haenszel for sex, diabetes mellitus, cardiac insufficiency, and hypertension. The maximum follow-up period was one year from the start of the interaction period. For control patients the follow-up was prolonged with the median of the lead time to the concomitant medication, which made the maximum 412 days. The first clopidogrel treatment periods were included in the survival analyses. The tested endpoints were overall mortality, thrombosis mortality, haemorrhage mortality, thrombosis complications, haemorrhage complications, combined thrombosis endpoints, and combined haemorrhage endpoints. They were analyzed separately by using Cox proportional

hazard with age, sex, diabetes mellitus, cardiac insufficiency, and hypertension as covariates in the model.

Statistical analyses were performed with GraphPad Prism version 3.03 and SPSS version 13 in Studies I–II, and with SAS System for Windows version 9.1 in Studies II–IV. P-values less than 0.05 were considered statistically significant.

4.4. Ethics and approvals

In general, all register data include information that is considered confidential according to the Finnish Constitution (the right to privacy). However, the Finnish legislation on data protection allows the use of administrative data for appropriate scientific, historical, and statistical research purposes. This legislation appoints enforcing authorities to make sure that individual rights are not violated when administrative data sources are exploited for research. Thus in order to obtain register data for research purposes an authorization from the register controller is needed. There is also a compulsory notification of the new created registers made by automatic data processing. Non-invasive studies do not need opinion from the ethics committee.

The study protocols of Studies I–IV were approved by the top management of Turku University Hospital responsible for all hospital registers and by the Office of the Data Protection Ombudsman for which also the register notifications were made. The nationwide data was collected with the help and permission of Kela, National Research and Development Centre for Welfare and Health (current name National Institute for Health and Welfare), Statistics Finland, and National Agency for Medicines (current name Finnish Medicines Agency).

No conflicts of interests have been expressed by the authors of the Studies I–IV.

5 RESULTS

5.1 Incidences of potential DDIs and demographics of the study subjects

In the hospital-based study population (described in Figure 4.1) CYP-mediated DDIs were seen in up to 23.5% (relating to glipizide) of the treatment periods (Table 5.1). In the patient level the incidence was highest in codeine users: 19.7%. Fibrates were used concomitantly with statins in 1.3% of the cases. (Table 5.1)

The calculations of nationwide DDI frequencies were based on The Prescription Register (chapter 4.1.2) covering 97% of the reimbursed prescriptions among the 5.2 million inhabitants (in 2004, www.stat.fi/index_en.html) in Finland. Among 19,655 patients, there were 26,302 reimbursed clopidogrel treatment periods in open care during the years 2002 to 2004 in Finland. After exclusions (see the criteria in chapter 4.2) 21,802 treatment periods remained, which were related to 19,654 patients (one patient died on the cohort entry day). The majority of these clopidogrel-treated patients had only one (76.0%) or two (17.3%) treatment periods; only 0.7% had five or more periods. Clopidogrel treatment was concomitant with CYP3A4 inhibitor use in 5.4% of the treatment periods, 0.9% with CYP3A4 inducer use, and 19.0% with atorvastatin (Table 5.1). During the three-month study period in Study II 72,024 and 19,632 patients received reimbursed simvastatin and lovastatin, respectively. In 5.2% of all statin-treated patients simvastatin or lovastatin was used concomitantly with a CYP3A4 inhibitor, in 1.0% with a CYP3A4 inducer, and in 0.6% with a fibrate.

During the study years the average treatment period was 5.6 days in the chosen wards at Turku University Hospital and 5.8 days according to the nationwide HILMO register. The most common interacting drugs in concomitant use with the study substrates were CYP2C9 inhibitors metronidazole and trimethoprim (the latter concerning sulphonylurea users in Study III only), CYP2D6 inhibitor hydroxychloroquine, CYP3A4 inhibitor diltiazem, and CYP3A4 inducer carbamazepine (Figures 5.1 a–d). Of the fibrates in Study II, bezafibrate was most commonly used with statins.

All statistical significances in sex distributions showed that DDIs occurred more often in women than in men with an exception in atorvastatin groups in the clopidogrel study (Study IV). Patients in the interaction groups were mainly older than control patients, but atorvastatin and CYP3A4 inhibitor (outpatients) treated clopidogrel patients, fibrate treated statin patients, and CYP2D6 inhibitor treated tramadol patients were younger than the respective controls. There were no clinically relevant differences in doses between the interaction and control groups, although some statistically significant differences were seen. (Table 5.1) Clopidogrel treatment follows uniform dosing; after a loading dose all patients receive 75 mg once a day.

Table 5.1 Characteristics of the study subjects

Substrate and group	Treatment periods, n (%)	Patients, n (%)	Male sex, n (%)	Age in years, mean ± SD (range)	Dose in mg, mean ± SD (range)
losartan					
CYP2C9 inhibitors	196 (19.4)	152 (15.7)	80 (40.8) **	65.6 ± 12.3 (30 - 88)	45 ± 14 (6.3 - 100) *
controls	815 (80.4)	815 (84.3)	428 (52.5)	64.4 ± 12.2 (17 - 94)	47 ± 15 (8.3 - 200)
codeine					
CYP2D6 inhibitors	953 (21.3)	792 (19.7)	313 (32.8) **	60.9 ± 14.9 (19 - 94) **	107 ± 45 (15 - 240)
controls	3519 (78.7)	3220 (80.3)	1582 (45.0)	62.5 ± 16.7 (16 - 97)	105 ± 44 (15 - 248)
tramadol					
CYP2D6 inhibitors	1273 (20.3)	1055 (19.0)	505 (39.7) **	61.6 ± 14.7 (19 - 94) **	167 ± 79 (20 - 600) *
controls	5008 (79.7)	4485 (81.0)	2422 (48.4)	63.5 ± 15.6 (16 - 99)	161 ± 77 (25 - 550)
simvastatin					
CYP3A4 inhibitors	348 (7.8)	331 (7.5)	199 (57.2) *	63.7 ± 11.8 (23 - 87)	14.0 ± 6.3 (5 - 40) *
CYP3A4 inducers	165 (3.7)	154 (3.5)	101 (61.2)	66.9 ± 10.4 (42 - 88) *	16.5 ± 10.8 (5 - 80)
fibrates	56 (1.3)	56 (1.3)	36 (64.3)	61.5 ± 8.8 (44 - 79)	22.4 ± 15.7 (8 - 80) **
controls	3878 (87.2)	3874 (87.7)	2523 (65.1)	64.3 ± 10.8 (16 - 96)	15.4 ± 7.9 (5 - 80)
lovastatin					
CYP3A4 inhibitors	120 (13.7)	111 (12.9)	67 (55.8)	64.2 ± 11.7 (29 - 89)	24.0 ± 12.9 (5 - 80)
CYP3A4 inducers	42 (4.8)	39 (4.5)	32 (76.2)	65.8 ± 8.3 (45 - 83)	22.1 ± 6.5 (10 - 40)
fibrates	13 (1.5)	13 (1.5)	11 (84.6)	59.5 ± 9.7 (43 - 80)	30.0 ± 19.1 (20 - 80)
controls	698 (80.0)	698 (81.1)	455 (65.2)	64.7 ± 9.6 (26 - 87)	23.6 ± 10.9 (5 - 80)
both statins					
CYP3A4 inhibitors	468 (8.8)	442 (8.4)	266 (56.8) **	63.9 ± 11.7 (23 - 89)	
CYP3A4 inducers	207 (3.9)	193 (3.7)	133 (64.3)	66.7 ± 10.0 (42 - 88) *	
fibrates	69 (1.3)	69 (1.3)	47 (68.1)	61.1 ± 8.9 (43 - 80) *	
controls	4576 (86.0)	4572 (86.6)	2978 (65.1)	64.4 ± 10.7 (16 - 96)	
glibenclamide					
CYP2C9 inhibitors	266 (16.9)	220 (14.4)	160 (60.2)	71 ± 10 (39 - 93) **	9.4 ± 4.4 (1.8 - 24.0) *
controls	1304 (83.1)	1304 (85.6)	807 (61.9)	68 ± 11 (21 - 98)	8.8 ± 4.3 (0.9 - 17.5)
glimepiride					
CYP2C9 inhibitors	302 (19.5)	232 (15.7)	157 (52.0) *	68 ± 12 (23 - 91) **	2.5 ± 1.6 (0.5 - 10.0) **
controls	1243 (80.5)	1243 (84.3)	758 (61.0)	64 ± 13 (23 - 98)	2.9 ± 1.7 (0.5 - 8.0)
glipizide					
CYP2C9 inhibitors	289 (23.5)	222 (19.1)	142 (49.1) *	72 ± 10 (45 - 93) *	11.3 ± 5.8 (2.5 - 25.0) *
controls	943 (76.5)	943 (80.9)	531 (56.3)	70 ± 12 (13 - 96)	12.2 ± 6.0 (1.7 - 20.0)

Substrate and group	Treatment periods, n (%)	Patients, n (%)	Male sex, n (%)	Age in years, mean ± SD (range)	Dose in mg, mean ± SD (range)
all sulphonylureas					
CYP2C9 inhibitors	857 (19.7)	627 (16.1)	459 (53.6) **	71 ± 11 (23 - 93) **	
controls	3490 (80.3)	3257 (83.9)	2096 (60.1)	67 ± 12 (13 - 98)	
clopidogrel inpatients					†
CYP3A4 inhibitor	33 (4.5)	33 (4.5)	18 (54.5)	66.1 ± 11.5 (32 - 90)	
CYP3A4 inducer	12 (1.7)	12 (1.7)	8 (66.7)	64.8 ± 16.8 (30 - 88)	
atorvastatin	127 (17.5)	127 (17.5)	100 (78.7) *	61.9 ± 9.9 (37 - 89) *	
controls	554 (76.3)	554 (76.3)	387 (69.9)	64 ± 11.1 (26 - 91)	
clopidogrel outpatients					†
CYP3A4 inhibitor	1432 (5.4)	1192 (6.1)	673 (47.0) **	66.5 ±11.4 (19 - 93) *	
CYP3A4 inducer	245 (0.9)	188 (0.9)	158 (64.5)	69.0 ± 11.9 (24 - 95)	
atorvastatin	4992 (19.0)	4087 (20.8)	3389 (67.9) **	64.9 ± 11.1 (14 - 94) **	
controls	19633 (74.7)	14187 (72.2)	12576 (64.1)	68.0 ± 11.6 (14 - 103)	

abbreviations: n, number; SD, standard deviation
* P < 0.05 compared with controls
** P < 0.001 compared with controls
† uniform dosing: 75 mg per day for all patients

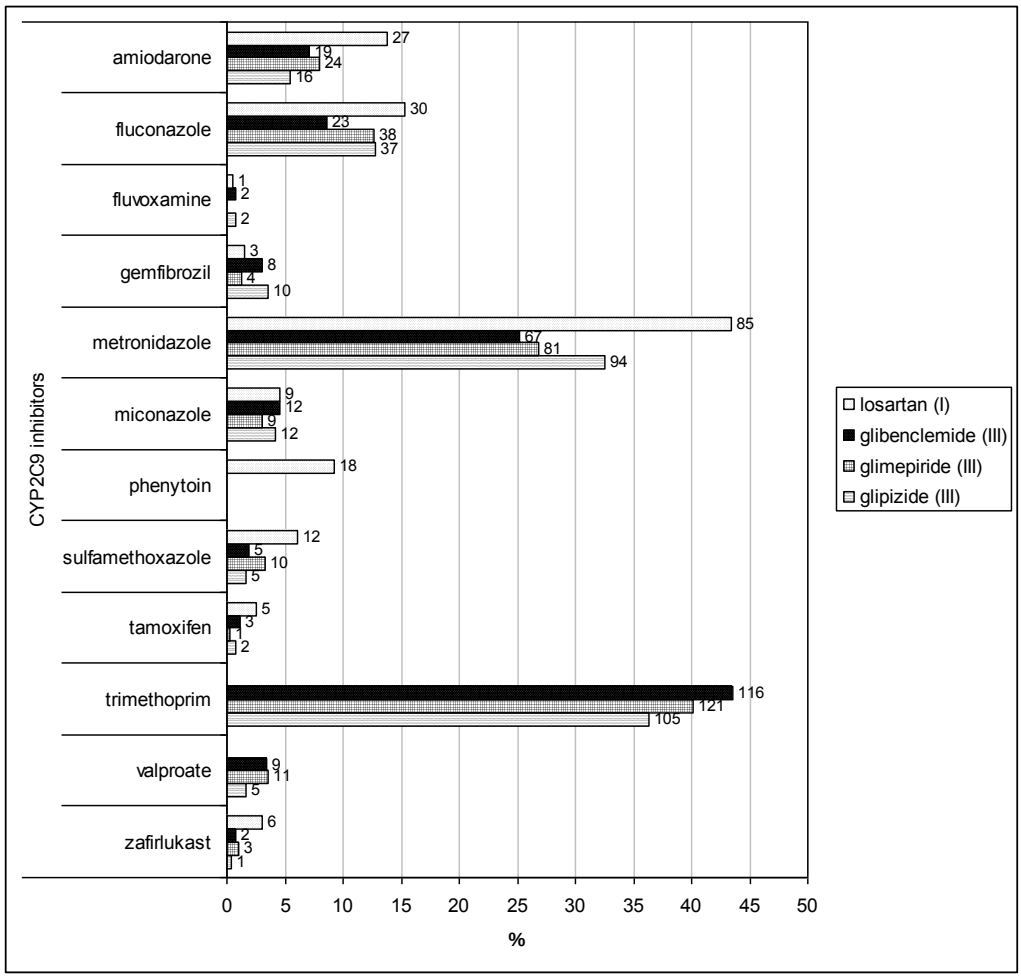


Figure 5.1 a The numbers and proportions of different CYP2C9 inhibitors in concomitant use with substrates in Studies I and III

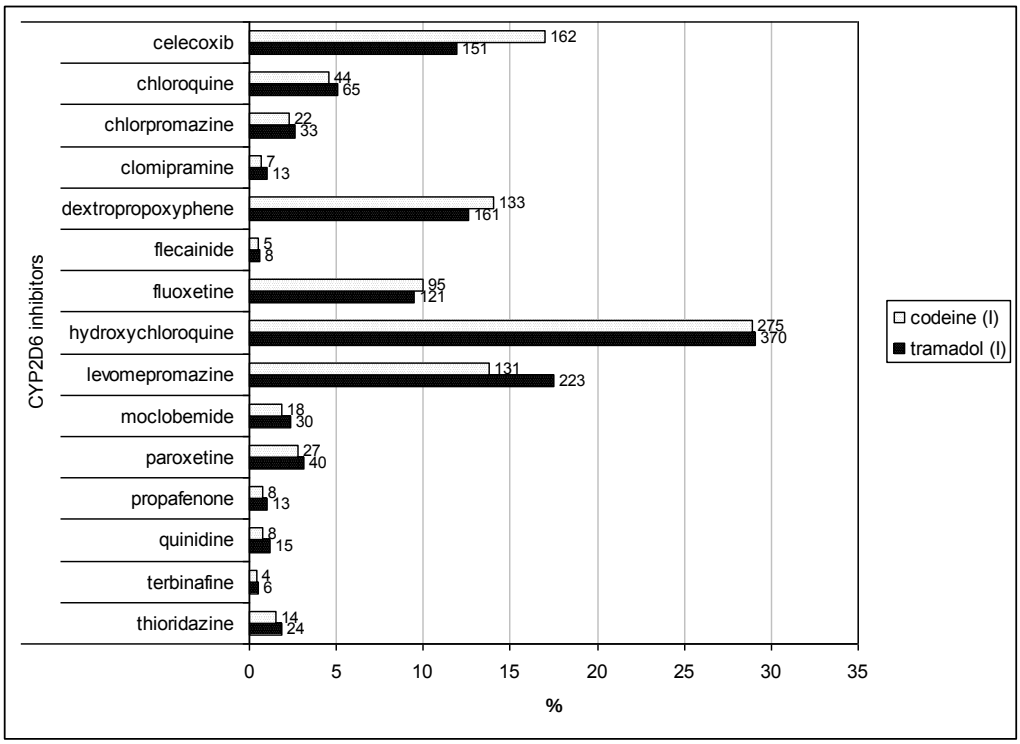


Figure 5.1 b The numbers and proportions of different CYP2D6 inhibitors in concomitant use with substrates in Study I

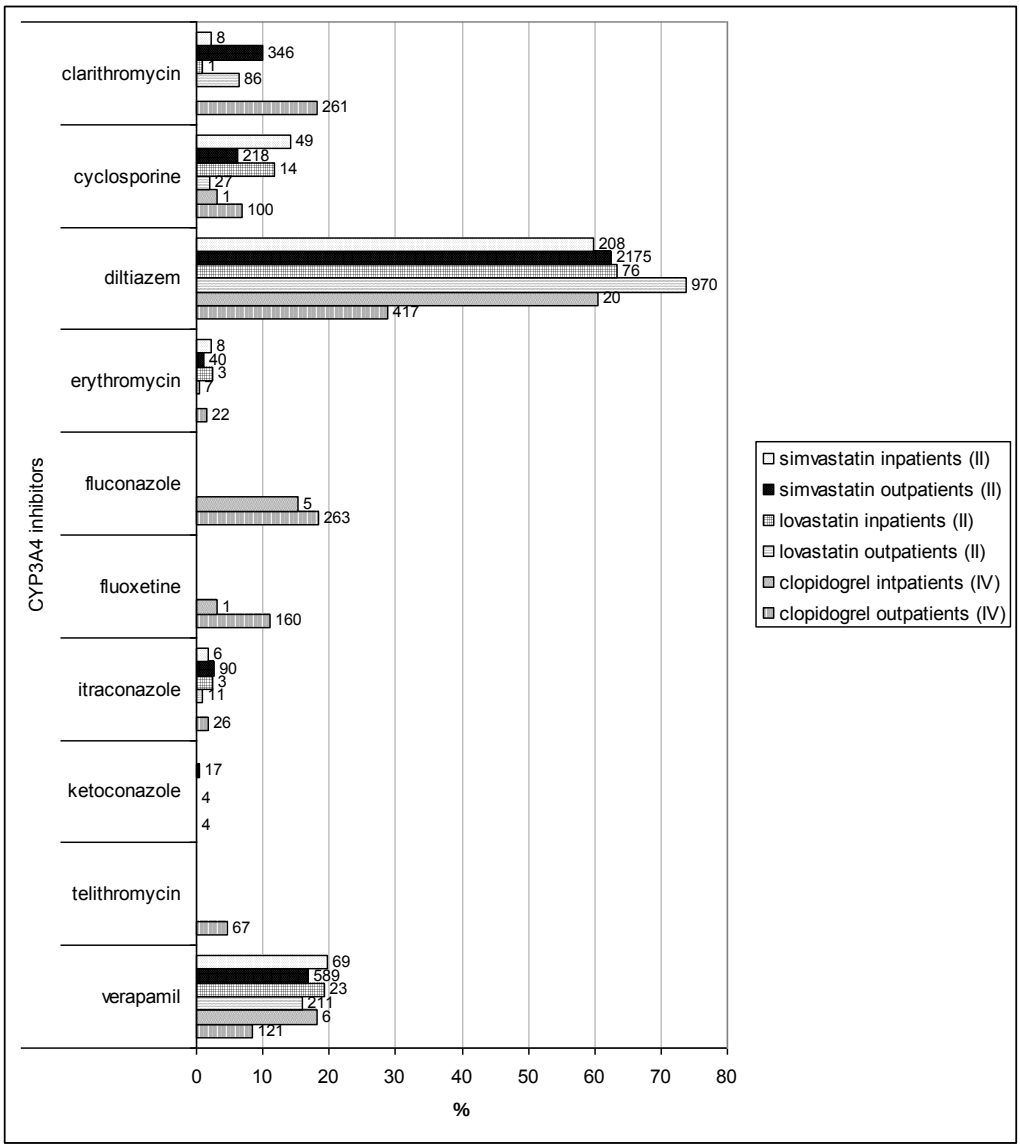


Figure 5.1 c The numbers and proportions of different CYP3A4 inhibitors in concomitant use with substrates in Studies II and IV

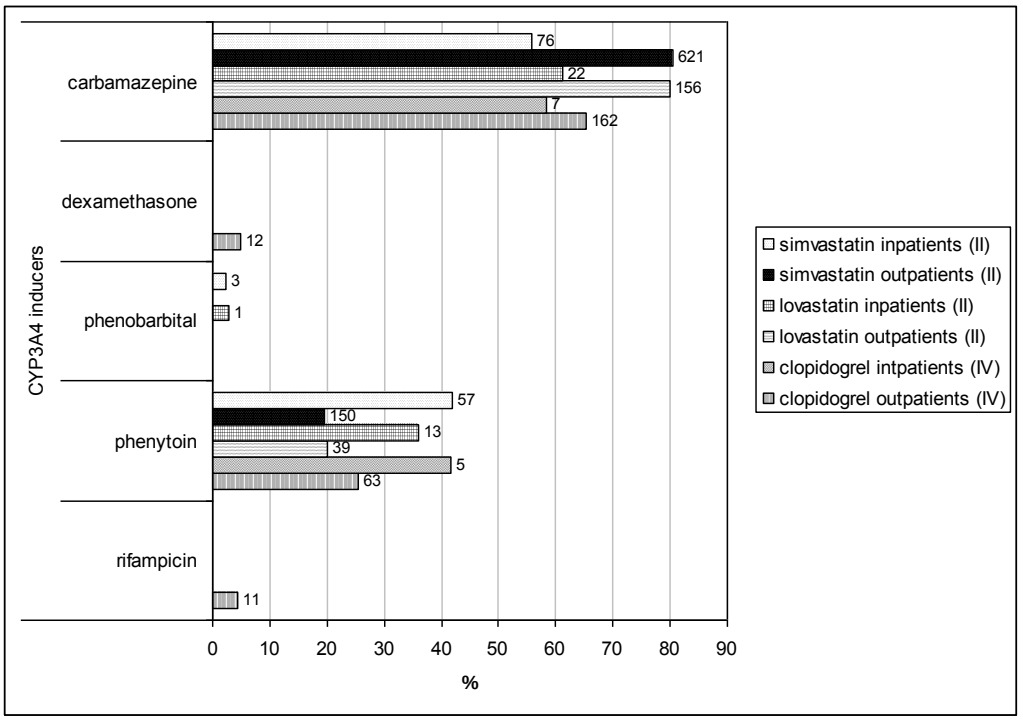


Figure 5.1 d The numbers and proportions of different CYP3A4 inducers in concomitant use with substrates in Studies II and IV

Based on the special reimbursement data, clopidogrel-treated patients in CYP3A4 inhibitor group had more often diabetes mellitus and hypertension and patients in CYP3A4 inducer group cardiac insufficiency compared with controls. In the atorvastatin group clopidogrel-treated patients had more hypertension and less cardiac insufficiency diagnoses than the controls. In the inhospital patients the only significant between-group difference was that hypertension diagnoses were more common in CYP3A4 inhibitor group than in the control group according to archived patient files. (Table 5.2) At Turku University Hospital the main indication for clopidogrel treatment was percutaneous transluminal coronary angioplasty (PTCA); 77.4% of the cases were PTCA patients.

Table 5.2 The incidence of predisposing diseases in clopidogrel-treated patients in Study IV

	CYP3A4 inhibitor	CYP3A4 inducer	atorvastatin	control
DM				
inpatients:				
n	6	1	20	90
%	18.2	8.3	15.7	16.2
outpatients:				
n	255	37	740	2578
%	21.4 *	19.7	18.1	18.2
CI				
inpatients:				
n	2	0	6	18
%	6.1	0	4.7	3.2
outpatients:				
n	118	25	258	1261
%	9.9	13.3 *	6.3 **	8.9
HA				
inpatients:				
n	21	3	58	209
%	63.6 *	25.0	45.7	37.7
outpatients:				
n	537	73	1502	4932
%	45.1 **	38.8	36.8 *	34.8

abbreviations: DM, diabetes mellitus; CI, cardiac insufficiency; HA, hypertension
* $P < 0.05$ compared with control
** $P < 0.001$ compared with control

There were no between-group differences in the numbers of concomitant drugs increasing or decreasing bleeding risk (see Table 4.3) in clopidogrel-treated inpatients (Table 5.3). Acetylsalicylic acid was used less often in CYP3A4 inhibitor group than in the control group (54.5 vs. 84.3%, $P < 0.001$). The distributions of concomitant medications affecting in blood and blood forming organs, cardiovascular system, infections (systemic antiinfectives), musculo-skeletal system, nervous system, and respiratory system are presented in Table 5.3.

Table 5.3 Concomitant medication other than CYP3A4 inhibitors, CYP3A4 inducers, and atorvastatin in clopidogrel-treated patients in Study IV

	CYP3A4 inhibitor	**CYP3A4 inducer**	**atorvastatin**	**control**
n of drugs increasing bleeding risk *				
mean ± SD	1.39 ± 0.86	1.17 ± 1.03	1.19 ± 0.64	1.16 ± 0.60
range	0 - 4	0 - 3	0 - 4	0 - 4
n of drugs decreasing bleeding risk *				
mean ± SD	0.12 ± 0.33	0.25 ± 0.45	0.14 ± 0.35	0.14 ± 0.34
range	0 - 1	0 - 1	0 - 1	0 - 1
acetylsalicylic acid				
mean ± SD	0.55 ± 0.51 †	0.75 ± 0.45	0.85 ± 0.36	0.84 ± 0.81
range	0 - 1	0 - 1	0 - 1	0 - 1
n of B-drugs				
mean ± SD	0.85 ± 0.62	1.00 ± 0.85	1.09 ± 0.51	1.03 ± 0.53
range	0 - 2	0 - 3	0 - 3	0 - 4
n of C-drugs				
mean ± SD	2.12 ± 1.60 †	2.17 ± 1.70 **	2.35 ± 1.25 †	3.24 ± 1.36
range	0 - 6	0 - 5	0 - 7	0 - 8
n of J-drugs				
mean ± SD	0.27 ± 0.57 **	0.08 ± 0.29	0.05 ± 0.28 **	0.11 ± 0.34
range	0 - 2	0 - 1	0 - 2	0 - 2
n of M-drugs				
mean ± SD	0.33 ± 0.60 †	0.08 ± 0.29	0.06 ± 0.24	0.09 ± 0.30
range	0 - 2	0 - 1	0 - 1	0 - 2
n of N-drugs				
mean ± SD	0.61 ± 0.90 †	1.00 ± 1.21 **	0.27 ± 0.65	0.25 ± 0.64
range	0 - 3	0 - 3	0 - 4	0 - 4
n of R-drugs				
mean ± SD	0.73 ± 1.13 †	0.08 ± 0.29	0.10 ± 0.42	0.15 ± 0.57
range	0 - 4	0 - 1	0 - 3	0 - 5

abbreviations: SD, standard deviation; B, blood and blood forming organs; C, cardiovascular system; J, antiinfectives for systemic use; M, musculo-skeletal system; N, nervous system; R, respiratory system

* See Table 4.3

** $P < 0.05$ compared with control

† $P < 0.001$ compared with control

5.2 Influence of potential DDIs on efficacy and safety laboratory parameters

Simvastatin and lovastatin users in all interaction groups had higher total fasting plasma cholesterol concentrations than the controls, P-value being significant when analyzing simvastatin and pooled statin groups (Table 5.4). In patients receiving CYP3A4 inhibitors or CYP3A4 inducers this difference was explained by significantly higher HDL cholesterol concentrations. No significant differences were then seen between these groups and the controls in the HDL cholesterol / total cholesterol ratio. Patients receiving fibrates had both lower HDL cholesterol concentration and lower HDL cholesterol / total cholesterol ratio than the controls. Mean fasting plasma LDL cholesterol concentrations were essentially similar in all study groups. In simvastatin and pooled statin groups the CYP3A4 inducer treated patients reached the statistical significance with LDL cholesterol values of 3.0 ± 0.9 mmol/l (mean ± SD) versus control values 2.8 ± 0.9 mmol/l (P = 0.010 and 0.009, respectively). Mean triglyceride concentrations were constantly higher in fibrate-treated patients than in controls receiving only simvastatin or lovastatin in Study II. Also CYP3A4 inhibitor receiving simvastatin patients had significantly different triglyceride concentrations from controls (1.8 ± 1.5 vs. 1.6 ± 0.9 mmol/l, respectively, P = 0.032). (Table 5.4)

In Study II the risk (odds ratio, OR) for elevation of total cholesterol concentration above the target value (see chapter 4.2.1) was 2.3 (95% confidence interval [CI] 1.5–3.3, P < 0.001) in patients receiving also CYP3A4 inducers and 2.1 (95% CI 1.2–3.7, P = 0.012) in patients receiving fibrates compared with the pooled control group. The HDL cholesterol concentration was more often within the target in patients in CYP3A4 inducer group than in controls (OR 0.6, 95% CI 0.4–0.9, P = 0.014). The opposite was seen in fibrate users who had 3.8-fold risk (95% CI 2.2–6.6, P < 0.001) of being outside of the target range of HDL cholesterol compared with controls. HDL cholesterol / total cholesterol ratio was also more often outside the target in fibrate users (OR 6.7, 95% CI 3.1–14.5, P < 0.001). These patients had 13.5-fold risk (95% CI 6.7–27.2, P < 0.001) of having triglyceride concentrations above the target value. A weaker (OR 2.0, 95% CI 1.1–3.6, P = 0.021) but statistically significant risk elevation was seen in LDL cholesterol in the fibrate group.

In Study IV the mean fasting plasma concentrations of total cholesterol and LDL cholesterol were lower in atorvastatin group, and HDL cholesterol concentration as well as the HDL cholesterol / total cholesterol ratio were higher in CYP3A4 inhibitor and inducer groups compared with controls receiving clopidogrel only (Table 5.4). The potential interactions with atorvastatin, CYP3A4 inhibitors, or CYP3A4 inducers did not affect haematological laboratory parameters in clopidogrel-treated patients (Table 5.5).

Table 5.4 Effect of potential DDIs on lipid values in simvastatin-, lovastatin-, and clopidogrel-treated patients

Substrate and group	fP-Chol, mmol/l mean ± SD	fP-HDL-Chol, mmol/l mean ± SD	HDL-Chol / Chol-ratio, % mean ± SD	fP-LDL-Chol, mmol/l mean ± SD	fP-Trigly, mmol/l mean ± SD
simvastatin					
CYP3A4 inhibitors	5.0 ± 1.1 *	1.33 ± 0.43 *	27 ± 9	2.9 ± 0.9	1.8 ± 1.5 *
CYP3A4 inducers	5.2 ± 1.2 **	1.45 ± 0.54 **	28 ± 9	3.0 ± 0.9 *	1.6 ± 0.8
fibrates	5.5 ± 1.1 **	1.05 ± 0.34 **	20 ± 5 **	3.0 ± 0.9	3.3 ± 2.6 **
controls	4.8 ± 1.0	1.25 ± 0.35	27 ± 8	2.8 ± 0.9	1.6 ± 0.9
lovastatin					
CYP3A4 inhibitors	5.1 ± 0.9	1.30 ± 0.39	26 ± 8	3.1 ± 0.8	1.7 ± 0.8
CYP3A4 inducers	5.3 ± 0.9	1.35 ± 0.46	26 ± 9	3.1 ± 0.7	1.7 ± 0.6
fibrates	5.2 ± 0.8	1.01 ± 0.26	20 ± 5	3.0 ± 0.9	2.8 ± 1.3 *
controls	5.0 ± 1.0	1.23 ± 0.33	25 ± 7	3.0 ± 0.8	1.8 ± 1.0
both statins					
CYP3A4 inhibitors	5.0 ± 1.1 *	1.32 ± 0.42 *	27 ± 9	2.9 ± 0.8	1.8 ± 1.3
CYP3A4 inducers	5.2 ± 1.1 **	1.43 ± 0.52 **	28 ± 9	3.0 ± 0.9 *	1.6 ± 0.8
fibrates	5.4 ± 1.1 *	1.04 ± 0.32 **	20 ± 5 **	3.0 ± 0.9	3.2 ± 2.4 **
controls	4.8 ± 1.0	1.25 ± 0.35	27 ± 7	2.8 ± 0.9	1.6 ± 0.9
clopidogrel					
CYP3A4 inhibitor	4.8 ± 0.8	1.58 ± 0.62 **	32 ± 11 *	2.7 ± 0.7	1.2 ± 0.4
CYP3A4 inducer	5.0 ± 0.5	1.84 ± 0.59 *	36 ± 6	3.0 ± 0.3	0.8 ± 0.2
atorvastatin	4.1 ± 1.2 *	1.09 ± 0.34	28 ± 8	2.3 ± 0.9 *	1.6 ± 1.2
controls	4.6 ± 0.9	1.13 ± 0.31	26 ± 8	2.7 ± 0.8	1.5 ± 0.9

abbreviations: f, fasting; P, plasma; Chol, total cholesterol; HDL-Chol, high-density lipoprotein cholesterol; LDL-Chol, low-density lipoprotein cholesterol; Trigly, triglycerides; SD, standard deviation

* $P < 0.05$ compared with controls in ANCOVA multivariate analysis (see chapter 4.3 for more details)

** $P < 0.001$ compared with controls in ANCOVA multivariate analysis (see chapter 4.3 for more details)

Table 5.5 Effect of potential DDIs on haematological laboratory parameters in clopidogrel-treated patients

	CYP3A4 inhibitor	CYP3A4 inducer	atorvastatin	control
B-HCT, proportion mean ± SD	0.36 ± 0.04	0.36 ± 0.04	0.37 ± 0.04	0.37 ± 0.04
B-Hb, g/l mean ± SD	124 ± 15	123 ± 12	126 ± 15	126 ± 14
B-Hb-min, g/l mean ± SD	117 ± 19	113 ± 21	119 ± 21	120 ± 18
fB-Leuc, E9/l mean ± SD	7.9 ± 2.7	7.8 ± 3.9	7.8 ± 2.5	7.5 ± 2.0
B-Eryt, E12/l mean ± SD	4.05 ± 0.51	3.88 ± 0.37	4.07 ± 0.49	4.06 ± 0.49
E-MCH, pg mean ± SD	31 ± 2	32 ± 2	31 ± 2	31 ± 2
E-MCV, fl mean ± SD	90 ± 6	93 ± 4	90 ± 4	91 ± 6
B-Trom, E9/l mean ± SD	268 ± 110	257 ± 81	227 ± 68	228 ± 78

abbreviations: B, blood; HCT, haematocrit; SD, standard deviation; Hb, haemoglobin; f, fasting; Leuc, leucocytes; Eryt, erythrocytes; E, erythrocyte; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; Trom, thrombocyte

In sulphonylurea-treated patients both mean and maximum fasting plasma glucose concentrations were significantly lower during the interaction periods compared with control periods. The minimum fasting plasma glucose values were statistically significantly more often under 4 mmol/l (lower limit of the target range 4–6 mmol/l) in patients with potential interactions compared with controls. The maximum values of glycosylated haemoglobin were statistically significantly lower and minimum values higher in CYP2C9 inhibitor group compared with controls but no difference was found in means. Only marginal differences were seen in plasma potassium and sodium concentrations. The proportions of periods with glucose or ketone bodies in the urine were significantly lower in CYP2C9 inhibitor users. (Table 5.6)

The results remained similar when only the CYP2C9 inhibitors whose interaction potential was considered best documented (see Table 4.2) were included in the analyses. The difference between the patients in the interaction group and controls became statistically strengthened in minimum fasting plasma glucose values under 4 mmol/l. (Table 5.6) When all CYP2C9 inhibitors were pooled together but the three sulphonylureas analysed separately the results remained essentially similar. However, statistical significance was not always reached due to the reduced number of treatment periods.

Table 5.6 Effects of potential DDIs with CYP2C9 inhibitors and pooled sulphonylureas on laboratory parameters indicating glucose homeostasis

	all CYP2C9 inhibitors	**well established CYP2C9 inhibitors †**	**controls**
fP-Gluc, mmol/l			
mean ± SD	8.5 ± 3.4 *	8.5 ± 3.4 *	9.1 ± 2.5
min ± SD	6.8 ± 3.2	6.5 ± 3.2	6.8 ± 2.6
max ± SD	10.7 ± 4.9 **	11.0 ± 5.1 **	12.2 ± 4.3
min fP-Gluc			
n (%) of values			
> 4 mmol/l (%)	421 (87.2) *	281 (84.6) **	2415 (91.1)
< 4 mmol/l (%)	62 (12.8) *	51 (15.4) **	237 (8.9)
3 – < 4 mmol/l (%) ‡	40 (8.3)	32 (9.7)	167 (6.3)
2 – < 3 mmol/l (%) ‡	20 (4.1)	17 (5.1)	60 (2.2)
< 2 mmol/l (%) ‡	2 (0.4)	2 (0.6)	10 (0.4)
B-GHb-A1C, %			
mean ± SD	7.8 ± 1.8	7.8 ± 2.0	8.0 ± 1.4
min ± SD	7.4 ± 1.7 *	7.5 ± 1.9 *	7.1 ± 1.4
max ± SD	8.4 ± 2.2 **	8.2 ± 2.3 **	9.2 ± 2.0
P-K, mmol/l			
mean ± SD	4.0 ± 0.5 *	4.0 ± 0.4 **	4.1 ± 0.3
min ± SD	3.7 ± 0.6 *	3.6 ± 0.6	3.6 ± 0.4
max ± SD	4.4 ± 0.6 **	4.4 ± 0.6 **	4.6 ± 0.6
P-Na, mmol/l			
mean ± SD	138.9 ± 4.0 **	138.9 ± 4.2 *	139.4 ± 2.8
min ± SD	136.4 ± 4.9 *	136.1 ± 5.2	136.0 ± 4.4
max ± SD	140.9 ± 6.0 **	141.1 ± 6.9 **	142.5 ± 3.7
U-Gluc			
n of measurements with positive finding (%)	105 (34.3) **	58 (34.3) **	1338 (55.6)
n of measurements with negative finding (%)	201 (65.7) **	111 (65.7) **	1068 (44.4)
U-Keto			
n of measurements with positive finding (%)	63 (20.6) *	30 (17.8) *	780 (32.4)
n of measurements with negative finding (%)	243 (79.4) *	139 (82.2) *	1626 (67.6)

abbreviations: f, fasting; P, plasma Gluc, glucose; B, blood; GHb-A1C, glycosylated haemoglobin; K, potassium; Na sodium; U-Gluc, urinary glucose bodies; U-Keto, urinary ketone bodies
* P < 0.05 compared with controls in ANCOVA multivariate analysis (see chapter 4.3 for more details)
** P < 0.001 compared with controls in ANCOVA multivariate analysis (see chapter 4.3 for more details)
† See Table 4.2
‡ Not statistically tested

Mean plasma CK activity was lower in patients receiving simvastatin or lovastatin concomitantly with CYP3A4 inhibitors compared with controls (the difference was statistically significant when analyzing simvastatin and pooled statin groups). γGT

values were significantly higher in all statin groups receiving CYP3A4 inducers compared with respective controls. Similarly, in sulphonylurea-treated patients the mean γGT activities were higher than in controls (P being > 0.05 in glimepiride group) as well as the minimum values, but there were no differences in maximum plasma γGT values. (Table 5.7)

There were no between-group differences in mean ALAT activities in simvastatin- and lovastatin-treated patients but patients in glibenclamide and pooled sulphonylurea groups had higher mean and minimum ALAT values, and glibenclamide-treated patients had also higher maximum ALAT activities compared with the respective controls. (Table 5.7)

Again, when comparing the results of the pooled sulphonylurea group between all CYP2C9 inhibitors and well-established CYP2C9 inhibitors they were essentially similar. Maximum ALAT values reached the statistical significance in the established CYP2C9 inhibitor group only. (Table 5.7)

In pooled statin analyses in Study II the odds ratio (OR) for mean CK above the target value (see chapter 4.2.1) was 2.0 (95% CI 1.0–4.0, P = 0.045) in patients receiving fibrates compared with controls. Mean plasma γGT values were more often above the target in patients in CYP3A4 inhibitor and CYP3A4 inducer groups (OR 1.4, 95% CI 1.0–2.0, P = 0.048; OR 4.6, 95% CI 3.1–6.8, P < 0.001, respectively). ALAT values above the target range were seen more often in patients in CYP3A4 inhibitor group than in control patients (OR 1.6, 95% CI 1.1–2.2, P = 0.010).

Table 5.7 Effects of potential DDIs simvastatin-, lovastatin-, glibenclamide-, glimepiride-, and glipizide-treated patients on safety laboratory parameters

Substrate and group	P-CK, U/l mean ± SD	P-γGT, U/l mean ± SD	P-γGT, U/l min ± SD	P-γGT, U/l max ± SD	P-ALAT, U/l mean ± SD	P-ALAT, U/l min ± SD	P-ALAT, U/l max ± SD
simvastatin							
CYP3A4 inhibitors	126 ± 156 *	119 ± 282 *			38 ± 70		
CYP3A4 inducers	230 ± 476	96 ± 86 **			27 ± 21		
fibrates	457 ± 1563	83 ± 86			31 ± 24		
controls	203 ± 462	65 ± 90			33 ± 56		
lovastatin							
CYP3A4 inhibitors	193 ± 263	64 ± 72			41 ± 90		
CYP3A4 inducers	189 ± 286	156 ± 95 **			27 ± 18		
fibrates	362 ± 292	64 ± 32			53 ± 38		
controls	236 ± 550	72 ± 127			33 ± 75		
both statins							
CYP3A4 inhibitors	146 ± 195 **	103 ± 42			39 ± 76		
CYP3A4 inducers	221 ± 442	110 ± 91 **			27 ± 21		
fibrates	433 ± 1351	80 ± 79			35 ± 29		
controls	209 ± 479	66 ± 97			33 ± 59		
glibenclamide							
CYP2C9 inhibitors		136 ± 161 *	112 ± 135 *	165 ± 214	106 ± 654 **	92 ± 577 **	120 ± 732 **
controls		88 ± 133	62 ± 90	129 ± 248	35 ± 82	25 ± 75	51 ± 115
glimepiride							
CYP2C9 inhibitors		127 ± 240	118 ± 238 *	139 ± 247	37 ± 51	29 ± 45	47 ± 60
controls		104 ± 222	72 ± 148	149 ± 356	37 ± 37	26 ± 27	56 ± 82
glipizide							
CYP2C9 inhibitors		149 ± 209 *	126 ± 198 *	174 ± 242	38 ± 69	31 ± 60	46 ± 80
controls		80 ± 128	53 ± 82	123 ± 305	34 ± 49	23 ± 39	54 ± 92
all sulphonylureas							
all CYP2C9 inhibitors		137 ± 209 *	119 ± 198 **	158 ± 236	56 ± 344 *	47 ± 303 **	67 ± 385
established CYP2C9 inhibitors †		158 ± 240 **	140 ± 232 **	179 ± 263	63 ± 404 **	53 ± 357 **	74 ± 452 *
controls		93 ± 172	64 ± 115	137 ± 307	36 ± 61	25 ± 53	54 ± 99

abbreviations: P, plasma; GT, glutamyl transferase; ALAT, alanine amino transferase; SD, standard deviation

* P < 0.05 compared with controls in ANCOVA multivariate analysis (see chapter 4.3 for more details)

** P < 0.001 compared with controls in ANCOVA multivariate analysis (see chapter 4.3 for more details)

† See Table 4.2

5.3 Endpoints in clopidogrel-, and lovastatin- and simvastatin-treated patients

In the nationwide setting in Study IV the overall mortality in clopidogrel-treated patients was 5.11%. The risk of death was increased by all tested confounding factors: age, male sex, diabetes mellitus, cardiac insufficiency, and hypertension.

Based on the survival analysis the hazard ratio (HR) of overall mortality was statistically significantly higher in CYP3A4 inducer group and smaller in atorvastatin group (HR 2.29, $P < 0.001$ and HR 0.74, $P = 0.003$, respectively) compared with controls. No significant differences were seen in thrombosis or haemorrhage mortalities. When these complications were estimated as hospitalizations, the HR of thrombosis events was significantly under one in CYP3A4 inhibitor group and above one in atorvastatin group, in haemorrhage endpoints the HR was significantly lower in CYP3A4 inhibitor and atorvastatin groups compared with the control group. The figures were similar for combined endpoints of hospitalizations and deaths. (Table 5.8)

During the first clopidogrel treatment periods (n = 19,654) the frequency of coronary artery reoperations (see Table 4.6) ranged from 0 to 14: in CYP3A4 inhibitor group 0–7, CYP3A4 inducer group 0–6, atorvastatin 0–8, and controls group 0–14. There was a significant P in Cochran Mantel-Haenszel statistics between CYP3A4 inhibitor and control groups. The median of reoperation number was 0 in all groups. The peripheral reoperations (see Table 4.6) ranged from 0 to 1 in CYP3A4 inhibitor and CYP3A4 inducer groups, 0–2 in atorvastatin group, and 0–3 in controls in Study IV. The median was 0 for all study groups. The distribution was statistically significant in CYP3A4 inhibitor and atorvastatin groups compared with control distribution ($P < 0.001$ for both).

During all the seven study years included in the hospital setting in Study II there were only 6 reported rhabdomyolysis cases in Finland. One patient used lovastatin and five simvastatin; one of the cases was caused by simvastatin alone, without any interacting medication. Three of the cases were associated with doubling the simvastatin dose from 40 to 80 mg per day. None of the rhabdomyolysis cases was fatal and all patients recovered. (www.laakelaitos.fi/instancedata/prime_product_julkaisu/laakelaitos/embeds/english_Publications_Tabu_tabu52002_eng.pdf and Palva E, personal communication, 2005)

Table 5.8 Results of survival analyses in Study IV

	CYP3A4 inhibitor	CYP3A4 inducer	atorvastatin	control
overall mortality				
events	63	22	116	804
follow-up, years	1157	176	4026	16171
risk / 1000 years	54.5	125	28.8	49.7
HR	1.3	2.29 **	0.74 *	reference
95% CI	1.00 - 1.69	1.50 - 3.50	0.61 - 0.91	
thrombosis mortality				
events	28	4	60	354
follow-up, years	1157	176	4026	16171
risk / 1000 years	24.2	22.7	14.9	21.9
HR	1.41	0.99	0.94	reference
95% CI	0.95 - 2.09	0.37 - 2.65	0.71 - 1.24	
haemorrhage mortality				
events	4	1	8	28
follow-up, years	1157	176	4026	16171
risk / 1000 years	3.5	5.7	2	1.7
HR	2.62	3.34	1.52	reference
95% CI	0.91 - 7.59	0.45 - 24.73	0.68 - 3.38	
thrombosis complication				
events	221	59	1717	4430
follow-up, years	1089	162	3589	14978
risk / 1000 years	202.9	364.2	478.4	295.8
HR	0.62 **	1.13	1.66 **	reference
95% CI	0.53 - 0.72	0.86 - 1.50	1.56 - 1.77	
haemorrhage complication				
events	21	4	75	689
follow-up, years	1089	162	3589	14978
risk / 1000 years	19.3	24.7	20.9	46
HR	0.31 **	0.54	0.50 **	reference
95% CI	0.18 - 0.52	0.20 - 1.44	0.39 - 0.66	
combined thrombosis endpoints †				
events	248	63	1766	4753
follow-up, years	1089	162	3589	14978
risk / 1000 years	227.7	388.9	492.1	317.3
HR	0.67 **	1.14	1.61 **	reference
95% CI	0.58 - 0.77	0.87 - 1.49	1.51 - 1.71	
combined haemorrhage endpoints †				
events	25	5	82	714
follow-up, years	1089	162	3589	14978
risk / 1000 years	23	30.9	22.8	47.7
HR	0.39 **	0.66	0.55 **	reference
95% CI	0.24 - 0.62	0.27 - 1.60	0.43 - 0.70	

abbreviations: HR, hazard ratio; CI, confidence interval

* P < 0.05 compared with controls in ANCOVA multivariate analysis (see chapter 4.3 for more details)

** P < 0.001 compared with controls in ANCOVA multivariate analysis (see chapter 4.3 for more details)

† combined endpoints include both deaths and hospitalizations

6 DISCUSSION

6.1 Methodological considerations

DDIs, especially pharmacokinetic DDIs, are traditionally studied with randomized controlled clinical trials whereas pharmacoepidemiological methods have been applied only in a limited amount. Polypharmacy and adverse drug reactions have been studied often with interviews and questionnaires making the data susceptible for recall bias. The advantage of observational studies is the setting of routine medical practice. However, register studies always include the bias source of human interface in data entry. The valid and comprehensive registers on drug use make Finland a good platform for pharmacoepidemiological studies. Turku University Hospital has been a forerunner in collecting electronic patient databases, especially drug and laboratory data. A problem associated with the hospital patient registers was the lack of exact starting and ending dates of the treatment of patients with long-term medication.

In the present studies all patients on whom information was available were included. Thus, the subjects included in the study and control groups were heterogeneous and adjustments with respect to confounders were performed only afterwards. An alternative approach would have been to select cohorts using algorithms making them more comparable with each other and applying prior sample size estimation. Studies II and III could have been conducted following the case-crossover design where the laboratory parameters of individual patients during exposure and non-exposure phases would have presented the cases and controls. The validity of the results could have been further strengthened by performing sensitivity analyses.

Due to the selection procedure, the study subjects could have been at different risk for clinical endpoints. The indication of medication remained unclear in many cases. This is, however, a typical drawback in most register-based studies. Other than CYP-related medication was taken into account only in hospitalized patients in Study II. Thus, other drugs may have contributed to the clinical endpoints. However, the study populations were not general patient populations but ones with well-defined conditions such as dyslipidemia, diabetes, or thromboembolic disease, which is supposed to reduce the variability between study and control groups.

The information on the drug concentrations in plasma was not available but only established CYP inhibitors and inducers were included in the studies (see Table 2.7). Furthermore, in many cases the pharmacokinetic consequences of the studied interactions have been reported in literature. Therefore, it is reasonable to assume that drug exposure to study substrates was affected by the interacting medications in the patients. Some changes in the definitions of interacting drugs took place during the

studies. Phenytoin was included as a CYP2C9 inhibitor in Study I but in Study III phenytoin users were excluded from the study due to its uncertain CYP2C9 inhibitory profile together with strong inducing effect on CYP3A4. To strengthen the results the data was reanalyzed with the most potent CYP2C9 inhibitors in Study III and atorvastatin was analyzed separately from CYP3A4 inhibitors in Study IV.

6.2 Studied drug-drug interactions

6.2.1 Prodrugs losartan, codeine, and tramadol

The harm resulting from DDIs inhibiting prodrug activation is not caused by increased toxicity but rather, by lack of efficacy. In hospitalized patients potential interaction occurred in more than 20% of the treatment periods. The findings suggest that inhibition of prodrug activation is an unrecognised source of irrational drug therapy even if lack of efficacy in the pharmacological treatment of hypertension or pain is a well acknowledged clinical problem (Flor *et al.* 1992, Mancia and Grassi 1999).

The efficacy of antihypertensive or analgesic effects was not measured in this study, but earlier evidence shows that low CYP2C9 activity reduces the antihypertensive effects of losartan (Munafo *et al.* 1992, Gradman *et al.* 1999, Sekino *et al.* 2003) and that reduced CYP2D6 activity diminishes the efficacy of codeine and tramadol in the treatment of pain (Sindrup *et al.* 1990, Sindrup *et al.* 1996, Poulsen *et al.* 1996a, Laugesen *et al.* 2005). Also the abuse of codeine is reported to be reduced during CYP2D6 inhibitor use (Fernandes *et al.* 2002).

Of 1273 tramadol interaction periods clomipramine, fluoxetine, or paroxetine was the CYP2D6 inhibitor in 174 cases. These drugs inhibit serotonin re-uptake and their concomitant use with tramadol may cause serotonin syndrome (Egberts *et al.* 1997, Lange-Asschenfeldt *et al.* 2002). The use of selective MAO-A inhibitor moclobemide together with tramadol is contraindicated for the same reason but nevertheless, there were 30 treatment periods where moclobemide and tramadol were combined during the six-year observation period.

The role of celecoxib as a CYP2D6 inhibitor is not the best-known of the included interacting drugs and there are no publications on its effects on codeine or tramadol pharmacokinetics. Celecoxib is often combined in analgesic treatment with codeine and tramadol, and therefore, further research on celecoxib interactions is warranted. In a Norwegian survey 25% of CYP2D6 substrate treatment periods were concomitant with celecoxib use, codeine being the most common substrate (Molden and Braathen 2005).

Concern about the costs of medical care in general and pharmaceuticals especially is common in all developed countries (Schulman *et al.* 2005). One hospital admission due to an adverse drug event is associated with costs of $16,000 in the US (Jha *et al.* 2001). The cost estimate of the ineffective therapy with prodrugs in Study I was made by calculating the waste of futile drugs in US dollars. Even without assessing the costs of potential consequent supplemental hospitalizations and other treatment the forfeited amount of money was strikingly large. (The subject is not reported elsewhere in this thesis summation but only in Study I publication.) Further studies with a comprehensive approach are needed to uncover all economic consequences of the interactions.

6.2.2 Simvastatin and lovastatin

The strongest CYP3A4 inhibitors increase the concentrations of lovastatin, simvastatin, and their active metabolites 10 to 20-fold (Neuvonen and Jalava 1996, Kivisto *et al.* 1998, Neuvonen *et al.* 1998). Strong CYP3A4 inducers have been shown to decrease simvastatin exposure by more than 75% and the same would be expected for lovastatin (Kyrklund *et al.* 2000, Ucar *et al.* 2004). Combined statin-fibrate use increases the risk of rhabdomyolysis compared with statin monotheropy; the worst scenario has been seen with cerivastatin combined with fibrate (Graham *et al.* 2004). Due to numerous rhabdomyolysis events cerivastatin was withdrawn from the market in 2002 (Charatan 2001, SoRelle 2001).

In the hospital setting in Study II 8.8% of all statin treatment periods with simvastatin or lovastatin were concomitant with CYP3A4 inhibitor medication, 3.9% with CYP3A4 inducer medication, and 1.3% treatment periods with fibrate. During the three-month survey in the nationwide setting 6338 patients (6.9%) on simvastatin or lovastatin were potentially exposed to a drug interaction with CYP3A4 inhibitor, CYP3A4 inducer, or fibrate. A statin-fibrate combination was seen in 581 cases (0.6%). However, only six rhabdomyolysis cases were reported in the whole of Finland during the seven years covering the time window of the hospital-based survey (Tokola *et al.* 2002) suggesting that clinical consequences with potential fatal outcome are very rare, which is consistent with other publications (Pedersen *et al.* 2005, Brown 2008). It is, however, important to remember that ADR reporting requires that physicians consistently take the time to file ADRs; even the most serious ADRs may be left unregistered. The incidence of rhabdomyolysis in patients using statins other than cerivastatin was 3.4 in 100,000 person-years the fatality being 10% (Law and Rudnicka 2006). The incidence was higher when analyzing only CYP3A4-metabolized statins (simvastatin, lovastatin, and atorvastatin), which refers to risk increment by potential DDIs.

In the Scandinavian Simvastatin Survival Study (4S) it has been reported that simvastatin reduced total cholesterol and LDL cholesterol 25% and 35%, respectively, and increased HDL cholesterol 8% over 5.4-year (median) follow-up (Scandinavian Simvastatin Survival Study Group 1994). In the present study total cholesterol was somewhat higher in all interaction groups when compared with controls. In patients either in CYP3A4 inhibitor group or CYP3A4 inducer group this difference was explained by higher HDL cholesterol values, whereas LDL cholesterol concentrations were similar and HDL cholesterol / total cholesterol ratio remained unaffected. Thus, even strong pharmacokinetic interactions potentially leading to marked decrease in simvastatin and lovastatin exposure seem to have a relatively small effect on the efficacy of statin treatment. In fact the use of CYP3A4-inducing agents *per se* is associated with higher HDL concentrations (Luoma *et al.* 1980, Nikolaos *et al.* 2004). However, there is one isolated case report suggesting that CYP3A4 induction could decrease the cholesterol lowering effect of simvastatin (Murphy and Dominiczak 1999). In the present study the most commonly used CYP3A4 inhibitor was diltiazem, which has been reported to increase simvastatin and lovastatin concentrations about 3.5-fold (Azie *et al.* 1998, Mousa *et al.* 2000, Watanabe *et al.* 2004). Despite potential increase in exposure to statins their lipid-lowering effect was not enhanced.

In an earlier study investigating the occurrence of myopathy during simvastatin treatment with its relationship to CYP3A4 inhibitor use the overall incidence of myopathy during simvastatin treatment was found to be 0.025% only. Proportional concomitant use ratio for myopathy was 9.1 for CYP3A4 inhibitor users and the association was higher for the cyclosporine group (23.6) but no association for increased risk to myopathy with calcium channel blockers diltiazem and verapamil was noted compared with controls using simvastatin only. (Gruer *et al.* 1999) In Study II cyclosporine used together with a statin did not cause more plasma CK, γGT, or ALAT alterations than other CYP3A4 inhibitors. In general, mean CK activities were lower and γGT activities higher in CYP3A4 inhibitor users compared with controls while no statistically significant difference was seen in ALAT values. In CYP3A4 inducer users a significant difference was seen only in higher γGT values. Mean statin doses in the present study were relatively low, and on the other hand, the therapeutic indexes of statins are quite wide. Thus, our results support the previous discussion that simvastatin and lovastatin can probably be used rather safely with CYP3A4 inhibitors if the statin doses are low and the patients are monitored carefully (Neuvonen *et al.* 2006).

Combined use of statins and fibrates was associated with significantly increased plasma CK activity indicating muscular toxicity. Also the statin doses were higher in fibrate groups. In hospitalized patients, on whom the laboratory values were collected, the fibrates used together with simvastatin or lovastatin were bezafibrate (n = 42), gemfibrozil (n = 23), and clofibrate (n = 4). When comparing bezafibrate with

gemfibrozil the mean CK values were almost three-fold higher in gemfibrozil-treated patients. Previously it has been demonstrated that gemfibrozil, but not bezafibrate, increases concentrations of active acid forms of simvastatin and lovastatin in plasma (Backman *et al.* 2000, Kyrklund *et al.* 2001). The increased CK activities may thus have, at least partly, a pharmacokinetic origin. The present results support also the previous information about 10 times greater incidence of rhabdomyolysis in patients receiving gemfibrozil with statins (other than cerivastatin) compared with statin mono-treatment (Law and Rudnicka 2006).

The present data indicate that co-administration of simvastatin and lovastatin with strong to moderate inhibitors and inducers of CYP3A4 enzyme as well as fibrates is common both in hospitalized patients and outpatients. Statins are not used optimally because the overall persistence of their use is low particularly among elderly patients (Linnarsson 1993, Benner *et al.* 2002, Perreault *et al.* 2005a, Perreault *et al.* 2005b). Patients with other cardiovascular risk factors such as diabetes and hypertension are the ones most likely to be persistent with statins (Perreault *et al.* 2005b). Also in Finnish patient material it has been seen that patients with at least one prescription for another cardiovascular medication are the most likely to continue statin therapy at least four years (Helin-Salmivaara *et al.* 2008). On the other hand, lower persistence is seen in patients who use the greatest number of prescribing physicians and pharmacies (Perreault *et al.* 2005a, Perreault *et al.* 2005b).

6.2.3 Sulphonylureas

The use of sulphonylureas carries a high risk of hypoglycaemia even with low doses (Holstein *et al.* 2003) and severe sulphonylurea-associated hypoglycaemia has a fatal outcome in up to 10% of the cases (Holstein and Egberts 2003). Of all glibenclamide, glimepiride, or glipizide treatment periods in Study III 19.7% were concomitant with CYP2C9 inhibitor use concerning 16.1% of sulpohonylurea-treated patients. The mean and maximum fasting plasma glucose concentrations and maximum glycosylated haemoglobin were lower during the interaction periods compared with control periods. Long-term sulphonylurea treatment decreases basal and postprandial plasma glucose levels by up to 3–5 mmol/l and glycosylated haemoglobin (GHb-A1C) by 20% (Graal and Wolffenbuttel 1999). Glibenclamide is considered to be the most problematic, in terms of hypoglycaemia, of the three substrates because of its active metabolites (Melander *et al.* 1998) and its ability to enhance target tissue insulin action (Kolterman 1992), but in the present study the glimepiride group had the lowest fasting plasma glucose levels and the glipizide group the lowest GHb-A1C proportions. This may be due to glibenclamide prescriptions to more serious or long-term (with secondary failure) type 2 diabetes mellitus cases. Also glucose or ketone bodies in urine were seen with decreased frequency during the interaction periods compared with controls.

Unfortunately, hypoglycaemic episodes were not recorded systematically in the electronic hospital database. The minimum fasting plasma glucose values were, however, significantly more often under the target in CYP2C9 inhibitor group than in the control group.

The risk of hypoglycaemia increases in relation to drug concentration, but in continuous exposure there is no simple relationship between the drug concentration and insulin or glucose concentrations in plasma. High doses may paradoxically cause lack of efficacy. The prescribed maximum daily doses are then considered to be often too high. The highest doses may also reduce β-cell function. (Stenman *et al.* 1993, Melander *et al.* 1998)

Systemic infections may affect glucose balance (McGuinness 2005), and within the group of CYP2C9 inhibitors 85% represented azole antifungals, sulphamethoxazole, and trimethoprim. The cases and controls were not adjusted for presence or absence of infections neither by using specific diagnostic codes nor the use of other antibiotics not affecting the CYP2C9 activity. However, systemic infections usually increase glucose concentration (McGuinness 2005) and then the results would rather underestimate than overestimate the effect of CYP2C9 inhibiting antimicrobial use on sulphonylurea effects.

Hypoglycaemia is the most important adverse effect of sulphonylureas but other toxic reactions, for example hyponatraemia, elevation of liver enzyme activities, and hepatocellular or cholestatic jaundice, have been described in some patients (Davis 2006). Mean and minimum plasma alanine amino transferase and gamma-glutamyl transferase activities were higher during the interaction periods compared with controls. This may refer to subclinical manifestations of sulphonylurea adverse effects.

6.2.4 Clopidogrel

Previous clopidogrel interaction studies have been based on the *in vitro* finding that atorvastatin inhibits clopidogrel metabolism by more than 90% (Clarke and Waskell 2003). However, neither atorvastatin nor pravastatin (independent from CYP metabolism) have been shown to influence clopidogrel-induced inhibition of platelet activation (Mitsios *et al.* 2004). Again, no differences have been seen in six-month mortality or morbidity in clopidogrel-treated patients with acute coronary syndrome when comparing concomitant use with CYP3A4-metabolized statins and non-CYP3A4 statins (Mukherjee *et al.* 2005). In similar patient material atorvastatin reduced primary endpoints (death from any cause, myocardial infarction, documented unstable angina requiring rehospitalization, revascularization with either percutaneous coronary intervention or coronary artery bypass grafting, or stroke) in clopidogrel-treated patients compared with pravastatin at two-year follow-up but no differences in bleeding endpoints were seen (Lotfi *et al.* 2008). However, as an inactive prodrug

clopidogrel needs to be converted to an active hydroxy metabolite form. The activation was first believed to be transformed by CYP3A (Clarke and Waskell 2003), which was the basis for the study plan in Study IV, but more recently CYP2C19 has also been found to play an important role (discussed more in detail later).

Due to the uncertain profile of atorvastatin in CYP3A4 inhibition and due to its potential to affect the measured endpoints *per se*, atorvastatin was studied as an independent interaction group in Study IV. In this study atorvastatin constituted the largest interaction study group (19.0% of the treatment periods in outpatients and 17.5% in the inpatient setting).

In the one-year follow-up atorvastatin use reduced and CYP3A4 inducer increased the overall mortality significantly compared with controls, although the inducer group was quite small, the hazard ratios (HR) being 0.74 and 2.29, respectively. The indications (epilepsy, bipolar disorders, and severe infections) of CYP3A4 inducer use may affect the high mortality in the group in question. Generally age, male sex, diabetes mellitus, cardiac insufficiency, and hypertension increased the risk of mortality. All the predisposing factors are so called life-style related diseases, like the indication for clopidogrel use.

In the hospital setting the fasting plasma concentrations of total cholesterol and low-density lipoprotein cholesterol were lower in the atorvastatin group compared with the control group. The number (mean) of other drugs affecting the cardiovascular system was lower in all interaction groups compared with controls but the number of statins (other than atorvastatin) was not taken into account. In the atorvastatin group the exposure to statin can be assumed to be 100% but in other study groups the exposure to statins may vary. High-density lipoprotein cholesterol concentration as well as the HDL cholesterol / total cholesterol ratio were higher in CYP3A4 inhibitor and inducer groups compared with the control group. The interacting drugs in these study groups are not associated with alterations in cholesterol levels but the use of CYP3A4 inducing agents in general has been connected with higher HDL concentrations (Luoma *et al.* 1980, Nikolaos *et al.* 2004). No laboratory values that could indicate myotoxicity (e.g. creatine kinase) were studied. However, there is one published case report about a stable heart transplant patient who developed rhabdomyolysis by the addition of clopidogrel to the existing regimen of cyclosporine and atorvastatin tolerated for longer than three years (Burton *et al.* 2007).

Acetylsalicylic acid has not been shown to modify the clopidogrel-mediated inhibition of ADP-induced platelet aggregation nor the prolongation of bleeding time induced by clopidogrel intake. However, clopidogrel may potentiate the effect of acetylsalicylic acid on collagen-induced platelet aggregation. A pharmacodynamic interaction between these two drugs is possible leading to increased risk of bleeding. (www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/

human/000174/WC500042189.pdf) In the inhospital material acetylsalicylic acid was used by 54.5% ($P < 0.001$ compared with control) of patients in CYP3A4 inhibitor group, 75.0% in CYP3A4 inducer group, 85.0% in the atorvastatin group, and 84.3% in the control group. In this type of situation, where the proportion of acetylsalicylic acid use was significantly lower in CYP3A4 inhibitor group, would rather emphasise the thrombosis endpoints in the present study population, but the opposite was seen and acetylsalicylic acid use as a bias source is of minor concern.

Atorvastatin was the most prevalent potentially interacting drug in the present study. After simvastatin it is the most commonly used statin in Finland (Finnish Statistics on Medicine 2002: www.fimea.fi). On the other hand, the therapeutic indications of clopidogrel have been extended to ST segment elevation acute myocardial infarction in combination with acetylsalicylic acid in medically treated patients eligible for thrombolytic therapy (www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000174/WC500042189.pdf), which increases also the concomitant use of statins and clopidogrel. In patients with acute coronary syndromes decreased long-term mortality and mortality + stroke as a combined endpoint have been seen in patients using clopidogrel concomitantly with atorvastatin compared with atorvastatin alone, but this difference was statistically significant only in univariate analysis (Wienbergen *et al.* 2003). In the second analysis with these endpoints atorvastatin did not differ from other statins (simvastatin, pravastatin, cerivastatin, lovastatin, and fluvastatin as one group).

According to the present study concomitant use of CYP3A4 inducers with clopidogrel was associated with increased overall mortality, but whether this was due to increased bioactivation of clopidogrel and thereby increased rate of bleedings could not be assessed. Concomitant administration of atorvastatin with clopidogrel may moderately attenuate the antithrombotic effect of clopidogrel, but the combination significantly reduced the overall mortality. While there was no difference in mortality between CYP3A4 inhibitor and control groups, the role of atorvastatin as CYP3A4 inhibitor is debatable. The positive results in co-treatment with a statin may relate to its lipid lowering effects. This also correlates with the previous study results showing that the consequences of atorvastatin use do not differ from non-CYP3A4 statins in clopidogrel-treated patients (Mukherjee *et al.* 2005, Lotfi *et al.* 2008).

The role of CYP2C19 in clopidogrel metabolism has been studied in healthy volunteers (Hulot *et al.* 2006, Brandt *et al.* 2007, Umemura *et al.* 2008) and in patients (Sibbing *et al.* 2009, Shuldiner *et al.* 2009, Collet *et al.* 2009) and it has been established that *CYP2C19*2* (loss-of-function polymorphism) is associated with increased platelet aggregation. The most recent *CYP2C19* genotype finding in clopidogrel treated patients shows that there is a significant association with *CYP2C19*17* and increased bleeding risk (Sibbing *et al.* 2010). A CYP2C19-mediated drug-drug interaction was first published by Gilard *et al.* in a letter where the association of diminished

clopidogrel activation by omeprazole was reported (Gilard *et al.* 2006). In contrast to this, pantoprazole or esomeprazole use was not associated with impaired response to clopidogrel (Siller-Matula *et al.* 2009). These studies were published later than the data collection of Study IV had been started and no CYP2C19-mediated interactions were taken into account in the study plan.

Based on the finding that CYP3A4 inhibitor use prevents thrombosis complications it would be reasonable to assume that the inhibition of CYP3A4 pathway would divert clopidogrel metabolism to the CYP2C19 direction. However, recently it has been defined *in vitro* that the formation of 2-oxo-clopidogrel is mediated by CYP2C19, CYP1A2, and CYP2B6 (in the order of contribution ratio) whereas the active metabolite, R-130964, is formed by CYP3A4, CYP2B6, CYP2C19, and CYP2C9 (Kazui *et al.* 2010). In the present study hospitalizations due to haemorrhages were less frequent both in the CYP3A4 inhibitor and atorvastatin groups when compared with the controls. Considering the roughly 40% contribution ratio of CYP3A4 in the second step of clopidogrel bioactivation (Kazui *et al.* 2010) these findings may reflect inhibition of CYP3A4 activity. However, in the CYP3A4 inhibitor group also thrombotic complications leading to hospitalizations were less common than in the control group. This may reveal selection of subjects with a smaller risk among those experiencing non-fatal thrombosis events, for both overall mortality and mortality due to thrombosis were higher in the CYP3A4 inhibitor group, the difference reaching almost statistical significance compared with controls.

As to the possible difference between the proton pump inhibitors (PPIs) in concomitant use with clopidogrel (Siller-Matula *et al.* 2009), a large study in 16,690 patients was published very recently on this topic (Kreutz *et al.* 2010). This study shows that the HR of major cardiovascular adverse events during a 12-month follow-up period after stent placement was 1.51 (95% CI 1.39–1.64, $P < 0.001$) in patients receiving PPIs with clopidogrel compared with clopidogrel alone, but the risk was similar between different PPIs (omeprazole, esomeprazole, pantoprazole, and lansoprazole).

6.3 Importance of drug-drug interactions and solutions to their avoidance

Patients often experience adverse effects from their medication and may stop the treatment prematurely. It has been estimated that the adherence of prescribed medication is only 50%, and for some type of medications even less, for example for antibiotics less than 40% (McDonald *et al.* 2002). DDIs represent a major clinical concern for health care professionals and patients. DDIs are estimated to cause 8% of all ADRs (Kelly 2001) and 26% of all hospitalizations (McDonnell and Jacobs 2002). The length of a hospital stay is associated with increased risk for DDIs (Moura *et al.*

2009). However, polypharmacy is found to be the main reason for DDIs. A potential DDI among patients receiving five or more drugs has been shown to be five-fold compared with patients taking less than five drugs (Moura *et al.* 2009).

Aging is a risk factor for both ADRs and polypharmacy (Egger *et al.* 2007) but in the present studies mean age was often higher in control groups than in interaction groups. Interaction patients receiving codeine or tramadol (Study I), statins with fibrates (Study II) or clopidogrel with atorvastatin (Study IV) were younger than respective controls.

One method to avoid DDIs is to choose another drug group member, which does not have similar interaction potential with patient's other medication. To remember all the interaction is, however, impossible for the clinicians. Even at the molecular structure level predicting interactions is difficult. Quinine, for example, is a levorotary diastereomer of quinidine but is not as potent CYP2D6 inhibitor as quinidine (Parkinson and Ogilvie 2008). In addition to this, patients often use over-the-counter (OTC) drug or herbal medicines that are not mentioned in the prescription situation. Patients believe that because herbal medicines are natural they are totally safe. It is reported that 15% of patients receiving conventional pharmacotherapy also take herbal products and, among these, potential adverse herb-drug interactions have been observed in 40% (Izzo and Ernst 2009).

It has been suggested that properly designed computer-based decision-support system would increase the awareness of clinically significant interactions and improve the quality of drug treatment (Linnarsson 1993). At Turku University Hospital there was no computerized DDI warning system integrated with the hospital data processing systems during the study periods. A year after the electronic medication database (see chapter 4.1.1) had been introduced it was reported that 6.8% of patients in internal medicine wards had one or several drug combinations potentially leading to serious clinical consequences (Gronroos *et al.* 1997). In the present results on metabolic CYP-related DDIs the prevalence was up to 20% and the risk for an interaction was higher expressly in internal medicine wards.

To control DDIs prescribing physicians can, for example, change the risky drug to another member of the same group, adjust the dosing, or monitor the patient by following the clinical status or by therapeutic drug monitoring. It has been reported that appropriate actions to avoid or handle DDIs and DDI-related ADRs are performed mainly when the actions could be regarded as routine checks relating to one drug or to a disease treated with that drug but not specifically from the DDI perspective (Linnarsson 1993). According to present data no clinically significant differences were seen in dosing between the interaction groups and controls.

7 CONCLUSIONS

On average one fifth of the inpatients receiving prodrugs losartan, codeine, and tramadol were exposed to potential CYP-mediated drug-drug interactions. DDIs inhibiting prodrug activation thus present a common source of irrational prescribing which may as an unrecognized phenomenon pose poor clinical efficacy of the prodrugs.

Sulphonylurea-treated inpatients were exposed to a potential CYP2C9-mediated interaction in 19% of the cases, mostly with antimicrobial agents. The mean and maximum fasting plasma glucose concentrations and maximum glycosylated haemoglobin were lower during the interaction periods compared with control periods. Clinically significant CYP2C9-mediated DDIs are thus commonly seen in hospitalized patients receiving glibenclamide, glimepiride, or glipizide.

Of simvastatin- or lovastatin-receiving outpatients and inpatients 6.2% and 12%, respectively, were exposed to potential DDIs with CYP3A4 inhibitors or CYP3A4 inducers. In clopidogrel-treated patients the potential CYP3A4-mediated DDIs were more common in open care than in hospital setting, prevalences being 7.0% and 6.2%, respectively. Based on laboratory data the DDIs between simvastatin and lovastatin together with CYP3A4 inhibitors and CYP3A4 inducers had much less clinical relevance that could have been hypothesized by their strong pharmacokinetic interactions. In low doses the use of simvastatin and lovastatin may then be safe with CYP3A4 inhibitors, especially the moderate ones. In clopidogrel-treated patients HDL cholesterol concentrations were higher in CYP3A4 inhibitor and CYP3A4 inducer users compared with controls. Overall mortality was more prevailing in the CYP3A4 inducer group than in the control group. Concomitant use of CYP3A4 inhibitor with clopidogrel did not affect mortality rates but non-fatal thrombosis and haemorrhage complications were rarer in this group than in the control group.

Concomitant use of fibrates was seen in 0.6–1.3% of simvastatin- and lovastatin-treated patients. Atorvastatin was used concomitantly with clopidogrel in 17% and 21% of inpatients and outpatients, respectively. DDIs between simvastatin and lovastatin with fibrates, with gemfibrozil in particular, carry a notable clinical impact by increasing the risk of muscular toxicity. This was seen in elevated creatine kinase activities in plasma. Thrombosis events were more common in clopidogrel-treated patients receiving also atorvastatin but total cholesterol and LDL cholesterol concentrations were significantly lower and overall mortality rarer in this group compared with the control group.

In summary, cytochrome P450-mediated drug-drug interactions are common among widely used drugs. They are difficult to recognize and may therefore inflict unforeseeable problems in everyday clinical work. Educational and other preventative methods are needed to decrease the extent of irrational drug prescribing.

8 ACKNOWLEDGEMENTS

This thesis was financially supported by Clinical Drug Research Graduate School, Helsinki, Finland; Turku University Hospital Grant EVOL3821; Orion-Farmos Research Foundation (formerly Research Foundation of Orion Corporation); Finnish Cultural Foundation, Regional Fund on Varsinais-Suomi; Aarne and Aili Turunen Foundation; Paulo Foundation. These are all greatly appreciated.

The present series of studies was carried out at the Department of Pharmacology, Drug Development and Therapeutics, Institute of Biomedicine, University of Turku, and the Unit of Clinical Pharmacology, Turku University Hospital over the period 2004 to 2010. I want to express my gratitude to Professors Mika Scheinin, Markku Koulu, and Liisa Kanerva for offering me the opportunity to perform the study. Professor Pertti Neuvonen, the director of the Clinical Drug Research Graduate School, is also acknowledged for providing me with excellent facilities for this research.

I owe my deepest gratitude to my supervisor, Professor Risto Huupponen. I admire not only his professional skills but also his way to handle things with calm and diplomacy. He emanates wisdom and experience, and it has been an honour for me to work under his guidance. Without Risto's support I could never have completed this thesis.

My supervisor Kari Laine, MD, PhD, has unintelligible enthusiasm for new things. By retelling my colleague's words, he erupts his ideas like dynamite. Thanks to Kari, during the study years I have seen different sights (from a hospital cellar to a lectern in an international congress) and sides of the science world.

I am very thankful to the reviewers of this thesis, Professor Mikko Niemi, MD, PhD, and Jorma Lahtela, MD, PhD, for their time and professional comments relating especially to their core competence, pharmacogenomics and internal medicine, respectively. I thank Pirkko Huuskonen, MA, for careful reviewing of the language.

Coauthors Professor Timo Klaukka, MD, PhD, Kerttu Irjala, MD, PhD, Anna Ryynänen, MD, Pekka Heikkilä, MSc, and Tero Vahlberg, MSc, are acknowledged for their valuable contribution to the articles included in this thesis. I want to thank Liisa Norman, MSc, for help in datamining at Turku University Hospital.

From the Unit of Clinical Pharmacology at Turku University Hospital I would like to express my thanks to the heads, Mika Scheinin, MD, PhD, Kari Laine, MD, PhD, and Risto Huupponen, MD, PhD, for giving me an opportunity to follow the consultation process in weekly meetings during my study years and have, even if only, a slight touch to the clinical work. I also thank all the specializing MDs for company during these years. Ms Marja-Liisa Heino and Ms Elina Kahra are acknowledged for their help relating to practical issues.

I want to thank the SFINX project for broadening my world of DDIs from CYP-mediated interactions to all types of pharmacokinetic and pharmacodynamic drug-drug

interactions. Especially Birgit Eiermann, PhD, and Marine Andersson, MSc, are warmly acknowledged for their colleague- and friendship.

All the collegues in the Epi-Pharma gang I want to thank for company and inspirational atmosphere during the journal club sessions. I warmly thank Maarit Korhonen, PhD, and Arja Helin-Salmivaara, MD, PhD, for teaching (and reteaching) me the methodology of pharmacoepidemiology.

I am truly grateful for all work mates for company in Pharma City, especially in room 3048. I have also nice memories from the congress trips in Turkey, Poland, China, and the Netherlands – thanks for your company. Ms Anja Similä and Ms Hanna Tuominen receive my warmest thanks for exellent secretarial assistance. I am happy that I have made friends with Ulriikka Jaakkola, MD, PhD, Päivi Ruokoniemi, MD, Susann Björk, MSc, and Milka Hauta-aho, MSc, so that we meet also outside work.

Thanks to you, course mates of Health Biosciences ’98 at the University of Turku: Anna Sahlber, Anna-Stina Tukiainen, Anne Timonen, Hanna Ryösä, Jonna Nevo, Jenni Bernoulli, Jenni Vaarno, Jonne Laurila, Jukka Rissanen, Karoliina Vuoriluoto, Liisa Gunnelius, Mari Vasama, Matias Scheinin, Niina Jalava, Riikka Oksala, Saku Ruohonen, and Tiina Ujula. You were the key to my presence at the university, the reason for me to get out of bed in the morning. I am happy that we are in touch even after completing the MSc studies. The boys I remember for giving me several darling nicknames. Jenni Bernoulli is specially acknowledged for being my PhD tutor.

However, even more warmly I want to thank my friends with whom I have been able to think about something else than pharmacology. Many of you are related to sports, and among others I want to high-five those I have met in water polo pools, volleyball fields, ski slopes, or scuba diving locations. Of the water polo team mates I want to give special thanks to Suski, Laura, and Kimmo, who made me run weekly and helped me to pass the marathons after my active career.

I also want to thank my singing buddies Pia and Jaana, members of Arrhythmics choir, and others that I have sung with more informally. The Polytech Choir is acknowledged for several serenades, but as important as this association, the “widow club” has become an important peer group for me.

I want to thank all the “Starlings”, Anna, Ansku, Eeva, Johku, Jonna, Karo, and Maarit, for the tireless quacking and ruffling during the student years and now as “adults”. Special thanks and warm hugs to Riina Kannisto, Ida Virkki, and Ulla Pekkinen for lifelong friendship and support. Besides Ida and Ulla, I want to thank all of those with whom I have visited numerous exotic places in the World.

All my relatives are very close to me. My grandmother, uncles, aunt, cousins, and their families constitute my extended family. We have always had extraordinary warm relationships and I want to thank you all, also those who are not with us anymore. Those living geographically close to me have someway been in a special position, but I want to emphasize that I think and care about you all very much, and, in fact, I see you

all too rarely. *Mummi ja Erkki sekä Pekka, Liisa, Paavo ja Hannu perheineen, haluan kiittää teitä kaikkia saamastani tuesta lapsuudestani tähän päivään saakka. Muistan myös sukulaisia, jotka eivät ole enää keskuudessamme. Sukumme erityisen läheiset välit ovat aina olleet minulle erittäin tärkeitä ja olen niistä ylpeä. Ajattelen teitä kaikkia paljon, vaikken näekään teitä niin usein kuin haluaisin, edes niitä, jotka asuvat maantieteellisesti lähellä. Olen todella iloinen serkusten ainutlaatuisista väleistä ja siitä, että keskitymme mielellämme juhlimiseen eivätkä "kokouksemme" ole mitään pönötystä vaan enemmänkin pokotusta. Toivon, että tämä työni antaa osaltaan aihetta juhlaan, ja että te kaikki voitte olla mukana iloitsemassa sen valmistumista.*

I want to thank Saara, my beloved sister, for giving an example of success. Having just completed my thesis project, surprisingly before you did yours, but now I know you can finalize your project too, even with Petra and Lauri running around. *Petra ja Lauri, tätä kirjaa tätsy on tehnyt työkseen viime ajat – eikä tulos ole tämän kummallisempi, tylsää tekstiä vain. Älkää siis olko kateellisia aikuisille, jotka 'saavat' istua tietokoneen ääressä. "Lue kirjaa, piirrä, mene ulos juoksemaan" on jotain, mitä minäkin tekisin paljon mieluummin.*

I warmly thank *Äiti* and *Isä*, my parents, Leena and Ilpo Tirkkonen, for the inspirational atmosphere in my childhood home and engouraging me for "everything". I want to thank Mom for influence on all sorts of natural sciences and Dad (in brief) balancing our lives. I also thank Dad for the opportunities to follow his international peacekeeping career even though you were not so much present at the time. You both know that I have learned my moral thinking from you, and that will last wherever I will reside.

There is a cliche that the Finns do not express their feelings, nor especially use the Finnish words for I love you. In my family, not only including my parents and sister, but also more widely, the love and nearness can be seen as unselfishness and sincerity. I am happy that we all can share this feeling. One of the best moments during the thesis years was when I once realized that the family could fit in one boat, literally, and we were all smiling and enjoying the silent ride in the Finnish Archipelago.

With Jouko I share the meaningful words *minä rakastan sinua* daily. I have found such a life-companion that I must make sure that he can never doubt my feelings to him. Jouko Prami is an unbelievable combination of energy and calm. When writing the thesis I often got tired when I realized all the tasks he had accomplished in one day relating to work, studies, and hobbies. In spite of all the duties he is always polite and stressless. I really admire your intelligence, skills, and efficiency. I want to thank you for supporting me when I have had difficulties in my career. It is wonderful that you love me as I am. I hope that in science there would be more such thinking that we have together: more rewarding than to compare or compete is to share.

In Espoo, September 2010

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