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CLINICAL STUDIES ON PHARMACOKINETIC DRUG-DRUG INTERACTIONS CAUSED BY CLOPIDOGREL

Focus on CYP2C8-mediated drug metabolism

MATTI ITKONEN

ACADEMIC DISSERTATION

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Every conundrum of life arises from cells that lie within the body or the mind.

-Anonymous, twenty-first century

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LIST OF ORIGINAL PUBLICATIONS

The following publications are the bases of this doctoral thesis

I Itkonen MK, Tornio A, Neuvonen M, Neuvonen PJ, Niemi M, and Backman JT. Clopidogrel has no clinically meaningful effect on the pharmacokinetics of the organic anion transporting polypeptide 1B1 and cytochrome P450 3A4 substrate simvastatin. *Drug Metab Dispos* (2015) 43:1655–1660.

II Itkonen MK, Tornio A, Neuvonen M, Neuvonen PJ, Niemi M, and Backman JT. Clopidogrel markedly increases plasma concentrations of CYP2C8 substrate pioglitazone. *Drug Metab Dispos* (2016) 44:1364–1371.

III Itkonen MK, Tornio A, Filppula AM, Neuvonen M, Neuvonen PJ, Niemi M, and Backman JT. Clopidogrel but not prasugrel significantly inhibits the CYP2C8-mediated metabolism of montelukast in humans. *Clin Pharmacol Ther* (2018) 104:495–504.

IV Itkonen MK, Tornio A, Lapatto-Reiniluoto O, Neuvonen M, Neuvonen PJ, Niemi M, and Backman JT (2019) Clopidogrel increases dasabuvir exposure with or without ritonavir, and ritonavir inhibits the bioactivation of clopidogrel. *Clin Pharmacol Ther* (2019) 105:219–228.

V Itkonen MK, Tornio A, Neuvonen M, Neuvonen PJ, Niemi M, and Backman JT. Clopidogrel and gemfibrozil strongly inhibit the CYP2C8-dependent formation of 3-hydroxydesloratadine and increase desloratadine exposure in humans. *Drug Metab Dispos* (2019) 47:377–385.

ABBREVIATIONS AND DEFINITIONS

3D	3-direct-acting antiviral
ABC	Adenosine triphosphate binding cassette superfamily
ADP	Adenosine diphosphate
AhR	Aryl hydrocarbon receptor
ANOVA	Analysis of variance
AUC	Area under the plasma concentration-time curve
BCRP	Breast cancer resistance protein
b.i.d.	Twice a day
BMI	Body mass index
c.	Nucleotide position in the coding DNA of a gene
CAR	Constitutive androstane receptor
CES	Carboxylesterase
CI	Confidence interval
CL	Clearance
C_{max}	Peak plasma concentration
CV	Coefficient of variation
CYP	Cytochrome P450
DDI	Drug-drug interaction
DNA	Deoxyribonucleic acid
DPYD	Dihydropyrimidine dehydrogenase
EDTA	Ethylenediaminetetra-acetic acid
EMA	European Medicines Agency
f_a	Absorbed fraction
FDA	United States Food and Drug Administration
f_m	Metabolized fraction
f_u	Unbound fraction
FXR	Farnesoid X receptor
GI	Gastrointestinal
GR	Glucocorticoid receptor
GWAS	Genome-wide association study
HCV	Hepatitis C virus
HEK293	Human embryonic kidney 293
HLA	Human leukocyte antigen

HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HNF	Hepatocyte nuclear factor
IC_{50}	Inhibitor concentration producing 50% inhibition
k_e	Elimination rate constant
K_m	Michaelis-Menten kinetic constant
LLQ	Lower limit of quantification
MATE	Multidrug and toxin extrusion protein
MBI	Mechanism-based inhibition
MDR	Multidrug resistance protein
MP	Methoxyphenacyl
mRNA	Messenger ribonucleic acid
MRM	Multiple reaction monitoring
MRP	Multi-drug resistance-associated protein family
NADPH	Nicotinamide adenine dinucleotide phosphate; reduced form
NTCP	Sodium/taurocholate contransporting polypeptide
OAT	Organic anion transporter
OATP	Organic anion-transporting polypeptide
OR	Odds ratio
p.	Amino acid position in the protein sequence
PBPK	Physiologically-based pharmacokinetic
PG	Prostaglandin
P-gp	P-glycoprotein
PPAR	Peroxisome proliferator-activated receptor
PRU	$P2Y_{12}$ reaction unit
PXR	Pregnane X receptor
q.d.	Once a day
RNA	Ribonucleic acid
rs	Reference SNV identification number in the NCBI database
SD	Standard deviation
SLC	Solute carrier superfamily
SNV	Single nucleotide variation
$t_{½}$	Elimination half-life
t_{max}	Time to peak plasma concentration
TRAP	Thrombin receptor activating peptide
UDP	Uridine 5’-diphosphate

UGT	Uridine 5’-diphospho-glucuronosyltransferase
V_d	Volume of distribution
VDR	Vitamin D receptor
VKORC1	Vitamin K epoxide reductase complex subunit 1

ABSTRACT

Clopidogrel, an adenosine diphosphate receptor subtype $P2Y_{12}$ antagonist, attenuates platelet activation via its active *cis*-thiol metabolite formed in two steps predominantly by cytochrome P450 (CYP) 2C19 and CYP3A4. It is widely used in acute treatment and secondary prevention of atherothrombotic events. A previous clinical study observed clopidogrel to markedly increase the exposure of CYP2C8 index substrate repaglinide, whose disposition is additionally dictated by CYP3A4 and the hepatic uptake transporter organic anion-transporting polypeptide (OATP) 1B1. *In vitro* experiments identified the secondary acyl-β-D-glucuronide metabolite of clopidogrel as a time-dependent inhibitor of CYP2C8, but they also suggested clopidogrel to inhibit OATP1B1 and CYP3A4.

The primary aim of this work was to characterize the drug-drug interaction (DDI) mechanisms of clopidogrel and their clinically relevant implications, while the secondary objective was to search for sensitive and selective CYP2C8 index substrates. This thesis consists of five prospective, clinical pharmacokinetic and pharmacodynamic DDI studies applying randomized, controlled, crossover design. In the first study, clopidogrel did not affect the pharmacokinetics of simvastatin, leading to the conclusion that clopidogrel is not a clinically relevant inhibitor of OATP1B1 or CYP3A4, which have paramount roles in the disposition of simvastatin. The second study reported clopidogrel to augment the exposure and trough concentration of the CYP2C8 substrate pioglitazone 2.1-fold and 4.5-fold, respectively. These observations implied that coadministering the two drugs might increase the risk for adverse reactions caused by pioglitazone due to the inhibition of its CYP2C8-mediated metabolism by clopidogrel. In the third study, clopidogrel doubled the exposure montelukast, whereas prasugrel did not significantly affect montelukast pharmacokinetics. These findings indicate that montelukast is less sensitive to CYP2C8 inhibition than suggested by previous studies with the strong CYP2C8 inhibitor gemfibrozil, and confirm that clopidogrel, but not prasugrel, is a potent inhibitor of CYP2C8. The fourth study found clopidogrel to increase the exposure of the CYP2C8 substrate dasabuvir ~4–5-fold and observed ritonavir to markedly decrease the exposure and antiplatelet effect of clopidogrel active metabolite. The results from the fourth study indicated that dasabuvir is a highly sensitive and specific CYP2C8 substrate and that combining clopidogrel with dasabuvir, or ritonavir with clopidogrel, may risk patient safety. In the fifth study, clopidogrel significantly inhibited, and gemfibrozil almost completely prevented, the CYP2C8-mediated biotransformation of desloratadine to its 3-hydroxy metabolite, strongly suggesting that CYP2C8 is a crucial enzyme in desloratadine metabolism *in vivo*.

In conclusion, this work provides several findings that are applicable to patient care and drug research. Most importantly, clopidogrel is a clinically relevant CYP2C8 inhibitor capable of causing potentially hazardous DDIs, and it can be employed as a selective index inhibitor of CYP2C8 in clinical DDI studies. Furthermore, dasabuvir can be applied as a CYP2C8 index substrate in subtherapeutic doses in clinical drug research. Moreover, the observations from this work corroborate the ability of ritonavir to disrupt CYP3A4-mediated bioactivation of prodrugs, including clopidogrel, which can compromise patient safety. Finally, these results highlight the ability of glucuronide metabolites to act as substrates and inhibitors of CYP enzymes, especially CYP2C8, and therefore they must be considered as potentially interacting compounds during drug development.

INTRODUCTION

Atherothrombotic events emerge as complications of atherosclerosis and are among the leading causes of death worldwide (Laslett et al. 2012). According to European data from 2015, of the total deaths due to all causes, coronary heart disease and stroke caused 19% and 9% among males, and 20% and 14% among females, respectively (Townsend et al. 2016). The rupture of an atherosclerotic plaque initiates a cascade that leads to platelet-rich thrombus formation that potentially causes ischemia in tissues distal to the lesion. Clopidogrel attenuates this process by inhibiting platelet aggregation by antagonizing the adenosine diphosphate (ADP) receptor subtype $P2Y_{12}$ via its active *cis*-5-thiol metabolite (Bristol-Myers Squibb/ Sanofi Pharmaceuticals 2018, Farid et al. 2010). Only under 10% of ingested clopidogrel is activated in two steps primarily by cytochrome P450 (CYP) 2C19 and CYP3A4 enzymes, and consequently, the bioactivation of clopidogel is susceptible to changes in CYP2C19 and CYP3A4 activity due to genetic factors and drug-drug interactions (DDIs) (**Figure 1**) (Farid et al. 2010, Gilard et al. 2008, Holmberg et al. 2014, Simon et al. 2009). However, the impact of the strong CYP3A4 inhibitor ritonavir on clopidogrel bioactivation has been investigated only in small groups of patients on concomitant and potentially interacting medications (Marsousi et al. 2018, Metzger and Momary 2014). Furthermore, approximately 85–90% of clopidogrel is hydrolyzed by the polymorphic carboxylesterase (CES) 1 enzyme to inactive carboxylic acid intermediate (Hagihara et al. 2009, Tang et al. 2006, Tarkiainen et al. 2015), which is a substrate for uridine 5'-diphospho-glucuronosyltransferase (UGT) 2B enzymes that form a secondary acyl-β-D-glucuronide metabolite (**Figure 1**) (Ji et al. 2018, Kahma et al. 2018).

In 2012, a pharmacoepidemiological study associated the concurrent use of clopidogrel with the increased risk for myotoxic adverse events of cerivastatin, which is a substrate of CYP2C8 and organic anion-transporting polypeptide (OATP) 1B1, a hepatic uptake transporter (Floyd et al. 2012, Shitara et al. 2004). Furthermore, clinical studies have shown that clopidogrel increases the area under concentration-time curve (AUC) of rosuvastatin (Pinheiro et al. 2012, Remsberg et al. 2013), an OATP1B1 substrate that is not significantly metabolized (Neuvonen et al. 2006). In a more recent study, clopidogrel augmented the exposure of the CYP2C8, OATP1B1, and CYP3A4 substrate repaglinide, and *in vitro* and *in silico* experiments identified clopidogrel acyl-β-D-glucuronide as a mechanism-based CYP2C8 inhibitor (**Figure 1**) (Tornio et al. 2014). Furthermore, clopidogrel has been shown to inhibit OATP1B1 and to a lesser extent CYP3A4 *in vitro* (Floyd et al. 2012, Tamraz et al. 2013, Tornio et al. 2014). Physiologically based pharmacokinetic (PBPK) simulations suggested OATP1B1 inhibition to complement CYP2C8 inhibition in the clopidogrel-repaglinide DDI in humans (Tornio et al. 2014). However, the clinical CYP2C8 and OATP1B1 inhibition potency of clopidogrel has required further clinical investigation.

The fibric acid derivative gemfibrozil is applied as a hypolipidemic agent, and its primary 1-O-glucuronide metabolite acts as mechanism-based inhibitor of CYP2C8 (Ogilvie et al. 2006), which almost completely obliterates the enzyme activity in common clinical doses of 600 mg b.i.d. (Honkalammi et al. 2012). Therefore, regulatory authorities recommend gemfibrozil as an index CYP2C8 inhibitor for clinical DDI studies (EMA 2012, FDA 2017a). However, gemfibrozil markedly diminishes the activity of OATP1B1 (Tornio et al. 2017), which poses limitations for data interpretation if the victim drug under examination is also a substrate of OATP1B1, such as repaglinide. Therefore, alternative CYP2C8 index inhibitors of high enzyme selectivity are needed, and clopidogrel is suggested due to its DDI profile.

Simvastatin is a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, the metabolism of which is mainly mediated by CYP3A4, whereas the role of CYP2C8 in its metabolism is less important (Lilja et al. 1998, Neuvonen et al. 1998, Prueksaritanont et al. 1997, Prueksaritanont et al. 2003). Furthermore, its active metabolite, simvastatin acid, is highly sensitive to alterations in

OATP1B1 activity (Niemi et al. 2011, Pasanen et al. 2006). Due to its pharmacokinetic profile, simvastatin is an excellent index substrate for determining whether a potential perpetrator disturbs the activity of OATP1B1 or CYP3A4. As clopidogrel was suggested to inhibit OATP1B1 and CYP3A4 (Tornio et al. 2014), the objective of Study I was to examine the effect of clopidogrel in simvastatin pharmacokinetics.

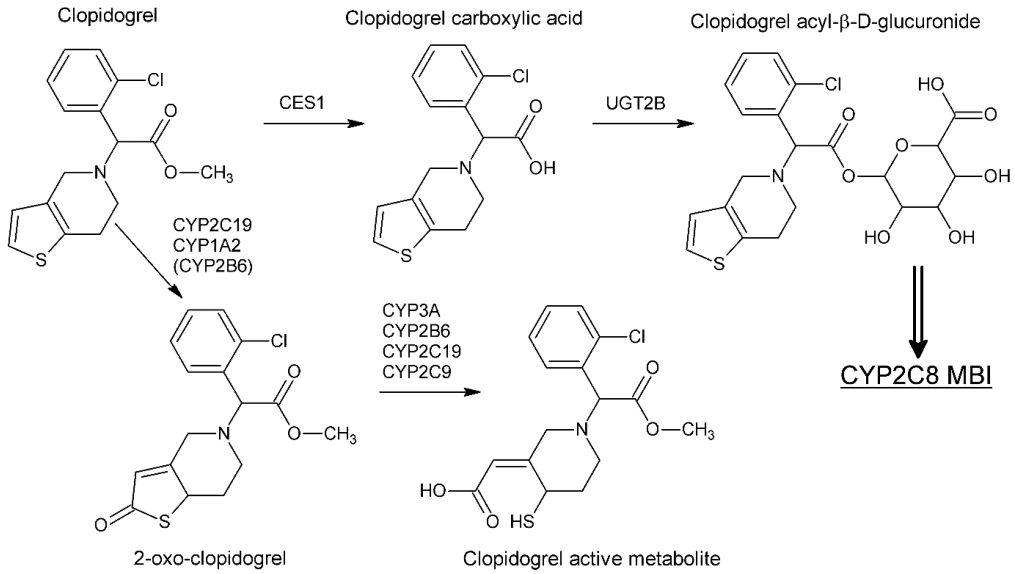

Figure 1. Biotransformation pathways of clopidogrel, and the chemical structures of parent clopidogrel and its 2-oxo-, active *cis*-5-thiol, carboxylic acid, and acyl-β-D-glucuronide metabolites. CES1, carboxylesterase 1; CYP, cytochrome P450; MBI, mechanism-based inhibition; UGT, uridine 5'-diphospho-glucuronosyltransferase.

Pioglitazone is a glucose-lowering agent of thiazolidinedione class that establishes its effect via peroxisome proliferator-activated receptor (PPAR) γ agonism (Waugh et al. 2006). CYP2C8 is responsible for both the biotransformation of pioglitazone to its major primary hydroxyl metabolite and for the further conversion to secondary keto metabolite, whereas other enzymes participate in pioglitazone metabolism only to a minor extent (Eckland and Danhof 2000, Jaakkola et al. 2005, Jaakkola et al. 2006a). Consistently, gemfibrozil has raised pioglitazone exposure approximately 3–5-fold, of which the highest average increases have been observed in carriers of the *CYP2C8*3* allele (Aquilante et al. 2013, Deng et al. 2005, Jaakkola et al. 2005). Due to the crucial role of CYP2C8 in the metabolism of pioglitazone, it can be applied as a CYP2C8 probe substrate, especially when considered that transporter-related mechanisms do not significantly participate in its disposition (Backman et al. 2016). Accordingly, Study II aimed to characterize the CYP2C8 inhibition potency of clopidogrel by examining its effect on pioglitazone pharmacokinetics.

Prasugrel belongs to the same thienopyrimidine class of ADP receptor antagonists as clopidogrel, and its platelet inhibition is mediated by the sequentially formed active metabolite that binds irreversibly to the $P2Y_{12}$ receptor (Farid et al. 2007). In contrast to clopidogrel, the CYP2C8 inhibition potential of prasugrel has not been examined in a clinical setting. As polypharmacotherapy is common in patients using clopidogrel or prasugrel, which have similar indications (Wiviott et al. 2007), comparing the

CYP2C8-inhibitory effect of clopidogrel to that of prasugrel provides clinically relevant data. Study III aimed to elaborate this matter by investigating how clopidogrel and prasugrel affect the pharmacokinetics of montelukast, which has been considered as a sensitive CYP2C8 substrate, as previously demonstrated by the ~4.5-fold increases in its exposure by gemfibrozil (Karonen et al. 2010, Karonen et al. 2012). Moreover, other mechanisms, such as inhibition of OATP1B1-mediated hepatic uptake, have been suggested to contribute to gemfibrozil-montelukast DDI (Varma et al. 2017), thereby requiring further elucidation of montelukast pharmacokinetics.

Dasabuvir is a nonstructural protein 5B inhibitor used as a component of a ritonavir-containing 3-direct-acting antiviral (3D) regimen in the treatment of hepatitis C virus (HCV) genotype 1 infection (Deeks 2015). The DDI profile of dasabuvir has been characterized in combination with the other drugs in 3D regimen (Menon et al. 2015, Polepally et al. 2016), but it has been individually understudied *in vivo*. Without ritonavir, the contribution of CYP2C8 and CYP3A4 in dasabuvir metabolism averages ~60% and ~30% *in vitro*, respectively, and the former is responsible for the formation of the *tert*-butyl hydroxyl metabolite (M1), the major metabolite in human plasma (King et al. 2017, Shebley et al. 2017a, Shen et al. 2016). When dasabuvir and ritonavir are administered concurrently *in vivo*, the metabolism of dasabuvir is highly CYP2C8-selective, which gemfibrozil exemplified by causing a ~11-fold increase in dasabuvir exposure (King et al. 2017, Menon et al. 2015, Polepally et al. 2016). Due to the increased risk of adverse events of dasabuvir, such as decreased hemoglobin levels and suggested potential for QTc prolongation, its concomitant administration with strong CYP2C8 inhibitors is contraindicated (FDA 2014a). Despite the unelucidated *in vivo* pharmacokinetics of dasabuvir, its DDI profile suggests that it could be applied as a CYP2C8 index substrate. Therefore, Study IV intended to examine the effect of clopidogrel on dasabuvir pharmacokinetics with and without ritonavir, while simultaneously characterizing the effect of ritonavir in clopidogrel pharmacokinetics and antiplatelet effect.

Desloratadine is a second-generation histamine H1 receptor antagonist indicated for the treatment of allergic rhinitis and chronic urticaria (FDA 2014c). *In vitro*, the formation of the major 3-hydroxy metabolite of desloratadine is highly dependent on CYP2C8 activity (Kazmi et al. 2015), but this finding has not been confirmed in clinical studies. The benign safety profile of desloratadine and proposed crucial role of CYP2C8 in its metabolism suggest that desloratadine could be utilized as a CYP2C8 probe substrate. In order to compare the CYP2C8 inhibition strength of clopidogrel with that of gemfibrozil, and to explore the contribution of CYP2C8 in desloratadine metabolism *in vivo*, Study V examined whether concurrent administration of clopidogrel or gemfibrozil could affect desloratadine pharmacokinetics.

This work includes five clinical pharmacokinetic DDI studies performed in randomized, controlled, crossover settings. The studies were primarily conducted in order to characterize the DDI mechanisms and profile of clopidogrel, and their implications for drug research. Furthermore, the secondary targets were to reveal important DDIs caused by clopidogrel, and to search for potential CYP2C8 index substrates.

REVIEW OF THE LITERATURE

1. Pharmacokinetics

The human body considers drugs to be xenobiotics, that is, foreign molecules or atoms. Pharmacokinetics examines how drugs are absorbed, distributed, metabolized, and eventually eliminated from the organism (Rowland and Tozer 2011). Every drug proves useless, if it cannot reach target tissues in required amounts to present its desired physiologic effects, i.e., "pharmacodynamics", or it accumulates in other tissues and causes unacceptable toxicity. Pharmacokinetic events often take place simultaneously and are affected by disease, age, organ function, body composition, sex, ethnicity, and numerous other factors. Therefore, it is crucial to study drug behavior in different populations. *In vitro* experiments, PBPK and other *in silico* modeling are preeminent procedures in studying compound properties, such as absorption, distribution, elimination, toxicity, and affinity to enzymes or transporters (FDA 2017b, Raunio et al. 2015). Only if these vital methods imply that safety and efficacy requirements are fulfilled, drug behavior can continue on to be studied in animals, and finally in humans. In order to give marketing authorization, regulatory authorities require pharmaceutical compounds to possess an adequate risk-benefit ratio, which includes a reasonable therapeutic window (EMA 2012, FDA 2017a). Of note, theories of sufficient practicality are prone to making oversimplifications when characterizing the complexities of physiological events, and pharmacokinetic theories are no exception.

Ingested drugs require absorption from the gastrointestinal (GI) tract that can take place by passive diffusion, transporter protein mediated uptake, or their combination. In contrast, efflux transporters can prevent xenobiotics from entering the body, or they may facilitate drug elimination to bile or urine, for example. The liver is the paramount organ in drug metabolism, but in certain cases, biotransformation begins already in the intestinal epithelial cells. Furthermore, some metabolic enzymes are expressed in significant quantities in other organs, such as the kidneys and the lungs (Hukkanen et al. 2002, Knights et al. 2013). Some drugs reach systemic circulation only in trace amounts, i.e., have low oral bioavailability, which can be caused by poor passive absorption, and transport and metabolic systems of the intestine and the liver. Even a drug with a low oral bioavailability can have metabolites that generate significant systemic exposure when released from hepatocytes, for instance. Drug metabolites are often physiologically inactive, but can also have their own pharmacologic effects. In addition, they may disturb enzyme and transporter protein activity or cause toxicity. Thus, investigating only the properties of the parent compound is insufficient to thoroughly characterize the net effect of a drug.

Primary pharmacokinetic variables, whereof other variables are derived, include clearance (CL), volume of distribution (V_d), bioavailability, and absorption rate. However, when reporting the physiological behavior of a drug, the most important pharmacokinetic parameters are AUC that reflects drug exposure, maximum concentration (C_{max}), elimination half-life ($t_{½}$), and time to reach C_{max} (t_{max}). When examining the pharmacokinetics of a drug administered as a single dose, its AUC extrapolated to infinity ($AUC_{0-\infty}$) corresponds to the dose interval AUC in steady-state conditions, if the drug exhibits linear pharmacokinetics. Therefore, $AUC_{0-\infty}$ is used as a standard pharmacokinetic parameter when describing drug exposure. Furthermore, several additional variables are often applied in describing pharmacokinetics, e.g., fraction metabolized by a certain enzyme (f_m), elimination rate constant (k_e) that is employed when determining $t_{½}$, and unbound fraction (f_u). In addition, it should be noted that the pharmacokinetic properties of a drug metabolite may radically differ from those of the parent drug.

1.1 Drug-drug interactions

Pharmacokinetic DDI is defined as an event where one drug called the "perpetrator" alters the absorption, distribution, metabolism, or elimination of a "victim" drug. Furthermore, concurrent administration of drugs with a similar or opposite pharmacodynamic profile often leads to potentiating synergism or abrogation of therapeutic response, respectively, which can have similar manifestations as pharmacokinetic DDIs. They can lead to increased risk of adverse reactions or diminished therapeutic efficacy, and especially if unrecognized, DDIs may increase mortality and morbidity, and burden healthcare services. Improved patient diagnostics and drug treatments, as well as population ageing, augment the prevalence of pharmaceuticals use. Polypharmacotherapy is a common practice, especially in patients with multiple medical conditions, which in turn may predispose individuals to relevant DDIs. Therefore, studying DDIs is paramount for improving patient safety.

Absorption can be interfered with, for example, by affecting the pH of the GI tract, which can modify the ionization ratio of another drug, as exemplified by the effect of proton pump inhibitors on rilpivirine pharmacokinetics (FDA 2011). A striking example of altered absorption kinetics is how activated charcoal binds to the majority of drugs and almost completely prevents them from entering the body (Rowland and Tozer 2011). Drug-induced changes in transporter protein function can modify all the aforementioned pharmacokinetic processes, depending on the location of the transporter (Shitara et al. 2006), which is discussed in more detail in section 5. Excluding transporter-mediated mechanisms, clinically relevant DDIs emerging from altered drug distribution are uncommon; however, the effects of certain chelating agents on the disposition of metals provide an example of this phenomenon (Andersen and Aaseth 2016). The majority of important DDIs are caused by changes in drug metabolism that result from enzyme inhibition or induction (Wilkinson 2005). Several mechanisms (e.g., competitive, uncompetitive, noncompetitive, quasi-irreversible, and reversible) of enzyme inhibition exist, but categorizing enzyme inhibition in a certain class can be challenging, because empirical observations on enzyme kinetics do not always fit in distinct theoretical models. Enzyme induction is predominantly based on increased gene expression via transcription factor activation, but also diminished enzyme degradation is considered to be an induction mechanism. Finally, drug elimination can be affected by changes in the physicochemical characteristics of urine, or the enterohepatic circulation, for example (Rowland and Tozer 2011).

When the DDI potential of a drug is evaluated, its abilities to act as a perpetrator or a victim drug must be characterized. If the capability of a drug to inhibit or induce the function of an enzyme or a transporter involved in drug disposition (i.e., to act as a perpetrator) is examined, a "probe" or "index" substrate is usually applied as the victim drug (Tornio et al. 2019). An ideal index substrate should possess dispositional qualities that are sensitive to alterations in the specific pharmacokinetic pathway under examination, while having an adequate safety profile to ensure the safety of studied subjects. Correspondingly, when the disposition mechanisms of a drug and its potential to act as a victim drug are characterized, a (pre)treatment with an index inhibitor or inducer is commonly utilized (FDA 2017a). Analogously to ideal index substrates, optimal index inhibitors or inducers should cause a strong and specific change in the function of a transporter or an enzyme of interest, and they should have a benign safety profile (EMA 2012). Consequently, the same pharmacokinetic qualities that make an ideal index perpetrator or a victim drug can cause problems in patient care and may even lead to contraindications in clinical practice, where dosage regimens often markedly differ from DDI studies.

1.1.1. Mechanism-based inhibition

Mechanism-based inhibition (MBI) is broadly defined as a process where the catalytic machinery of the enzyme converts an enzyme substrate to a reactive species that inactivates the enzyme before leaving the active site (Silverman 1995). Commonly, MBI is irreversible due to the covalent nature of the bond formed between the enzyme active site and the reactive intermediate, but slowly reversible reactions and non-covalent pairings have also been reported (Silverman and Hoffman 1984). More specifically, seven original criteria have been established to characterize MBI of enzymes: (i) the inhibition has to be time-dependent, that is, the lost enzyme activity should increase over time and ideally exhibit pseudo first-order kinetics; (ii) the rate of inactivation should saturate with respect to inhibitor concentration; (iii) the presence of another substrate slows down the inhibition reaction; (iv) not including rare exceptions, enzyme activity does not return upon dialysis or gel filtration; (v) the inactivation should result in a 1:1 stoichiometry of labeling of the enzyme by the inactivator; (vi) catalytic transformation by the targeted enzyme is an obligatory requirement for the creation of the reactive intermediate; and (vii) enzyme inactivation takes place before release of the reactive species from the active site (Silverman and Hoffman 1984, Silverman 1995). The first five criteria could be used to define any inhibition process by covalent or tightly bound slow-binding non-covalent inhibitor, whereas the sixth criterion is unique to MBI. In addition, MBI needs to involve nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor, and the presence of added reactive species scavengers should not interfere with the inhibition reaction (Fontana et al. 2005). It has been suggested that mechanism-based inhibitors exhibit more pronounced enzyme specificity than competitive inhibitors due to the multiple requirements imposed on them (Hollenberg et al. 2008). Moreover, immunological adverse reactions and hepatotoxicity have been associated with drugs that cause MBI (Kalgutkar et al. 2007, Masubuchi and Horie 2007).

Concerning human physiology, MBI causes practically permanent enzyme inactivation, and therefore newly synthesized protein is required to restore enzymatic function. Therefore, the time needed to reach baseline enzyme activity depends on the degradation half-life of the enzyme and on the degree of net enzyme inactivation achieved by the inhibitor, which is dependent on the inhibitor exposure. The MBI-related slow recovery rate of enzyme activity has important clinical implications, because the DDI risk persists longer than in the case of competitive inhibition that is only dependent on the inhibitor concentrations on the enzyme site at any given time. In addition to causing longer lasting risk of harmful DDIs, this phenomenon can be beneficial when a mechanism-based inhibitor is applied as a pharmacokinetic enhancer to achieve more infrequent dosing and consistent exposure of other drugs.

2. Pharmacogenomics

Many major drug classes exhibit vast variability in drug response and toxicity between individuals. Instead of receiving the desired drug response, a marked proportion of patients shows only a partial response or experiences adverse drug reactions. Plasma concentrations of a drug can vary substantially between two individuals of the same weight using the same dose, as exemplified by the pharmacokinetics of alfentanil, desloratadine, midazolam, nortriptyline, and paritaprevir (Eichelbaum et al. 2006, Menon et al. 2017, Rowland and Tozer 2011). On average, genetics is estimated to account for 15–30% of variability in drug metabolism and response, but for certain drugs, genetic factors can explain up to 95% of the interindividual variation in disposition and response (Eichelbaum et al. 2006, Evans and McLeod 2003). Even though knowledge regarding the impact of genetic variance on drug

response has expanded enormously in recent years, a large fraction of inherited pharmacokinetic and pharmacodynamic traits remain uncharacterized.

Pharmacogenomics studies the differences in drug response owing to genetic variation and gene expression, while its subset pharmacogenetics examines the relation of deoxyribonucleic acid (DNA) sequence variations and drug response (EMA 2007). Several genetic variants have already been determined to pronouncedly affect drug response, but the significance of interindividual differences in gene expression is still understudied. "Genetic polymorphism" is defined as the occurrence in the same population of two or more alleles at one locus, each with appreciable frequency (traditionally at least 1%) (Cavalli-Sforza and Bodmer 1971). Despite the fact that the terms "variation" and "polymorphism" are often used interchangeably, the former is currently preferred due to the lack of allele frequency limitations that concern the latter, especially when single nucleotide variations (SNVs) are discussed. Variation in DNA sequence encoding drug targets, transporters, metabolizing enzymes, can significantly contribute to variability in drug response between individuals (Evans and Relling 1999). This applies not only to variation in the DNA sequence of normal tissues but also to changes seen in tumor DNA, which can predict responses to antineoplastic treatments. Aside from human genomic traits, the genomics of pathogens can also affect drug responses in the form of antimicrobial resistance, for example (Hughes and Andersson 2015).

The link between heritable traits and altered drug response was first elucidated in the 1950s when primaquine-induced hemolysis was found to be more common in populations with a high prevalence of glucose-6-phosphate deficiency (Beutler 1969), and when prolonged suxamethonium-induced muscle relaxation was associated with inherited low plasma pseudocholinesterase (butyrylcholinesterase) activity (Lehmann and Ryan 1956). Concurrently, isoniazid metabolism and adverse effects were found to have hereditary characteristics (Evans et al. 1960). In the early 2000s, biotechnological advancements and the sequencing of the human genome caused an exponential increase in pharmacogenomics research (Lander et al. 2001). Contemporarily, a wide array of specific genetic variations have been associated with differences in drug response, e.g., *CYP2D6* genotype and response to various antidepressants, or analgesia and toxicity produced by codeine and tramadol (Eichelbaum et al. 2006, Ingelman-Sundberg et al. 2007); however, routine applications of this knowledge remain scarce. To be accepted as standard of care, clinical implementations of pharmacogenomics must meet the criteria of analytical validity, and clinical validity and utility (Relling and Evans 2015). These matters have been addressed by clinical guidelines on how to implement pharmacogenomics knowledge in order to improve treatment outcomes (Relling and Klein 2011), and pharmacogenomics testing is likely to be widespread in clinical practice in the not-so-distant future when the amount, availability, validity, and costs of the tests reach sufficient levels.

DNA sequence variation can vary from SNVs to gene copy-number variations and chromosomal reorganization (Feuk et al. 2006), and epigenetic modifications include DNA methylation and hydroxymethylation, noncoding RNA-mediated gene regulation, histone modification, and changes in nucleosome positioning (Huang et al. 2014). Genomic variation can influence gene expression and alter phenotype in multiple ways; however, a large portion of genomic variation related to drug response is considered to have almost neutral phenotype effects (Sadee and Dai 2005). DNA sequence variation may have phenotype effects by altering gene expression regulation, mRNA stability and processing (including splicing), and protein structure and function, for example. Furthermore, genetic variation can cause augmented levels of a translated protein, which in turn may lead to accelerated drug metabolism, as is the case with the *CYP2C19*17* allele and various CYP2C19 substrates (Sim et al. 2006). Nucleotide sequence variations may also create a functionally deficient enzyme, or it can decrease the level of metabolic enzyme expression that may raise both the drug concentrations and risk of toxicity. For example, certain *DPYD* variants are associated with increased exposure of fluoropyrimidines and probability of their toxic effects (Amstutz et al. 2018, Henricks et al. 2018, Meulendijks et al. 2015).

Moreover, interindividual differences in drug response can take place via genetic variation that results in alternative forms of a pharmacodynamic target, as demonstrated by certain *VKORC1* variants and warfarin resistance (Rost et al. 2004), and *CFTR* genotype and ivacaftor response (Accurso et al. 2010). In addition, variation in DNA sequence can have profound effects on drug response that are unrelated to the pharmacodynamic and pharmacokinetic characteristics of a drug. For example, many immunologic adverse drug reactions are strongly associated with certain *HLA* alleles (Redwood et al. 2018).

3. Phase I metabolism

Most drugs are relatively lipophilic, which facilitates their penetration across biological membranes but also limits their elimination. Thus, many drugs require biotransformation to a more hydrophilic form to be efficiently excreted from the body. Furthermore, some drugs, such as clopidogrel and prostaglandin (PG) analogs used to treat glaucoma (Bean and Camras 2008, Farid et al. 2010), are prodrugs, that is, without inherent pharmacological efficacy, and they need to be bioactivated often via same enzymatic pathways that facilitate the elimination of xenobiotics. Commonly, the first step in drug metabolism creates a functional group in the molecule, for example via oxidation, reduction, hydrolyzation, or hydration reaction by one or several enzymes, such as cytochrome P450 (CYP), esterases, and amine oxidases. These processes are traditionally categorized under the term “phase I metabolism”. The resulting metabolite usually acts as an intermediate for the next metabolic stage. However, dividing drug metabolism into distinct stages is imprecise, because it falsely implies a universal sequentiality in metabolic events (Josephy et al. 2005).

3.1. Cytochrome P450 enzymes

CYPs were discovered in the 1950s when cytochrome pigments were isolated from rabbit liver microsomes, and their light absorption peak wavelength was measured at 450 nm, when bound to carbon monoxide (Klingenberg 1958). Accordingly, a few years later they were labeled as “cytochrome P450” hemoproteins (Omura and Sato 1962). The CYP superfamily deserves special attention, because it accounts for approximately 75% of drug clearance (Guengerich 2008, Wrighton and Stevens 1992). Furthermore, CYPs play a key role in the mitochondrial electron transfer chain (Hannemann et al. 2007), and they participate in the metabolism of several endogenous substances, such as steroid hormones and eicosanoids (Rendic and Di Carlo 1997). CYPs are highly abundant and are found in most eukaryotic and prokaryotic cells, and even in some viruses (Lamb et al. 2009). Those CYPs that participate in drug metabolism are transmembrane proteins located in the smooth endoplasmic reticulum with their active site on the cytosolic side of the membrane (Cribb et al. 2005). While the great majority of CYPs catalyze oxidative reactions (Guengerich 2001), some also catalyze uncommon reactions, such as reduction, complex dimerization, and ring formation (Isin and Guengerich 2007). As CYPs predominantly add a functional group on their substrate molecules, the CYP-mediated reactions are suspected to more often produce a species that can act as a mechanism-based enzyme inhibitor, when compared to reactions by most other enzymes. Quantitatively, CYPs involved in drug metabolism are mainly expressed in the liver but can also be found in large variety of extrahepatic tissues, especially the small intestine (Ding and Kaminsky 2003). The protein structure of many CYPs is highly adaptive, and their active site cavities

are able to change their size and conformation depending on the ligand they are accommodating (Ekroos and Sjogren 2006, Zhao et al. 2006).

In humans, 57 functional genes are identified to encode individual CYP enzymes, which are subsumed under 18 families and 42 subfamilies based on their amino acid sequence (Nebert and Russell 2002, Nelson et al. 2004). All CYPs have the same heme complex as the catalytic center (Meunier et al. 2004), but the amino acid sequence is ≥ 40% similar between CYP family members and ≥ 55% similar between members of subfamilies (Nelson et al. 2004). In addition to genetic differences, multiple other factors including age, sex, body weight, and disease, explain the marked interindividual variability in enzyme activity exhibited by many CYPs (Tracy et al. 2016). Due to the increased risk of diminished efficacy or adverse reactions, dosing sensitive substrates of polymorphic CYPs is problematic, especially if the substrate has a narrow therapeutic index. Therefore, *CYP* genotyping is recommended when prescribing numerous drugs (Tornio and Backman 2018). In particular, CYP2C19, CYP2C9, and CYP2D6 are highly polymorphic, whereas variant alleles leading to diminished enzyme activity of clinical relevance are rare in *CYP1A2* and *CYP2E1* (Ingelman-Sundberg et al. 2007, Zanger and Schwab 2013, Zhou et al. 2017). It should be noted, however, that although *CYP* pharmacogenomics has been investigated intensively, a marked share of inherited variance in CYP activity is still inexplicable. For example, CYP3A4, the most important CYP enzyme in xenobiotic metabolism, demonstrates a vast variance in activity between individuals. *CYP3A4* harbors the **22* allele that results in decreased metabolism of several CYP3A4 substrates, but it accounts for only a small portion of heritable variation in CYP3A4 activity, which cannot be explained by other known *CYP3A4* variants, either (Zanger and Schwab 2013). Furthermore, functional consequences of many identified *CYP* SNVs or haplotypes are yet undetermined.

Most of the known clinically relevant DDIs are CYP-mediated, and therefore drugs under development are routinely screened for potential to act as CYP substrates, inhibitors, or inducers (FDA 2017a). The United States Food and Drug Administration (FDA) states that strong, moderate, and weak CYP inhibitors are compounds that increase the AUC of a sensitive probe substrate by ≥ 5-fold, ≥ 2- to < 5-fold, and ≥ 1.25- to < 2-fold, respectively (FDA 2017a). This classification is somewhat problematic as some enzymes (e.g., CYP2B6) lack sensitive index or probe substrates, or because the probe substrate may have additional disposition mechanisms.

3.1.1. Cytochrome P450 2C8

CYP2C8 contributes to 6–7% of total hepatic CYP content (Rostami-Hodjegan and Tucker 2007, Totah and Rettie 2005), and it is also expressed in the small intestine, kidneys, and adrenal glands, for example (Klose et al. 1999, Lapple et al. 2003). CYP2C8 is the most abundant hepatic CYP2C isoenzyme during prenatal development (Johansson et al. 2014), and its expression reaches adult levels in early childhood (Naraharisetti et al. 2010). Although the amino acid sequence of CYP2C8 is about 74% identical to those of CYP2C19 and CYP2C9, these CYP2C isoenzymes have largely distinct substrate specificities (Chen and Goldstein 2009, Johnson and Stout 2005, Ridderström et al. 2001). The expression of CYP2C8 is regulated by multiple transcription factors including constitutive androstane receptor (CAR), pregnane X receptor (PXR), glucocorticoid receptor (GR), vitamin D receptor (VDR), and hepatocyte nuclear factor (HNF) 4α (Ferguson et al. 2005). Concerning DDIs, CAR and PXR are the most important routes of CYP2C8 induction by xenobiotics. Dimeric CYP2C8 has a molecular weight of 110 kDa (Schoch et al. 2004), and its degradation half-life is approximated to 22 hours (Backman et al. 2009). The active site cavity is trifurcated and large in volume, which explains its capability to accommodate structurally diverse molecules (Schoch et al. 2008).

CYP2C8 contributes to the metabolism of more than 100 clinically applied drugs (Backman et al. 2016). It has a major role in the biotransformation of a host of widely used drugs including amodiaquine, dasabuvir, enzalutamide, ibuprofen, montelukast, paclitaxel, pioglitazone, repaglinide, and selexipag (Backman et al. 2016, Bruderer et al. 2017). In addition, several drugs, e.g., desloratadine, diclofenac, and estradiol, have glucuronide metabolites that are substrates of CYP2C8 (Delaforge et al. 2005, Kazmi et al. 2015, Kumar et al. 2002). Contemporarily, repaglinide is the CYP2C8 index substrate for *in vivo* and *in vitro* studies most often recommended by the European and American regulatory authorities (EMA 2012, FDA 2017a, FDA 2017b). However, the hypoglycemia-inducing potential and additional disposition mechanisms (OATP1B1-mediated hepatic uptake and metabolism by CYP3A4) of repaglinide limit its practicality as a CYP2C8 probe substrate (Niemi et al. 2003, Niemi et al. 2005), and thus an urge for more specific and safe CYP2C8 index substrates exists. Besides repaglinide, cerivastatin and estradiol 17-β-glucuronide are also dual substrates of CYP2C8 and OATP1B1 (Kanai et al. 1996, Muck 1998).

Gemfibrozil and clopidogrel are the recommended index CYP2C8 inhibitors for *in vivo* DDI studies, and both have a glucuronide metabolite that acts as mechanism-based inhibitor of CYP2C8 (FDA 2017a, Ogilvie et al. 2006, Tornio et al. 2014). Multiple drugs, for example candesartan cilexetil, zafirlukast, and felodipine, markedly inhibit CYP2C8 *in vitro* (Walsky et al. 2005). However, they are unlikely to cause clinically significant CYP2C8-mediated DDIs because candesartan cilexetil is rapidly systemically hydrolyzed, while zafirlukast and felodipine are highly-bound to plasma proteins. These discordances highlight the need for clinical studies when the DDI potential of different pharmacological compounds is being evaluated.

In concomitant use, rifampicin, a CYP2C8 inducer mainly via PXR activation (Chen and Raymond 2006), has decreased the exposure of several CYP2C8 substrates (Jaakkola et al. 2006b, Niemi et al. 2000, Niemi et al. 2004). DDI studies between other CYP2C8 inducers and substrates are scarce, but phenobarbital, a CAR activator, has caused a pronounced induction of CYP2C8 *in vitro* (Madan et al. 2003).

The *CYP2C8 *2, *3,* and *4* alleles account for most of the nonsynonymous variance in the *CYP2C8* gene (Backman et al. 2016). Depending on the substrate, a *CYP2C8* polymorphism can have different or even opposite effects in drug clearance. For example, the *CYP2C8*3* allele has been associated with decreased exposure of rosiglitazone and repaglinide (Aquilante et al. 2008, Niemi et al. 2005), whereas the same allele has been associated with lower ibuprofen and paclitaxel clearance (Garcia-Martin et al. 2004) and increased risk for paclitaxel-induced neurotoxicity in ovarian and breast cancer patients (Green et al. 2011, Hertz et al. 2013). The frequency of the *CYP2C8* alleles associated with decreased enzyme activity (expressed as decreased paclitaxel clearance) varies between major populations ranging from < 1% in East Asians to 17.2% and 19.2% in Africans and Europeans, respectively (Zhou et al. 2017). Currently, no consensus guidelines are established for implementing pharmacogenomic *CYP2C8* testing.

4. Phase II metabolism

In general, phase II metabolism refers to a metabolic process where a charged species such as glucuronic acid, sulfate, glycine, or glutathione, is conjugated to a drug or its metabolite producing a compound that is usually more easily eliminated from the body than the original species. Examples of enzymes involved in phase II metabolism include sulfotransferases, glutathione S-transferases, and methyltransferases. Many drugs require no preceding biotransformation by a phase I enzyme and can be directly subject to phase II metabolism, as exemplified by the metabolism of lorazepam, lamotrigine, and morphine (Guillemette et al. 2014). Furthermore, phase II metabolites can possess their own

pharmacological activity (Paul et al. 1989), cause toxicity (Spahn-Langguth and Benet 1992), or be further metabolized (Delaforge et al. 2005, Ingelman-Sundberg et al. 1975).

4.1. Uridine 5'-diphospho-glucuronosyltransferase enzymes

UGTs form a crucial enzyme superfamily in phase II metabolism (Ritter 2000). They catalyze covalent bonding of a functional group in a substrate molecule and a glucuronic acid residue from the cofactor uridine 5'-diphosphate (UDP) glucuronic acid yielding solely β-D-glucuronides (Rowland et al. 2013), excluding rare cases when they conjugate other sugar residues (Mackenzie et al. 2003, Senafi et al. 1994). UGTs govern the inactivation of nearly 35% of the drugs currently on the market (Guillemette et al. 2014), and a large variety of their substrates are related to their ability to catalyze the addition of a glucuronic acid residue to multiple functional sites, such as amine, hydroxyl, carboxyl, and sulfuryl groups (Radominska-Pandya et al. 1999). Contemporary classification divides the 19 functional UGTs into two families, UGT1 and UGT2, with the latter being further subdivided to UGT2A and UGT2B (Guillemette et al. 2014). UGTs are closely related two other UDP-glycosyltransferase families, UGT3 and UGT8, which consist of enzymes that catalyze the conjugation of glycosyl residues other than glucuronic acid (Mackenzie et al. 2005). The members of the UGT families share more than 50% amino acid sequence identity to each other, but less than 50% to members of other families, and subfamily members share > 60% sequence homology (Burchell et al. 1991). Mature UGTs are transmembrane proteins of about 505 amino acid residues that are found almost exclusively in the endoplasmic reticulum (Radominska-Pandya et al. 2005), and they exist mostly as oligomers (Meech and Mackenzie 1997a, Radominska-Pandya et al. 2005). The luminal domain consists of about 95% of the peptide chain, while the transmembrane and cytosolic domains include 17 and about 20 amino acid residues, respectively (Radominska-Pandya et al. 2005). The carboxyl terminal end of UGTs is conserved, which has led to the suggestion that this domain binds to the cofactor UDP-glucuronic acid common to all UGT isoforms (Meech and Mackenzie 1997b). The hypothesis has been corroborated by the determination of the UGT2B7 crystal structure (Miley et al. 2007, Radominska-Pandya et al. 2010); however, the crystal structures of other UGT isoenzymes remain unsolved (Fujiwara et al. 2016). UGTs are mainly expressed in the liver and some isoforms are found in the intestinal epithelium, but various other tissues express UGTs in smaller quantities (Stingl et al. 2014). The UDP-glucuronic acid concentrations partly determine the UGT activity in different tissues because they are below the K_m of UGTs in many cell types (Ritter 2000).

Aside from xenobiotics, UGTs also metabolize endogenous substances such as bilirubin, steroid hormones, and biliary acids (Guillemette 2003), and defects in UGT function can cause diseases, e.g., Crigler-Najjar syndrome (Wells et al. 2004). Furthermore, genetic variants that lead to altered UGT activity may modify susceptibility to diseases and the aggressiveness of certain cancers, independent of drug response (Guillemette et al. 2014). Although UGTs generally facilitate the elimination of endo- and xenobiotics by forming a conjugate that is usually less reactive than the original species, certain glucuronides possess biological activity. For example, morphine-6β-glucuronide is a more potent μ-opioid receptor antagonist than parent morphine (Paul et al. 1989), and 3-glucuronides of buprenorphine and its metabolite, norbuprenorphine, exhibit pharmacological activity distinct from the parent compound (Brown et al. 2011). In addition, some glucuronides have been observed to cause toxicity, or act as membrane transporter and enzyme inhibitors (Regan et al. 2010, Shitara et al. 2004, Tornio et al. 2014). The proximity of UGTs and CYPs in the endoplasmic reticulum enables the interplay of these enzymatic systems, and accordingly, if a glucuronide acts as a CYP inhibitor, its plasma concentrations do not always reflect its concentrations at the enzyme site.

In the liver, HNF1 is one of the key regulators of UGT expression (Mackenzie et al. 2005). Furthermore, many UGTs and CYPs involved in xenobiotic metabolism share common induction mechanisms, for example activation of aryl hydrocarbon receptor (AhR), CAR, and PXR (Hu et al. 2014, Lin 2006, Yang et al. 2017), which provides another demonstration of the functional interconnectedness of the two enzyme superfamilies. The amount of known and relevant drug interactions mediated by UGT-involved mechanisms is substantially smaller than that by CYP-related mechanisms (Kiang et al. 2005, Lin and Lu 1998). Examples of clinically important UGT-mediated DDIs include alterations in lamotrigine glucuronidation by the UGT inhibitor valproate or UGT-inducing antiepileptics that require dosage adjustments of lamotrigine, when used concurrently (Perucca 2006, Yuen et al. 1992). In general, when contrasted with common *CYP* variants, important common *UGT* variants are rare (Stingl et al. 2014). Among the most clinically relevant *UGT* variants are the *UGT1A1* alleles leading to decreased enzyme activity that are associated with the increased risk for irinotecan-induced toxicity in colorectal cancer treatment (Liu et al. 2014), and hyperbilirubinemia by antiretroviral protease inhibitor atazanavir (Gammal et al. 2016).

5. Membrane transporters

Facilitated or active transport mechanisms can complement passive diffusion in assisting the movement of endobiotics and drugs through biological membranes, sometimes even against concentration gradients. Membrane transporters can participate in protecting the whole organism, or specific vulnerable tissues or pharmacokinetic compartments, e.g., the central nervous system and fetus when expressed in blood-brain barrier and placenta, respectively. In the human genome, approximately 2,000 genes encode transporter proteins, which reflects the importance of transporter function in cellular and systemic homeostasis (Brunton et al. 2006).

Membrane transporters can be categorized in several ways, for example to influx and efflux transporters, or based on their energy source or transmembrane structure, e.g., adenosine triphosphate binding cassette (ABC) or solute carrier (SLC) transporters, respectively (He et al. 2009, Hediger et al. 2013, Jones and George 2004). Influx transporters facilitate cellular drug uptake, and they include organic anion transporters (OATs), organic cation transporters (OCTs), and OATPs. In contrast, efflux transporters are involved in moving different compounds out of cells, and moreover, their increased expression can contribute to resistance against antineoplastic agents, and against antimicrobials when localized in microbial membranes. Clinically important efflux transporters include P-glycoprotein or multidrug resistance protein 1 (P-gp or MDR1), the multi-drug resistance-associated proteins (MRPs), multidrug and toxin extrusion proteins (MATEs), and breast cancer resistance protein (BCRP). In the liver, most efflux transporters are expressed in the canalicular membranes, but certain MRP transporters are located in the sinusoidal membranes where they are thought to export compounds to the bloodstream for urinary elimination (Gu and Manautou 2010).

One cell can express multiple transporter proteins, which in turn can have common substrates and inhibitors (Giacomini et al. 2010). This complicates the evaluation of individual transporter participation in pharmacokinetics based on *in vivo* data. Several transporter-related drug interactions of high clinical significance exist and thus transporter contribution to drug disposition is contemporarily examined *in vitro* and *in vivo* during drug development (EMA 2012, FDA 2017a, FDA 2017b). Regarding relevant pharmacokinetic interactions, the most important transporters are located in the epithelium of the small intestine, liver, and kidneys (Gessner et al. 2019). Moreover, genetic polymorphisms may influence transporter activity and drug exposure, thereby altering the risk of adverse drug reactions and

the magnitude of DDIs (Zolk and Fromm 2011). Membrane transporters can also act as drug targets, of which the inhibition of the serotonin transporter by antidepressants provide one the most prominent examples (Coleman et al. 2016).

5.1. Organic anion transporting polypeptide 1B1

Organic anion transporting polypeptide (OATP) 1B1 is a transporter protein that is expressed on the basolateral membrane of hepatocytes, where it facilitates the uptake of several compounds from portal venous circulation (König et al. 2000). OATP1B1 consists of 691 amino acids with a molecular weight of ~84 kDa (König et al. 2000), and the amino acid sequence of OATP1B1 is ~80% identical with that of OATP1B3, the other member of the OATP1B subfamily (Hagenbuch and Meier 2003). Typical OATP1B1 substrates are anionic, amphipathic, and highly bound in albumin, with a molecular weight of > 350 Da (Hagenbuch and Meier 2004). OATP1B1 contributes to hepatic uptake of several endogenous substances, such as bile salts, bilirubin, eicosanoids, and steroid conjugates (Hagenbuch and Meier 2003, Xiang et al. 2009). In addition, it participates in the disposition of multiple drugs, such as repaglinide, anticancer agent irinotecan, benzylpenicillin, methotrexate, bosentan, and many HMG-CoA reductase inhibitors (statins) (Niemi et al. 2011).

The *SLCO1B1* gene, which has over 45 identified nonsynonymous variants, encodes OATP1B1 (Lee and Ho 2017), and its transcription is regulated by several transcription factors including HNF1, HNF3, FXR, and PXR (Jung et al. 2001, Niemi et al. 2011). The coordinate regulation of CYPs, UGTs, and several transporters including OATP1B1 (Congiu et al. 2009), reflects the importance of the interplay between these physiological components in protecting the organism against chemical stressors. Rotor Syndrome, an autosomal recessive disorder, is associated with certain rare coexisting *SCLO1B1* and *SLCO1B3* variants that lead to conjugated hyperbilirubinemia and coproporphynuria (van de Steeg et al. 2012). The c.521T>C (rs4149056) SNV in exon 5 that produces p.Val174Ala substitution, leads to decreased transport activity and membrane expression of OATP1B1 (Tirona et al. 2001). The frequency of this SNV ranges from 1.9% in sub-Saharan populations to 24% in Native American populations (Pasanen et al. 2008), and it has substantial implications involving numerous drugs (Oshiro et al. 2010).

OATP1B1 inhibition is considered to be an important mechanism that contributes to many clinically relevant DDIs, involving various OATP1B1 substrates, such as multiple statins, repaglinide, and bosentan (Shitara 2011). According to current knowledge, no clinically relevant DDIs are caused solely by OATP1B1 induction. In primary human hepatocytes, the PXR agonist rifampicin induced SLCO1B1 mRNA levels 2.4-fold (Jigorel et al. 2006). However, rifampicin also inhibits OATP1B1, as demonstrated by its augmenting impact on the exposure of relatively sensitive and specific OATP1B1 substrate pitavastatin, when rifampicin is administered in a single dose (Prueksaritanont et al. 2014). Many OATP1B1 inhibitors, such as cyclosporine A, gemfibrozil, and ritonavir, have inhibitory effects on metabolic enzymes and/or other transporters. Moreover, the disposition of most OATP1B1 substrates depends on additional pharmacokinetic mechanisms such as CYP-mediated metabolism (Niemi et al. 2011). These factors complicate the evaluation of OATP1B1 contribution in pharmacokinetics based on DDI data, and therefore, pharmacogenetic studies provide the most reliable means to examine it, especially if the studied drug is a sensitive OATP1B1 substrate.

6. Clopidogrel

Clopidogrel irreversibly antagonizes ADP receptor subtype $P2Y_{12}$ and subsequently inhibits the ADP-induced activation of glycoprotein IIb–IIIa integrin complex and its binding to fibrinogen, thus preventing platelet aggregation and thrombus formation (Gachet et al. 1990, Hollopeter et al. 2001, Schrör 1998). Clopidogrel belongs to the same thienopyridine class as ticlopidine and prasugrel, whereas the reversible $P2Y_{12}$ inhibitor ticagrelor is a cyclopentyl-triazolopyrimidine derivative (Angiolillo et al. 2017). The molecular weight of clopidogrel base is 321.8 g/mol (FDA 2010).

Due to the good efficacy and tolerable safety profile, clopidogrel is widely used in acute care and secondary prevention of strokes and myocardial infarction, and in peripheral artery disease (EMA 2008, FDA 2010). In one study of 19,185 patients, clopidogrel demonstrated a marginal benefit in reducing the risk of a composite outcome cluster of ischemic stroke, myocardial infarction, or vascular death, when compared to aspirin (CAPRIE Steering Committee 1996). This finding has not been replicated, but the clopidogrel-aspirin combination has exhibited additional efficacy contrasted with aspirin monotherapy (FDA 2010). Owing to its pharmacodynamic action, bleeding is the most prominent adverse event caused by clopidogrel. Moreover, rash, nausea, constipation, and pruritus have been associated with clopidogrel use.

6.1. Pharmacokinetics

At least 50% of orally administered clopidogrel is absorbed, based on urinary elimination of clopidogrel metabolites (FDA 2010). The parent clopidogrel is inactive, and two distinct routes dominate its extensive metabolism. The predominant pathway is the CES1-mediated hydrolysis to inactive carboxylic acid metabolite, which is subsequently glucuronidated to acyl-β-D-glucuronide by several UGT2B isoenzymes, namely UGT2B7, UGT2B17, and UGT2B4 (about 85–90% of the absorbed dose) (Ji et al. 2018, Kahma et al. 2018, Tang et al. 2006, Tarkiainen et al. 2015). CES1 is mainly expressed in the endoplasmic reticulum of hepatocytes (Satoh and Hosokawa 2006). The *CES1* c.428G>A (p.Gly143Glu, rs71647871), a single nucleotide variation leading to decreased enzyme function, impairs clopidogrel hydrolysis and increases its antiplatelet efficacy (Tarkiainen et al. 2015). The minor metabolism route is the two-step bioactivation by CYP enzymes, which involves less than 10% of the ingested dose (**Figure 1**) (Farid et al. 2010). Based on *in vitro* data, the intermediate in clopidogrel bioactivation, 2-oxo-clopidogrel, is formed by CYP1A2, CYP2C19, and CYP2B6, whereas the formation of the active *cis*-5-thiol metabolite is mediated by CYP2B6, CYP2C19, and CYP3A4 (Kazui et al. 2010). In 2011, Bouman and colleagues suggested a significant role for paraoxonase 1 in clopidogrel bioactivation (Bouman et al. 2011), but other studies, including a meta-analysis that combined results from 13 reports, have failed to support this hypothesis (Mega et al. 2016). Contemporarily, CYP2C19 and CYP3A4 are considered to be the essential enzymes in clopidogrel bioactivation *in vivo*.

Clopidogrel exhibits nonlinear metabolite kinetics: raising the dose from 75 mg to 300 mg results in 2.7-fold increase in the AUC of clopidogrel active metabolite (Bristol-Myers Squibb/ Sanofi Pharmaceuticals 2018). Clopidogrel bioactivation is a very rapid process and both the 2-oxo-intermediate and the active metabolite are extremely unstable, which has posed analytical challenges in determining their exposure. In 2010, a publication presented a derivatization method for stabilizing the active *cis*-5-thiol metabolite (Delavenne et al. 2010), which facilitated the reliable quantification of active metabolite exposure, whereas the determination of 2-oxo-clopidogrel has been unsuccessful *in vivo*. The platelet aggregation

inhibition by clopidogrel can be measured with several methods, but the most commonly applied system (VerifyNow $P2Y_{12}$; Accumetrics, San Diego, USA) is based on a turbidimetric optical detection (Jeong et al. 2012, Lordkipanidze et al. 2008). Furthermore, the antiplatelet effect of clopidogrel measured with VerifyNow $P2Y_{12}$ system correlates well with its therapeutic efficacy (Price et al. 2008).

Carriers of loss-of-function *CYP2C19* alleles have exhibited an impaired clopidogrel antiplatelet response that has led the manufacturer to alert healthcare providers about diminished antithrombotic effect in poor CYP2C19 metabolizers (Bristol-Myers Squibb/ Sanofi Pharmaceuticals 2018, Simon et al. 2009). The association between *CYP2C19* loss-of-function alleles and diminished clinical efficacy of clopidogrel has been reasonably well-documented, but the benefits of genotyping-based antiplatelet therapy has been questioned by a systematic review of 11 overlapping meta-analyses (Osnabrugge et al. 2015). Although most included meta-analyses reported significant associations between diminished clinical efficacy and *CYP2C19* loss-of-function alleles, between-study heterogeneity and publication bias were handled differently the across meta-analyses. Therefore, the meta-analyses drew partly discordant conclusions concerning the advantages of antiplatelet therapy based on *CYP2C19* genotype (Osnabrugge et al. 2015). In contrast to carriers of *CYP2C19* loss-of-function alleles, those of the *CYP3A4*22* or *CYP3A4*5* alleles have not demonstrated diminished clopidogrel bioactivation (Holmberg et al. 2019).

6.2. Drug-drug interactions

Consistent with the impact of *CYP2C19* genotype on clopidogrel response, the CYP2C19 inhibitor omeprazole has decreased clopidogrel bioactivation in humans (Gilard et al. 2008). Furthermore, the CYP1A2, CYP2C19, and CYP2D6 inhibitor fluvoxamine has impaired clopidogrel's platelet aggregation inhibition, with the most relevant mechanism being interpreted as CYP2C19 inhibition (Hirsh-Rokach et al. 2015). In addition, grapefruit juice, which contains furanocoumarins that act as mechanism-based inhibitors of intestinal CYP3A4 (Edwards et al. 1996, Fukuda et al. 1997, Schmiedlin-Ren et al. 1997), has markedly decreased the exposure and platelet inhibition of clopidogrel active metabolite (Holmberg et al. 2014). The CYP3A4 inhibitor ketoconazole has attenuated the antiplatelet effect of clopidogrel active metabolite and decreased its AUC and C_{max} to 71–78% and to 39–52%, respectively (Farid et al. 2007). Decreased clopidogrel response has been observed in individual HIV patients on ritonavir-containing antiretroviral therapy (Marsousi et al. 2018, Metzger and Momary 2014), but sufficient systematic pharmacokinetic data have been lacking.

In addition to the aforementioned CYP2C8 MBI, clopidogrel causes pharmacokinetic DDIs also via other mechanisms. *In vitro* studies have reported that parent clopidogrel inhibits CYP2C19 and acts as a mechanism-based inhibitor of CYP2B6 (Nishiya et al. 2009, Richter et al. 2004). In humans, clopidogrel has inhibited the CYP2B6-mediated hydroxylation of bupropion (indicated by a 68% decrease in hydroxybupropion-bupropion AUC ratio), which corroborates the clinical relevance of its CYP2B6 inhibition (Turpeinen et al. 2005). As clopidogrel decreases the activity of CYP2B6 that is involved in its own bioactivation, its pharmacokinetics are possibly less prone to changes by CYP2B6-mediated DDIs or *CYP2B6* loss-of-function genotypes. Furthermore, clopidogrel has decreased the 5-hydroxyomeprazole-omeprazole AUC ratio (that reflects the CYP2C19-mediated metabolism of omeprazole) by 29%, which implies that the CYP2C19 inhibitory properties of clopidogrel seem to be of small clinical relevance (Turpeinen et al. 2005).

7. Other perpetrator drugs investigated with clopidogrel

7.1. Prasugrel

Similar to clopidogrel, prasugrel is an irreversible ADP-receptor subtype $P2Y_{12}$ antagonist of thienopyridine class, and it is indicated for the reduction of thrombotic cardiovascular events in patients that have experienced an acute coronary event (FDA 2013). Compared to clopidogrel, prasugrel has demonstrated superior efficacy in preventing cardiovascular complications, but its impact on overall mortality has been similar with a higher rate of bleeding events (Wiviott et al. 2007). After oral administration, CES2 completely hydrolyzes prasugrel to an inactive intermediate species, R-95913, which then undergoes biotransformation to the active metabolite, R-138727, by CYP3A4, CYP2B6, CYP2C19, and CYP2C9 (Farid et al. 2007, Rehmel et al. 2006). However, the CYP inducer rifampicin and the CYP3A4 inhibitors ritonavir, ketoconazole, and grapefruit juice furanocoumarins have only a negligible to modest impact on the exposure and/or antiplatelet effect of R-138727 (Ancrenaz et al. 2013, Farid et al. 2007, Farid et al. 2009, Holmberg et al. 2015, Marsousi et al. 2018). Moreover, studies have not found an association between *CYP2C19* or *CYP2B6* loss-of-function alleles or the *CYP3A4*22* allele, and diminished antiplatelet efficacy of prasugrel (Holmberg et al. 2019, Mega et al. 2009). In the premarketing studies, the potential of prasugrel inhibit CYP2C8 was not examined *in vivo*, as opposed to most other CYP-mediated DDI mechanisms (EMA 2009). As the predictable antithrombotic effect and low DDI liability of prasugrel has made it a possible alternative to clopidogrel in certain patient populations that are often at risk for polypharmacy, the CYP2C8 inhibition potential of prasugrel requires clarification.

7.2. Ritonavir

Ritonavir was one of the first HIV protease inhibitors brought to market, but its antiviral doses caused a high rate of dose-dependent adverse reactions, such as hypertriglyceridemia (AbbVie 2018). The pharmacokinetic profile of ritonavir is highly complex as it alters the activity of several enzymes and transporters. Ritonavir is metabolized by CYP3A4, and to a lesser extent by CYP2D6 (Hsu et al. 1998). It acts as a potent mechanism-based inhibitor of CYP3A while weakly inducing CYP3A in continuous administration, the net effect being inhibition (Koudriakova et al. 1998). Moreover, ritonavir causes up to 2-fold increase in the exposure of CYP2D6 substrates, but no dosage adjustment of the victim drug metabolized by CYP2D6 is usually required when ritonavir is applied in low doses (Aarnoutse et al. 2005, AbbVie 2018). Owing to its strong CYP3A4 inhibitory effect, contemporary antiviral combinations apply ritonavir as a pharmacokinetic enhancer that permits consistent effects and hence standardized dosing of other antiviral drugs (Rathbun and Rossi 2002). Furthermore, ritonavir alters the activity of the efflux transporter P-gp by causing a mixed inhibition-induction effect, the net result being inhibition (Kharasch et al. 2008, Kirby et al. 2012). Accordingly, ritonavir has increased the AUC of P-gp substrates digoxin and fexofenadin (Kharasch et al. 2008, Kirby et al. 2012), whereas it has demonstrated negligible to slightly diminishing effect on the exposure of dabigatran (which is the active metabolite of P-gp substrate dabigatran etexilate) (Kakadiya et al. 2018, Kumar et al. 2017). Furthermore, ritonavir has induced the metabolism of several substrates of CYP1A2, CYP2B6, CYP2C9, CYP2C19, and UGTs (Foisy et al. 2008). The most notable enzyme induction mechanism of ritonavir is PXR activation (Gupta et al. 2008), while a weak AhR activation has also been observed (Frotschl et al. 1998). As discussed in section 6.3., two individual reports have observed diminished clopidogrel response in HIV patients using

antiviral medications. Therefore, the possibility of ritonavir-clopidogrel interaction supports the pursuit of a prospective clinical DDI study.

7.3. Gemfibrozil

The fibric acid derivative gemfibrozil modifies plasma lipid concentrations primarily by activating the PPAR-α (Backes et al. 2007). Gemfibrozil decreases serum triglycerides and increases high-density lipoprotein levels while having a varying effect on low-density lipoproteins (FDA 2016), but it only has a mediocre impact on cardiovascular mortality (Backes et al. 2007). Gemfibrozil is subject to extensive oxidative metabolism and glucuronidation, and *in vitro* studies imply that its primary glucuronide metabolite, gemfibrozil 1-O-β-D-glucuronide, is formed mainly by UGT2B7 (Mano et al. 2007). The concomitant use of gemfibrozil has been associated with greatly increased risk for the CYP2C8 and OATP1B1 substrate cerivastatin-induced rhabdomyolysis (Farmer 2001, Jones and Davidson 2005), and gemfibrozil was found to increase cerivastatin exposure 5.6-fold (Backman et al. 2002). This DDI remained long unrecognized, as the actual perpetrator, gemfibrozil 1-O-β-D-glucuronide, was identified as mechanism-based inhibitor of CYP2C8 five years after the market withdrawal of cerivastatin (Ogilvie et al. 2006). In clinical doses, gemfibrozil causes more than 98% inhibition of CYP2C8, as demonstrated by its pronounced effect on the pharmacokinetics of the CYP2C8 index substrate repaglinide, for example (Table 10) (Backman et al. 2009, Honkalammi et al. 2012, Niemi et al. 2003). Therefore, regulatory authorities recommend it as a CYP2C8 index inhibitor for clinical DDI studies (EMA 2012, FDA 2017a). In addition, both the parent gemfibrozil and its glucuronide metabolite inhibit certain membrane transporters *in vitro*, most notably OATP1B1, but also OATP1B3, OATP2B1, OAT3, and sodium/taurocholate cotransporting polypeptide (NTCP) (Tornio et al. 2017). The OATP1B1 inhibition has been considered to be a clinically relevant DDI mechanism of gemfibrozil, which also limits the applicability of gemfibrozil as a CYP2C8 index inhibitor. Furthermore, gemfibrozil has inhibited UGT1A1 and UGT1A3 *in vitro* (Gan et al. 2010), but these results have not been confirmed *in vivo*. It is worth noting that the sales and use of gemfibrozil have declined due to its modest clinical efficacy, in addition to its hazardous transporter- and CYP2C8-mediated DDIs, especially with statins (Graham et al. 2004).

8. Studied victim drugs

8.1. Simvastatin

Simvastatin is a HMG-CoA reductase inhibitor that is applied widely in the primary and secondary prevention of cardiovascular diseases (Merck 2018). In general, simvastatin is well-tolerated, but its most conspicuous adverse effects include myopathies and hepatic transaminase elevations. While the therapeutic daily dose of simvastatin ranges from 10 to 40 mg, premarketing trials observed that it exhibits linear pharmacokinetics with doses as high as 120 mg. Simvastatin is administered in inactive lactone form, which is subject to reversible nonenzymatic and CES-mediated bioactivation to simvastatin acid (Vickers et al. 1990a, Vickers et al. 1990b). Furthermore, *in vitro* studies suggest that CYP2C8 has a minor contribution in simvastatin acid formation (Prueksaritanont et al. 2003), but the clinical relevance of CYP2C8 in simvastatin pharmacokinetics is unconfirmed. The metabolism of simvastatin acid and the formation of other metabolites from simvastatin lactone are mainly mediated by CYP3A4 *in vitro* (Prueksaritanont et al. 1997, Prueksaritanont et al. 2003). In clinical studies, the

CYP3A4 inhibitor itraconazole has increased the AUC of simvastatin lactone and acid more than 10-fold (Neuvonen et al. 1998), and grapefruit juice has augmented those 16- and 6.8-fold, respectively (Lilja et al. 1998). Furthermore, ritonavir augmented simvastatin acid exposure 32-fold, when given in combination with another CYP3A4-inhibiting protease inhibitor, saquinavir (Fichtenbaum et al. 2002). The concomitant administration of the CYP3A4 inductor rifampicin caused 87% and 93% decreases in the AUCs of simvastatin lactone and acid, respectively (Kyrklund et al. 2000).

Unlike parent simvastatin, simvastatin acid is highly susceptible to alterations in OATP1B1 function and accordingly, it is one of the most sensitive of known OATP1B1 substrates (Niemi et al. 2011). A pharmacogenetic study in 32 healthy volunteers reported that the average exposure of simvastatin acid was 220% higher in individuals homozygous for the *SLCO1B1* c.521T>C variant, when compared to those carrying the c.521TT genotype (Pasanen et al. 2006). In a genome-wide association study (GWAS) with patients using simvastatin 80 mg q.d., individuals exhibiting the c.521CC genotype were observed to have an increased risk for simvastatin-induced myopathy with an odds ratio (OR) of 16.9, when compared with c.521TT carriers (Link et al. 2008). Therefore, high-dose simvastatin treatment is not recommended for individuals with c.521CC genotype (Ramsey et al. 2014). Furthermore, cyclosporine, an inhibitor of CYP3A4 and several membrane transporters including OATP1B1, has increased the simvastatin acid exposure 6–8-fold (Neuvonen et al. 2006). In addition, gemfibrozil caused a 2.9-fold increase in simvastatin acid exposure, which demonstrated the clinical relevance of OATP1B1-mediated hepatic uptake in simvastatin acid disposition as gemfibrozil does not inhibit CYP3A4 (Backman et al. 2000). However, the CYP2C8 inhibitory role of gemfibrozil could have been contributing to this DDI to a minor extent. For these reasons, simvastatin is commonly applied as an index substrate for investigating whether a perpetrator compound is a clinically relevant inhibitor of CYP3A and/or OATP1B1.

8.2. Pioglitazone

Thiazolidinedione pioglitazone is a glucose-lowering drug that mediates its effect by activating the PPAR-γ and thereby improves insulin sensitivity and inhibits gluconeogenesis (Waugh et al. 2006). When applied in secondary prevention, pioglitazone lowers the risk of recurrent major cardiovascular events; however, it has no beneficial effect on overall mortality (de Jong et al. 2017). Dose-related fluid retention is a common adverse effect of pioglitazone, which can lead to exacerbation of congestive heart failure. Pioglitazone promotes lipid storage and increases body weight, while redistributing body fat from visceral to subcutaneous deposits. It has an oral availability of approximately 80% and presents linear pharmacokinetics in the 2 to 60 mg dose range (Eckland and Danhof 2000). Pioglitazone undergoes extensive biotransformation to active and inactive metabolites by CYP2C8 and to a minor extent by CYP3A4 *in vitro* and *in vivo* (**Figure 2**) (Eckland and Danhof 2000, Jaakkola et al. 2005, Jaakkola et al. 2006a). The main metabolites, primary hydroxypioglitazone (M-IV), and its secondary metabolite, ketopioglitazone (M-III), are active and contribute to the therapeutic effect (Eckland and Danhof 2000). Moreover, CYP2C8 has been indicated to have a dominant role in the oxidation of M-IV to M-III (Jaakkola et al. 2005). *SLCO1B1* c.521T>C genotype has not affected pioglitazone pharmacokinetics, which implies a small to nonexistent role for OATP1B1 in its disposition (Kalliokoski et al. 2008). Furthermore, pioglitazone has not been reported to impact CYP or transporter activity suggesting a low potential to act as a DDI perpetrator (Eckland and Danhof 2000, Gillies and Dunn 2000).

Gemfibrozil has raised pioglitazone exposure over 3-fold (Deng et al. 2005, Jaakkola et al. 2005), and interestingly one study reported that the mean increase was over 5-fold in *CYP2C8*3* carriers, when that was only 3.3-fold in *CYP2C8*1* homozygotes (Aquilante et al. 2013). Other studies have also associated higher pioglitazone clearance with carrying the *CYP2C8*3* allele (Kadam et al. 2013, Tornio

et al. 2008). These findings strongly imply that carrying the *CYP2C8*3* allele increases the $f_{mCYP2C8}$ of pioglitazone. Owing to the increased risk for concentration-dependent adverse effects, the regulatory authorities have recommended caution when combining gemfibrozil with pioglitazone (EMA 2010). In contrast, itraconazole has not significantly altered pioglitazone disposition in humans indicating that CYP3A4 has a negligible contribution in its metabolism *in vivo* (Jaakkola et al. 2005). Using pioglitazone as a CYP2C8 probe substrate is supported by the dominant role of CYP2C8 in its metabolism, and by its good tolerability in single doses.

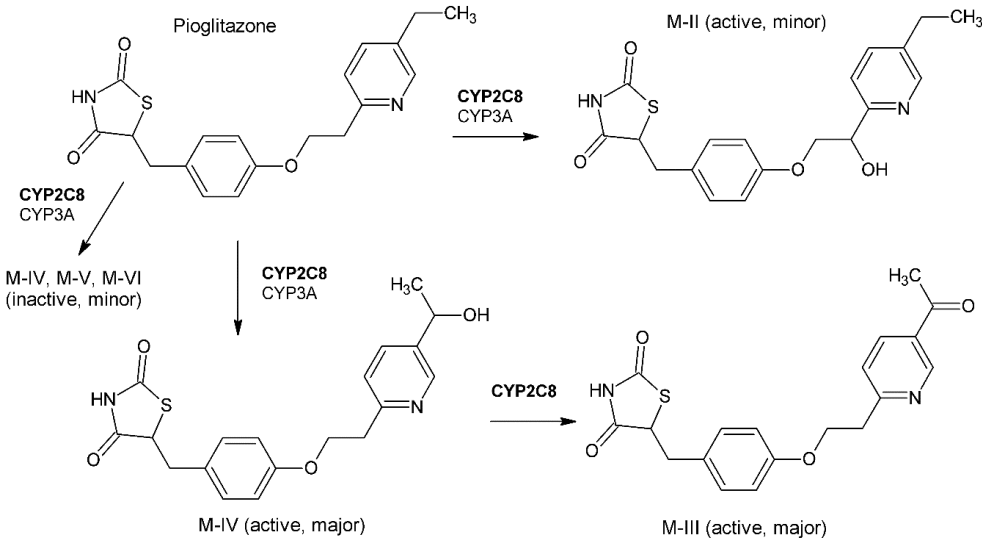

Figure 2. Biotransformation pathways of pioglitazone, and the chemical structures of pioglitazone, and its M-IV, M-III, and M-II metabolites. CYP, cytochrome P450.

8.3. Montelukast

Montelukast is a potent and selective cysteinyl leukotriene receptor 1 antagonist that inhibits mucus hypersecretion and smooth muscle contraction in the bronchi, thereby providing therapeutic efficacy in the treatment of asthma (Lipworth 1999, Reiss et al. 1998). Montelukast has an oral bioavailability of approximately 60–70%, exhibits linear pharmacokinetics in therapeutic doses, and is approximately 99.8% bound to plasma proteins (Cheng et al. 1996, FDA 1998). Montelukast is tolerated relatively well, however, increased incidence of neuropsychiatric symptoms, headache, and GI problems have been reported (Benard et al. 2017, Haarman et al. 2017, Lipworth 1999). The major route in the extensive metabolism of montelukast is the formation of its 36-hydroxy metabolite (M6) and further oxidation to dicarboxylic acid metabolite (M4) by CYP2C8 (**Figure 3**) (Balani et al. 1997, Cheng et al. 1996, FDA 1998, Filppula et al. 2011, Karonen et al. 2010). Furthermore, minor montelukast metabolites include the acyl glucuronide (M1) formed by UGT1A3 (Cardoso Jde et al. 2015, Hirvensalo et al. 2018), and the 21-hydroxy metabolite (M5) formed by CYP3A4 (Chiba et al. 1997, Karonen et al. 2012).

Two individual papers (a clinical DDI study and a paper incorporating data from PBPK simulations, *in vitro* investigations, and *in vivo* experiments in rats and cynomolgus monkeys) have suggested a role for

OATP1B1 in montelukast disposition (Hegazy et al. 2012, Varma et al. 2017). The clinical study found that clarithromycin increased the AUC of montelukast 2.4-fold, whereas fluconazole decreased it by 31% (Hegazy et al. 2012). The authors attributed both observed DDIs to transporter-mediated mechanisms but offered no mechanistic explanation that would fit the known DDI characteristics of fluconazole or clarithromycin, and montelukast. In the second study, single-dose rifampicin reduced the cellular uptake of montelukast into human hepatocytes, whereas inconsistently, another potent OATP1B1 inhibitor, cyclosporine A, significantly increased its uptake (Varma et al. 2017). Moreover, in human embryonic kidney (HEK) 293 cells transfected with individual human transporters, the uptake fold-ratio of montelukast to negative (propranolol) and positive (rosuvastatin) controls were 1.4–1.9 and 0.08–0.1 (for OATP1B1), and 1.1–1.3 and 0.28–0.36 (for OATP1B3), respectively, and the montelukast to rosuvastatin uptake fold-ratio was 0.05–0.09 (for OATP2B1), depending on the concentration and the incubation time of montelukast. Furthermore, single-dose rifampicin decreased montelukast clearance in rats and monkeys, but the corresponding alterations in the disposition of pitavastatin, the prototypical OATP1B1 substrate, were markedly more pronounced (Varma et al. 2017). A GWAS associated the *UGT1A3*2* variant allele (that leads to augmented UGT1A3 expression) with amplified montelukast clearance and increased exposure of its M1 metabolite, but it found no evidence indicating that montelukast is a sensitive OATP1B1 substrate (Hirvensalo et al. 2018). To summarize, the role of OATP1B1 in montelukast disposition is not fully established, but it is mediocre at best. In addition, the common missense variants of *SLCO1B3* or *SLCO2B1* genes, which encode OATP1B1 and OATP2B1, respectively, have not been associated with altered pharmacokinetics of parent montelukast (Hirvensalo et al. 2018, Tapaninen et al. 2013).

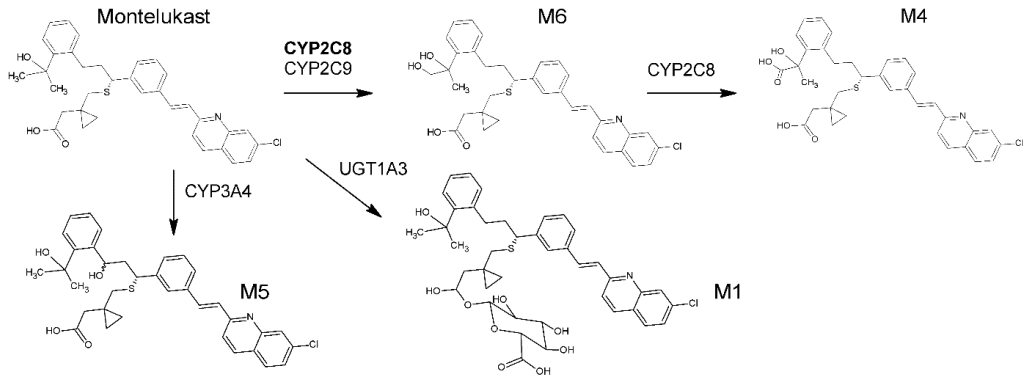

Figure 3. Biotransformation pathways of montelukast and the chemical structures of montelukast and its metabolites M6, M4, M5, and M1. CYP, cytochrome P450; UGT, uridine 5'-diphospho-glucuronosyltransferase.

Early *in vitro* papers identified montelukast as a CYP2C8 inhibitor, but in clinical studies, it failed to affect the pharmacokinetics of the CYP2C8 substrates pioglitazone, repaglinide, and rosiglitazone (Jaakkola et al. 2006c, Kajosaari et al. 2006, Kim et al. 2007). Further *in vitro* experiments performed with physiologically relevant free drug concentrations explained the low CYP2C8 inhibitory effect by montelukast with its low f_u in human plasma (Filppula et al. 2011). However, X-ray crystallography has demonstrated that CYP2C8 heme interacts with the montelukast benzyl ring near the site of oxidation in the biotransformation of montelukast to its M6, M4, and M3 metabolites (Chiba et al. 1997, Filppula et al. 2011, Schoch et al. 2008). The observed ligand-protein interaction of montelukast and CYP2C8 prompted research into the role of CYP2C8 in montelukast metabolism. The clinical studies found that

gemfibrozil increases montelukast AUC 4.3–4.5-fold, whereas itraconazole has only a negligible effect on it (Karonen et al. 2010, Karonen et al. 2012). The CYP2C8-inhibitory effect of gemfibrozil primarily accounted for the augmented montelukast exposure, thus making montelukast a potential CYP2C8 index substrate for DDI studies.

8.4. Dasabuvir

Dasabuvir inhibits nonstructural protein 5B, an RNA polymerase of HCV, and thereby disrupts the replication of viral RNA (FDA 2014a). Dasabuvir is routinely used in the treatment of HCV 1 infection in conjunction with three other drugs in 3D regimen: paritaprevir, ombitasvir, and ritonavir (Deeks 2015). Depending on the patient population, a sustained virological response has been achieved with rates of 90–100% by a twelve-week treatment with 3D regimen, but its use is currently limited by its high cost (Klibanov et al. 2015). The adverse effects of the 3D combination include pruritus, skin reactions, insomnia, and elevated serum alanine aminotransferase levels. Dasabuvir has an oral bioavailability of about 70% and is more than 99.9% bound to plasma proteins. It exhibits linear pharmacokinetics in clinically relevant dose ranges and has a mean terminal $t_{½}$ of about 7 hours (King et al. 2017). *In vitro* and *in silico*, CYP2C8 metabolizes 60% of dasabuvir to the primary *tert*-butyl hydroxyl metabolite (M1), the most abundant metabolite in human plasma, whereas CYP3A4 contribution in dasabuvir biotransformation is about 30% (King et al. 2017, Shebley et al. 2017b, Shen et al. 2016). Based on clinical data, the role of CYP2C8 in dasabuvir metabolism seems to be more pronounced *in vivo* than *in vitro*, and conversely, that of CYP3A4 appears to be of lesser importance (Menon et al. 2015). In addition, dasabuvir has been suggested to be a substrate of P-gp and BCRP, and the 3D regimen has been observed to inhibit UGT1A1 and BCRP.

Most clinical research that focused on characterizing the pharmacokinetics and DDI potential of dasabuvir has been performed with concurrently administered paritaprevir, ombitasvir, and ritonavir. This has complicated the data interpretation and has required examination of the individual pharmacokinetic characteristics of dasabuvir. When given concurrently with ritonavir, gemfibrozil has increased the C_{max} and AUC of dasabuvir 2.0- and 11.3-fold, respectively, while prolonging its $t_{½}$ from 5 to 90 hours (Menon et al. 2015). The CYP3A4 and P-gp inhibitor ketoconazole has caused only a modest 1.4-fold increase in the AUC of dasabuvir with an insignificant effect on the C_{max}. Furthermore, the CYP inducer carbamazepine has decreased dasabuvir C_{max} and AUC to 45% and 30%, respectively. For these reasons, studies have concluded that concomitant use of dasabuvir and potent CYP2C8 inducers or inhibitors is contraindicated (FDA 2014a). The dominant role of CYP2C8 in dasabuvir metabolism has raised questions concerning the safety of coadministered clopidogrel and dasabuvir; however, a PBPK study suggested only a “limited” DDI between the two drugs (Shebley et al. 2017a, Stark 2015). As supratherapeutic dasabuvir exposure is associated with decreased hemoglobin levels and potential to prolong the QTc interval (FDA 2014b), additional clinical examinations with other CYP2C8 inhibitors and dasabuvir were urged (Stark 2015).

8.5. Desloratadine

Desloratadine is a nonsedating histamine 1 receptor inverse agonist indicated for the symptomatic treatment of allergic rhinitis and urticaria (FDA 2014c, Henz 2001). It exhibits linear pharmacokinetics

in the therapeutic dose range and is extensively metabolized (Henz 2001, Molimard et al. 2004, Murdoch et al. 2003). The safety profile of desloratadine resembles that of placebo, albeit pharyngitis, dry mouth, and fatigue have been 1–2% more common in allergic rhinitis patients using desloratadine. In normal doses, desloratadine has not significantly altered the QTc interval (FDA 2014c, Henz 2001), and its dosages exceeding nine times the normal therapeutic upper limit have prolonged QTc by 8.1 and 0.4 ms, when Bazett and Friedricia corrections were applied, respectively (FDA 2014c). CYP3A4, CYP2D6, and CYP2C19 convert desloratadine to its minor 5-hydroxy and 6-hydroxy metabolites, but the enzymatic mechanism responsible for the formation of the major 3-hydroxy metabolite was long unclear (Barecki et al. 2001). In 2015, an *in vitro* study observed that CYP2C8 could form 3-hydroxydesloratadine, but only if coincubated with UGT2B10 (**Figure 4**) (Kazmi et al. 2015).

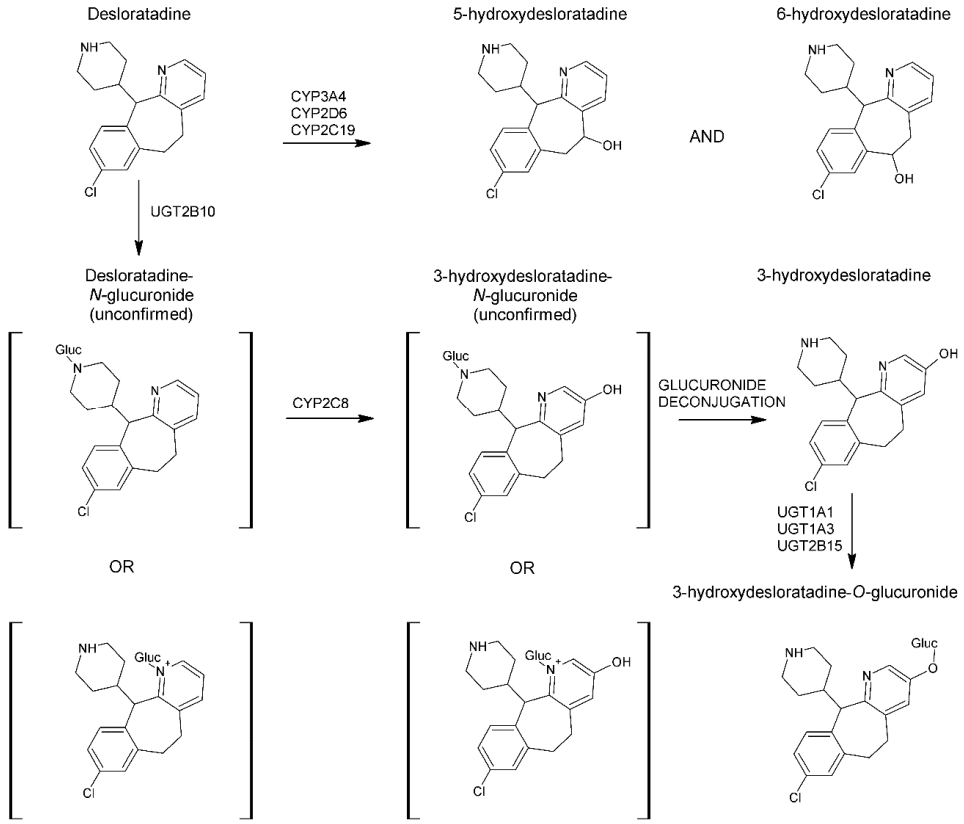

Figure 4. The proposed biotransformation pathways and the chemical structures of desloratadine and its 5-hydroxy, 6-hydroxy, *N*-glucuronide, 3-hydroxy, and 3-hydroxy-O-glucuronide metabolites (Kazmi et al. 2015). CYP, cytochrome P450; UGT, uridine 5'-diphospho-glucuronosyltransferase.

Accordingly, four distinct steps were proposed for the dominant metabolism route of desloratadine. First, UGT2B10 forms an *N*-glucuronide, which then undergoes a rapid CYP2C8-mediated 3-hydroxylation (Kazmi et al. 2015). Subsequently, the produced 3-hydroxy metabolite is further glucuronidated to 3-hydroxydesloratadine-O-glucuronide by UGT1A1, UGT1A3, and UGT2B15, which was reported soon after desloratadine marketing approval (Ghosal et al. 2004). Kazmi and colleagues

were unable to detect desloratadine-*N*-glucuronide in their experiments, however, which was explained by its hypothetically unstable nature (Kazmi et al. 2015). A subset of the general population (ranging from 2% in Caucasians and Hispanics to 17% in African Americans) has a decreased capacity to form 3-hydroxydesloratadine. These individuals are described as desloratadine "poor metabolizers", whose desloratadine exposure and 3-hydroxydesloratadine:desloratadine AUC ratio are 600% and 21% of those of "normal desloratadine metabolizers", respectively (FDA 2014c, Prenner et al. 2006). Furthermore, membrane transporters have not been observed to participate in desloratadine disposition to a clinically relevant extent (Henz 2001, Murdoch et al. 2003). In addition, desloratadine has not exhibited significant effects on CYP enzyme or drug transporter activity. In clinical DDI studies, ketoconazole has augmented desloratadine exposure 1.4-fold, whereas azithromycin, grapefruit juice, erythromycin, fluoxetine, and cimetidine have caused a smaller to nonexistent alterations desloratadine pharmacokinetics. To summarize, the proposed 3-hydroxylation mechanism of desloratadine could make it a potential CYP2C8 index substrate, but this question requires addressing with clinical data.

AIMS OF THE WORK

The ADP-receptor subtype $P2Y_{12}$ antagonist clopidogrel markedly increases the exposure of repaglinide, which is a CYP2C8, OATP1B1, and CYP3A4 substrate. However, the interaction profile of clopidogrel has not been characterized in detail. In addition, specific, safe, and sensitive CYP2C8 index substrates are lacking, although they are highly warranted for DDI studies. Clopidogrel is a prodrug bioactivated partly by CYP3A4, but the effect of CYP3A4 inhibitors on its pharmacokinetics, such as ritonavir, has remained partly unexplored. This work primarily aimed to examine the potential of clopidogrel to alter the pharmacokinetics of different CYP2C8, OATP1B1, and CYP3A4 substrates, and to screen clinically relevant pharmacokinetic interactions by clopidogrel. Moreover, the target was to search for potential CYP2C8 index substrates to be utilized in DDI studies.

Specific aims of individual studies:

Study I To investigate the effect of clopidogrel in loading and maintenance doses on the pharmacokinetics of simvastatin, a sensitive CYP3A4 and OATP1B1 substrate.

Study II To examine whether clopidogrel alters the pharmacokinetics of the CYP2C8 substrate pioglitazone.

Study III To characterize the effects of clopidogrel and prasugrel on the pharmacokinetics of montelukast, a CYP2C8 substrate.

Study IV To explore the impact of clopidogrel on the pharmacokinetics of the CYP2C8 substrate dasabuvir with or without concurrently administered CYP3A4 inhibitor ritonavir. Secondarily, the aim was to examine the ability of ritonavir to modify clopidogrel bioactivation.

Study V To investigate whether clopidogrel and the strong CYP2C8 inhibitor gemfibrozil affect the pharmacokinetics of desloratadine, a proposed CYP2C8 substrate.

MATERIALS AND METHODS

1. Subjects

Twelve healthy Finnish volunteers took part in each study, except for Study II, in which ten subjects participated (**Table 1**). One subject withdrew from Study V due to personal reasons, but all other participants completed the studies. One male subject participated in two studies (Studies II and IV) totaling 56 subjects (27 female, 29 male). In each study, the number of participants was estimated to be sufficient to detect at least a 30% difference in the AUC of investigated drugs between phases or groups, with a power of 80% (α-level 5%). The subjects' health was confirmed by medical history, clinical examination, the following routine laboratory tests, and electrocardiography (only in Study IV) before entering the studies. All subjects had normal blood pressure, glomerular filtration rate, plasma sodium and potassium levels, blood thrombocyte counts, and hemoglobin values. Only clinically insignificant divergences were approved in plasma alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, and creatine kinase (only in Study I) levels. Before participation, plasma human chorionic gonadotropin was measured from every female participant to exclude pregnancy. None of the subjects used continuous medication, hormonal contraception, or tobacco products. Use of other drugs or omega-3 fatty acid dietary supplements were not permitted from 1 week before to 1 week after each study. Furthermore, consumption of grapefruit-derived products was not allowed one week before and during the studies. Strenuous exercise and use of alcohol were prohibited the day before, and on the days when the victim drugs were administered. Participating in other trials or donating blood within three months before and after each study was also prohibited.

Table 1. Characteristics of participants

Study number (n)	Sex (f/m)	Age (years)	BMI (kg/m^2)
I (12)	4/8	22; 19–27	22.7; 18.6–25.9
II (10)	4/6	26; 20–35	22.4; 19.2–28.9
III (12)	7/5	25; 19–31	24.6; 20.2–26.8–(33.0*)
IV (12)	6/6	25; 20–33	22.4; 19.4–26.6
V (11)	6/5	23; 20–29	23.1; 18.7–27.3

Age and body mass index (BMI) data are presented as arithmetic mean with range; f, females; m, males. In Study III, one subject (indicated with *) had a BMI of 33.0 due to athletic body composition.

2. Study Design

These placebo-controlled pharmacokinetic studies with clopidogrel and possible additional drugs as perpetrator pretreatments (prasugrel, ritonavir, and gemfibrozil in Studies III, IV, and V, respectively) were carried out with a randomized crossover design. The victim drugs were simvastatin, pioglitazone, montelukast, dasabuvir and clopidogrel, and desloratadine in Studies I, II, III, IV, and V, respectively (**Tables 2**, **3**, **4**, **5**, and **6**). Ritonavir and gemfibrozil pretreatments were partly performed by the subjects at home, unsupervised, but the volunteers were instructed to be punctual with the dosing schedule and document the time when gemfibrozil or ritonavir were ingested. On the actual study days, the victim drugs were administered one hour after the pretreatment to allow dissolution and disintegration of

perpetrator tablets. All drugs were ingested with 150 ml of water, and depending on the study, the subjects spent the following 9–12 hours under medically supervised conditions. Standardized warm meals were served 3 hours, and snacks 7 hours, and 9 hours (Study IV) or 10 hours (Studies I–III and V) after the administration of the victim drugs. Depending on the pharmacokinetic profiles of the administered drugs, study phases were separated by a wash-out period of 2–3 weeks in each study.

Table 2. Design of Study I

Pretreatment phase	Day 1	
	8 AM	9 AM
Placebo	Placebo	Simvastatin 40 mg
Clopidogrel 300 mg	Clopidogrel 300 mg	Simvastatin 40 mg
Clopidogrel 75 mg	Clopidogrel 75 mg	Simvastatin 40 mg

Table 3. Design of Study II

Pretreatment phase	Day 1		Days 2 and 3
	8 AM	9 AM	8 AM
Placebo	Placebo	Pioglitazone 15 mg	Placebo
Clopidogrel	Clopidogrel 300 mg	Pioglitazone 15 mg	Clopidogrel 75 mg

Table 4. Design of Study III

Pretreatment phase	Day 1		Day 2
	8 AM	9 AM	8 AM
Placebo	Placebo	Montelukast 10 mg	Placebo
Clopidogrel	Clopidogrel 300 mg	Montelukast 10 mg	Clopidogrel 75 mg
Prasugrel	Prasugrel 60 mg	Montelukast 10 mg	Prasugrel 60 mg

Table 5. Design of Study IV

Pretreatment phase	Days 1 and 2		Day 3			Days 4 and 5	
	8 AM	8 PM	8 AM	9 AM	8 PM	8 AM	8 PM
Placebo	–	–	Placebo	DAS 250 mg	–	Placebo	–
Ritonavir	RIT 100 mg	RIT 100 mg	RIT 100 mg	DAS 250 mg	RIT 100 mg	RIT 100 mg	RIT 100 mg
Clopidogrel	–	–	CLOP 300 mg	DAS 250 mg	–	CLOP 75 mg	–
Clopidogrel and ritonavir	RIT 100 mg	RIT 100 mg	CLOP 300 mg and RIT 100 mg	DAS 250 mg	RIT 100 mg	CLOP 75 mg and RIT 100 mg	RIT 100 mg

CLOP, clopidogrel; DAS, dasabuvir; RIT, ritonavir.

Table 6. Design of Study V

Pretreatment phase	Days 1 and 2		Day 3			Days 4 and 5	
	8 AM	8 PM	8 AM	9 AM	8 PM	8 AM	8 PM
Placebo	–	–	Placebo	DES 5 mg	–	Placebo	–
Clopidogrel	–	–	CLOP 300 mg	DES 5 mg	–	CLOP 75 mg	–
Gemfibrozil	GEM 600 mg	GEM 600 mg	GEM 600 mg	DES 5 mg	GEM 600 mg	GEM 600 mg	GEM 600 mg

CLOP, clopidogrel; DES, desloratadine; GEM, gemfibrozil.

The following drugs used in the studies were supplied by the Pharmacy of Helsinki University Central Hospital: clopidogrel (Plavix 300 mg and 75 mg tablets; Sanofi- Aventis, Paris, France); prasugrel (Efient 10 mg tablets; Eli Lilly, Nederland BV, Houten, The Netherlands); ritonavir (Norvir 100 mg tablets; AbbVie, Ludwigshafen am Rhein, Germany); gemfibrozil (Lopid 600 mg tablets; Pfizer Manufacturing Deutschland GmbH, Freiburg, Germany); simvastatin (Zocor 40 mg tablets; MSD, Hoddeson, United Kingdom); pioglitazone (Actos 15 mg tablets; Takeda Europe, London, UK), dasabuvir (Exviera 250 mg tablets; AbbVie); desloratadine (Aerius 5 mg tablets; MSD); and placebo (Placebo tablets; University Pharmacy, Helsinki, Finland).

3. Sampling

For the pharmacokinetic and pharmacodynamic measurements, timed blood samples were drawn from a cannulated forearm vein, or by venipuncture according to a predetermined schedule, which differed from one study to another, depending on the pharmacokinetic profiles of the used drugs. In Study I, samples were taken before the administration of pretreatment, and 5 minutes before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 7, 9, and 12 hours after administering simvastatin. In Study II, blood samples were drawn before the administration of pretreatment, and 5 minutes before and 1, 2, 3, 4, 5, 7, 9, 12, 24, 48, and 72 hours after the administration of pioglitazone. In Study III, samples were taken before the administration of pretreatment, and 5 min before and 0.5, 1, 2, 3, 4, 5, 7, 9, 12, 24, and 48 h after the administration of montelukast. In Study IV, blood samples were drawn before the administration of pretreatment, and 5 minutes before and 0.5, 1, 2, 3, 4, 6, 9, 23, 47, and 71 hours after the dasabuvir ingestion. In Study V, samples were taken before pretreatment, and 5 minutes before and 0.5, 1, 2, 3, 4, 5, 7, 9, 11, 23, 47, and 71 hours after desloratadine administration. For the determination of the clopidogrel antiplatelet effect in Study IV, anticoagulated whole-blood samples were collected in citrate tubes at just before, and 55 minutes, 2, 4, 10, and 24 hours after clopidogrel dosing.

Blood samples were collected into EDTA-containing tubes, which were placed on ice immediately after sampling. Plasma was separated within 30 minutes and stored at -70 ºC until analysis. The samples for the determination of clopidogrel active metabolite, and prasugrel metabolites R-138727 and R-95913, were treated with 2-bromo-3'-methoxyacetophenone within 30 seconds of blood sample collection in whole blood EDTA samples for the derivatization of the active metabolite of clopidogrel and prasugrel, as originally described by Delavenne and colleagues, and Farid and colleagues (Delavenne et al. 2010, Farid et al. 2007).

4. Determination of drug concentrations

The lower limits (LLQ) of quantification and the day-to-day coefficients of variation (CV) of all analyzed compounds are given in **Table 7**. The bioanalytical methods are described in more detail in the original publications. The matrix used was human plasma, and no interfering peaks were observed at the mean retention times of analytes, except in Study I (see section 7). All analyses were performed with liquid chromatography-tandem mass spectrometry.

Table 7. Summary of bioanalytical variables in each study

Study	Analyte	LLQ (ng/ml or S/N*)	Day-to-day CV (%)
I–V	Clopidogrel	0.05	< 10
I–V	Clopidogrel active metabolite	0.05	< 15
I–IV; V	Clopidogrel carboxylic acid	100; 20	< 15
I–IV; V	Clopidogrel acyl-β-D-glucuronide	100; 25	< 15
I	Simvastatin	0.04	< 15
I	Simvastatin acid	0.05	< 15
II	Pioglitazone	0.2	< 15
II	Hydroxypioglitazone (M-IV)	0.5	< 15

II	Ketopioglitazone (M-III)	0.5	< 15
III	Prasugrel R-138727 metabolite	0.1	< 15
III	Prasugrel R-95913 metabolite	0.2	< 15
III	Montelukast	2.0	< 9
III	Montelukast acyl-β-D-glucuronide (M1)	0.5	< 8
III	Montelukast 1,2-diol (M6)	0.2	< 8
III	21(S)-hydroxy montelukast (M5a)	0.25	< 6
IV	Ritonavir	5.0	< 9
IV	Dasabuvir	0.8	< 10
IV	*Tert*-butyl hydroxyl dasabuvir (M1)	10:1*	–
V	Gemfibrozil	50	< 10
V	Gemfibrozil-1-O-glucuronide	50	< 10
V	Desloratadine	0.05	≤ 6
V	3-hydroxydesloratadine	0.025	< 5
V	3-hydroxydesloratadine-O-glucuronide	3:1*	–

The LLQ data are given as nanograms per milliliter, or as a signal-to-noise ratio (indicated with *).

5. Platelet aggregation measurements

In Study IV, the inhibition of platelet aggregation by clopidogrel was tested using the VerifyNow $P2Y_{12}$ turbidimetric optical detection system (Accumetrics, San Diego, USA) (Jeong et al. 2012, Lordkipanidze et al. 2008) within 2 hours of sampling. Whole blood samples were injected to a standard cartridge composed of two channels. One channel contained a mixture of ADP and PGE1, the latter being included to improve the specificity of the PRU result to reflect the antiplatelet impact of clopidogrel taking place via $P2Y_{12}$ receptor activation. The second channel contained thrombin receptor activating peptide (TRAP) to induce platelet aggregation with no ADP receptor activation. As thrombocyte aggregation occurred, the system converted luminosity transmittance results into arbitrary $P2Y_{12}$ reaction units (PRU) for the ADP/PGE1 channel and arbitrary BASE units for the TRAP channel. In addition to reporting the results in arbitrary units, a platelet inhibition percentage was given. The average inhibition values were calculated by dividing the area under the effect-time curve from time 0 to 24 hours by the corresponding time interval.

6. Genotyping

Buffy coats were prepared from 9 ml whole-blood EDTA samples after plasma separation. Genomic DNA was extracted from the buffy coats using the Maxwell 16 LEV Blood DNA Kit on a Maxwell 16 Research automated nucleic acid extraction system (Promega, Madison, USA). Depending on the study, participants were genotyped for some of the following alleles (**Table 8**): *CYP2C8*2* (rs11572103) **3* (rs10509681 and rs11572080), and **4* (rs1058930); *CYP2C19*2* (rs4244285), **3* (rs4986893), **8* (rs41291556), and **17* (rs12248560); *UGT1A3*2* (rs3821242 and rs6431625), **3* (rs3821242), and **6* (rs3821242, rs6431625, and rs45449995); *CES1* c.428G>A (rs71647871); and *SLCO1B1* c.521T>C (rs4149056). Genotyping was performed with commercially available or custom TaqMan assays with

OpenArray technology on a QuantStudio 12K Flex real-time polymerase chain reaction system (Life Technologies, Carlsbad, USA).

Table 8. Genotypes of the study subjects

Study number (n)	Genotype (n)	Number of subjects
I (12)	*SLCO1B1* c.521TT	9
	SLCO1B1 c.521TC	3
II (10)	*CYP2C8*1/*2*	1
	*CYP2C8*1/*3*	3
	*CYP2C8*1/*4*	2
	*CYP2C8*1/*1*	4
III (12)	*CYP2C8*1/*1*	9
	*CYP2C8*1/*2*	1
	*CYP2C8*1*3*	2
	*UGT1A3*1/*1*	1
	*UGT1A3*1/*2*	8
	*UGT1A3*1/*3*	1
	*UGT1A3*2/*2*	1
	*UGT1A3*3/*3*	1
	*CYP2C19*1/*1*	5
	*CYP2C19*1/*17*	7
	CES1 c.428G/G	11
	CES1 c.428G/A	1
IV (12)	*CYP2C8*1/*1*	9
	*CYP2C8*1/*2*	1
	*CYP2C8*1/*3*	1
	*CYP2C8*1/*4*	1
	*CYP2C19*1/*1*	1
	*CYP2C19*1/*2*	3
	*CYP2C19*1/*17*	6
	*CYP2C19*2/*17*	2
	CES1 c.428G/G	10
	CES1 c.428G/A	2
V (11)	*CYP2C8*1/*3*	2
	*CYP2C8*1/*4*	2
	*CYP2C8*1/*1*	7

7. Pharmacokinetic calculations

The pharmacokinetics of all analyzed compounds were characterized by C_{max}, t_{max}, $t_{½}$, $AUC_{0-\infty}$, and appropriate partial AUCs. Pharmacokinetics were calculated by noncompartmental analysis using MK-Model, version 5.0 in Study I (Biosoft, Cambridge, UK), and Phoenix WinNonlin, version 6.4 in Studies II–V (Certara, Princeton, USA). The terminal log-linear part of each concentration-time curve was identified visually. The k_e was determined by linear regression analysis of the log-linear part of the plasma concentration time curve. The $t_{½}$ value was calculated by the equation $t_{½} = \ln 2/k_e$. The AUC values were

calculated by using a combination of the linear (for increasing concentrations) and log-linear (for decreasing concentrations) trapezoidal rules, with extrapolation to infinity, when appropriate, by dividing the last measured concentration by k_e.

When the effect of clopidogrel 75 mg on simvastatin pharmacokinetics was examined two days after exploring the effect of the 300 mg dose in Study I, certain individuals had residual concentrations of simvastatin and simvastatin acid from the preceding phase. These were taken into account by subtracting the residual AUC from the observed AUC. The residual AUC was calculated by dividing the residual concentration by the k_e estimated in the preceding phase. Residual concentrations were observed in 8 and 10 of the individuals for simvastatin and simvastatin acid, respectively. The geometric mean residual $AUC_{0-\infty}$ of simvastatin and simvastatin acid were 2% and 4% of the total $AUC_{0-\infty}$ observed, respectively.

8. Statistical analyses

Based on the pharmacokinetic results of previous drug interaction studies with the victim drugs, 10 (Study II) or 12 (Studies I and III-V) subjects were estimated to be adequate to detect about 30% change in the AUC of victim drugs between the placebo and perpetrator phases, with a power of at least 80% (α level 5%). The pharmacokinetic results were expressed as geometric means and geometric mean ratios with geometric CV or 90% CIs, excluding t_{max}, which were given as median with range. In Study IV, the results of the platelet aggregation measurements were presented as arithmetic means with standard deviation. Logarithmic transformation was used for pharmacokinetic variables, except for t_{max}, before statistical analysis. The pharmacokinetic parameters were compared by repeated-measures analysis of variance with treatment phase as a within-subjects, and treatment sequence as a between-subjects factor in Studies I and II, with pairwise comparisons with the Fisher's least significant difference method. Because Studies IV and V compared the pharmacokinetic parameters of dasabuvir and its M1 metabolite, and desloratadine and its metabolites, respectively, between all the pretreatment phases, they applied Bonferroni correction. The t_{max} data were compared using the Wilcoxon signed rank test. In Studies II–V, the correlations between the $AUC_{0-\infty}$ of clopidogrel acyl-β-D-glucuronide, and the changes in the pharmacokinetics of the victim drugs (metabolite:parent drug AUC ratio and AUC fold-changes) were quantified as Kendall's (Study IV) or Pearson's correlation coefficients and tested using Kendall's test (Study IV) or t-test (Studies II, III, and V). In all the studies, P-values below 0.05 were considered significant. Statistical analyses were performed using SPSS Statistics for Windows, version 22.0 (IBM, Armonk, NY).

9. Static *in vivo-in vitro* predictions and physiologically-based pharmacokinetic simulations

In Studies II and V, previously published *in vitro* inhibitor concentrations that support half maximal rate of inactivation, and maximal inactivation rate values of clopidogrel acyl-β-D-glucuronide for CYP2C8 were applied to predict the *in vivo* clopidogrel-pioglitazone, clopidogrel-desloratadine, and gemfibrozil-desloratadine interactions with the following equation (Mayhew et al. 2000):

$$\frac{AUC_I}{AUC} = \frac{1}{\frac{fm_{CYP2C8}}{1+\frac{k_{inact}\times[I]}{K_I \times k_{\deg}}} + (1 - fm_{CYP2C8})}$$

where k_{deg} is the rate constant of hepatic P450 degradation in the absence of the inhibitor, k_{inact} is the maximum inactivation rate, I is the unbound inhibitor concentration at the enzyme site, and K_I is the inhibitor concentration needed to cause half of k_{inact}. The values used were obtained from previous studies (Backman et al. 2009, Ogilvie et al. 2006, Tornio et al. 2014).

To elucidate the mechanisms of the clopidogrel-montelukast interaction and to predict the magnitude of the clopidogrel-montelukast DDI in steady-state caused by the clinical dosing of clopidogrel, PBPK models were constructed in the Simcyp Population-Based Simulator version 15.1 (Simcyp, Sheffield, UK). The montelukast and perpetrator drug models were validated by simulating previous DDI studies available in the literature. Two clopidogrel models were refined and validated for the PBPK simulations. The clopidogrel models differed with respect to the unbound hepatocyte-to-plasma ratio and OATP1B1 K_I of clopidogrel acyl-β-D-glucuronide, which were 8 and 0.1 µM, and 25 and 5.45 µM. Thereafter, two montelukast models were constructed: a perfusion-limited liver model, and a permeability-limited liver model with OATP1B1 transport. In both montelukast models, CYP2C8 was the main enzyme involved in the metabolism of montelukast. To examine the clopidogrel–montelukast interaction, the $f_{mCYP2C8}$ was varied between 0.55 and 0.80.

10. Ethical and safety considerations

The Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District, and the Finnish Medicines Agency approved the study protocols. All participants received both oral and written information and signed an informed consent prior to participating in the studies. All the administered drugs are considered to possess a relatively benign safety profile with low risk for serious adverse reactions, especially in short-term use. The potential pharmacokinetic interactions were not estimated to markedly change the risk profile of the victim drugs when they are administered in single doses to healthy volunteers. The only invasive procedures done to the subjects were forearm vein cannulation and venipuncture, which pose only minimal risk for serious complications. The volunteers were under medical supervision 10–12 hours after the victim drugs were administered, and they were given a card that contained the names, dates, and dosages of the drugs that were administered. In case of emergencies, two of the researchers were available via telephone around the clock during the studies. In Study I, one subject developed a minor atraumatic hematoma in the left arm after clopidogrel pretreatment, and in Study IV, two subjects experienced transient and spontaneously resolved nausea after the first ritonavir dose, which did not reappear after the following doses. No other or major adverse effects were observed.

RESULTS

1. Effect of clopidogrel on the pharmacokinetics of simvastatin (Study I)

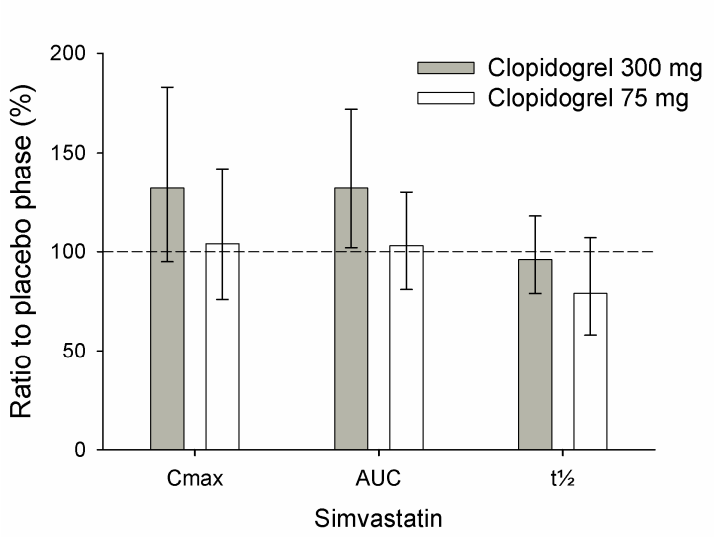

Figure 5. Effects of clopidogrel 300 mg and clopidogrel 75 mg on the C_{max}, $AUC_{0\text{-}12h}$, and $t_{½}$ of simvastatin. Bars represent geometric mean ratios to the placebo phase (%) with 90% confidence intervals.

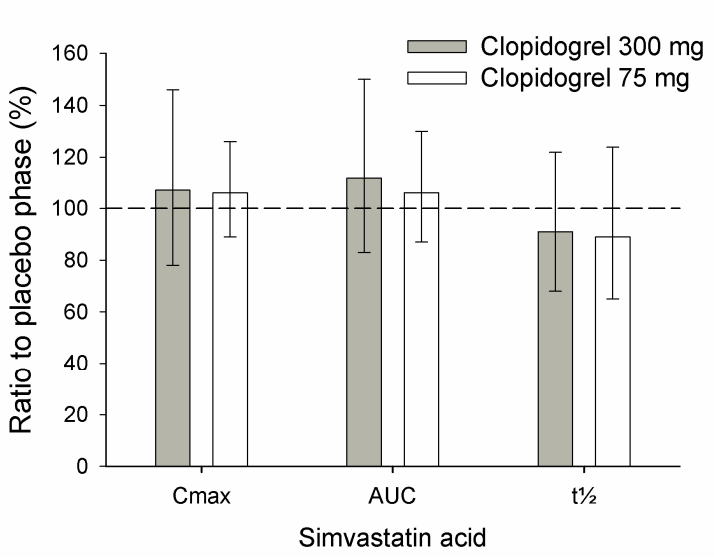

Figure 6. Effects of clopidogrel 300 mg and clopidogrel 75 mg on the C_{max}, $AUC_{0\text{-}12h}$, and $t_{½}$ of simvastatin acid. Bars represent geometric mean ratios to the placebo phase (%) with 90% confidence intervals.

By clopidogrel 300 mg, the $AUC_{0\text{-}2h}$ of simvastatin and simvastatin acid were 156% (P = 0.02, 90% CI of the geometric mean ratio 116–209%) and 148% (P = 0.04, 90% CI 109–201%) of those when placebo

was coadministered. The lower clopidogrel dose did not affect the $AUC_{0\text{-}2h}$ of simvastatin or simvastatin acid. No significant changes were observed in the C_{max}, t_{max}, $AUC_{0\text{-}12h}$, $AUC_{0\text{–}\infty}$, or $t_{½}$ of simvastatin or simvastatin acid by either clopidogrel dose (**Figures 5** and **6**). Three of the subjects were heterozygous for the *SLCO1B1* c.521T>C SNV and none were homozygous, while other subjects carried the c.521TT genotype.

2. Effect of clopidogrel on the pharmacokinetics of pioglitazone (Study II)

In the clopidogrel phase, the $AUC_{0\text{–}\infty}$ of pioglitazone, its primary hydroxy metabolite (M-IV) and secondary keto metabolite (M-III) were 214% ($P = 9{\cdot}10^{-5}$, 90% CI of the geometric mean ratio 176–261%), 105% (P > 0.9, 90% CI 90–122%), and 105 % (P > 0.9, 90% CI 89–124%) of those in the placebo phase (control), respectively (**Figure 7**). Thereby, clopidogrel decreased the M-IV:pioglitazone $AUC_{0\text{-}\infty}$ ratio, an index of the CYP2C8-mediated clearance of pioglitazone, to 49% (P = 0.0001, 90% CI 40–59%) of that when placebo was coadministered. The pioglitazone C_{max} remained unchanged, whereas the C_{max} of M-IV and M-III were 61% (P = 0.007, 90% CI 48–79%) and 58% (P = 0.003, 90% CI 46–74%) of control, respectively. The C_{24} of pioglitazone, which reflects its trough concentration, was 452% ($P = 5{\cdot}10^{-5}$, 90% CI 317–645%), when compared to control. Furthermore, clopidogrel prolonged the $t_{½}$ of pioglitazone from 6.7 to 11 hours (P = 0.002; 90% geometric mean ratio CI 135–204%). In addition, the $t_{½}$ of M-IV and M-III were prolonged from 21 to 38 hours (P = 0.007, 90% geometric mean ratio CI 130–232%) and from 22 to 40 hours (P = 0.006, 90% geometric mean ratio CI 136–248%) by clopidogrel, respectively. Moreover, the t_{max} of pioglitazone and both of its metabolites were significantly prolonged by clopidogrel.

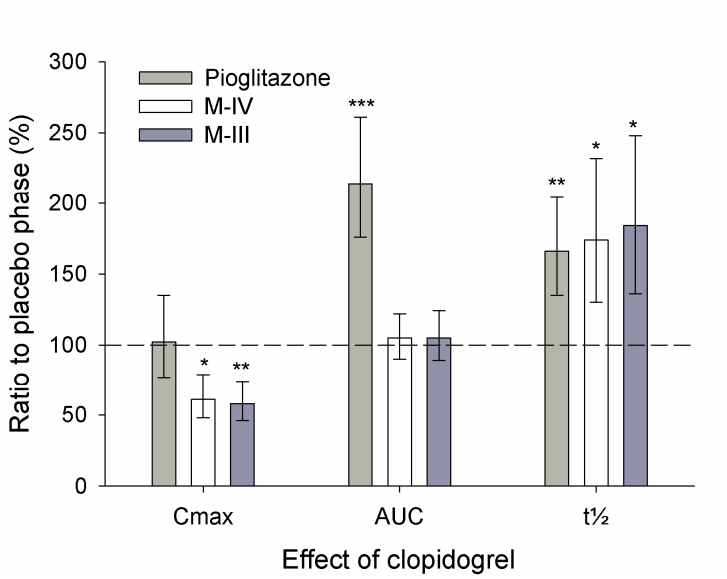

Figure 7. Effect of clopidogrel on the C_{max}, $AUC_{0\text{-}\infty}$, and $t_{½}$ of pioglitazone, and its primary hydroxy (M-IV) and secondary keto (M-III) metabolites. Bars represent geometric mean ratios to the placebo phase (%) with 90% confidence intervals. * P < 0.05, ** P < 0.005, *** P < 0.001 compared with the placebo phase.

The three individuals carrying the *CYP2C8*1/*3* genotype had the highest M-IV:pioglitazone $AUC_{0-\infty}$ ratios in the placebo phase (P = 0.0007). They also demonstrated greater decreases in the M-IV:pioglitazone $AUC_{0-\infty}$ ratio by clopidogrel, than did the noncarriers of *CYP2C8*3* (P = 0.02), and no significant differences in this ratio existed in the clopidogrel phase between the genotypes (P = 0.7). There were no significant correlations between the AUC_{0-13h} of clopidogrel, or its metabolites, and the fold-increase in pioglitazone $AUC_{0-\infty}$. Furthermore, the static prediction model of Study II suggested that for substrates with an fm_{CYP2C8} of 60–80%, lower than 1 µM static concentrations of clopidogrel acyl-β-D-glucuronide could cause a 210% increase in their $AUC_{0-\infty}$, similar to the effect of clopidogrel on pioglitazone exposure in this Study. Assuming a 10% unbound fraction, the median $C_{max,u}$ and median $C_{avg,u}$ (0–13 hours) of clopidogrel acyl-β-D-glucuronide after clopidogrel 300 mg were 0.85 and 0.26 µM, respectively.

3. Effect of clopidogrel and prasugrel on montelukast pharmacokinetics (Study III)

Clopidogrel increased the $AUC_{0-\infty}$ of montelukast to 198% (P = $1 \cdot 10^{-5}$, 90% CI 172–228%) and its primary 1,2 diol metabolite (M6) to 160% (P = $6 \cdot 10^{-5}$, 90% CI 140–183%) (**Figures 8** and **9**), when compared with those in the placebo phase. Moreover, clopidogrel decreased the M6:montelukast AUC_{0-7h} ratio to 45% (P = $6 \cdot 10^{-7}$, 90% CI 40–50%) of that in the placebo phase. In addition, clopidogrel prolonged the $t_{½}$ of montelukast from 4.5 to 8.2 hours (P = $6 \cdot 10^{-8}$, 90% geometric mean ratio CI 170–195%), and that of M6 from 5.0 to 9.9 hours (P = $2 \cdot 10^{-7}$, 90% geometric mean ratio CI 183–218%). Clopidogrel lacked an impact on the pharmacokinetics of the 21(S)-hydroxyl metabolite of montelukast (M5a) but caused increases in the $t_{½}$ and $AUC_{0-\infty}$ of montelukast-acyl-β-D-glucuronide (M1) that were secondary to the slowed elimination and augmented exposure of the parent montelukast. Prasugrel caused a minor prolongation from 4.5 to 5.2 hours (P = 0.03; geometric mean ratio 90% CI 104–127%) in montelukast $t_{½}$, whereas other pharmacokinetic variables of montelukast and its metabolites were unaffected by it.

The interaction with the clopidogrel PBPK model applying higher unbound hepatocyte-to-plasma ratio and OATP1B1 *Ki* of clopidogrel acyl-β-D-glucuronide could be adequately predicted when the $f_{mCYP2C8}$ of montelukast was 0.55–0.70 with both montelukast models. However, while the clopidogrel model, which used lower values for the aforementioned parameters, performed equally well with the perfusion-limited montelukast liver model, it overpredicted the interaction with the permeability-limited montelukast liver model with OATP1B1 transport model using the same $f_{mCYP2C8}$ values. Simulations of the effects of long-term dosing of 75 mg clopidogrel once daily on the plasma concentrations of montelukast predicted on average a 1.5–2-fold increase in montelukast dose interval AUC during steady-state conditions.

The *CYP2C8* genotype did not significantly affect montelukast pharmacokinetics or the increase in the AUC of montelukast by clopidogrel. In all three study phases, there was a trend for higher montelukast $AUC_{0-\infty}$ in the three *UGT1A3*2* noncarriers than in the nine subjects carrying the *UGT1A3*2* allele, and the largest montelukast exposures were observed in two *UGT1A3*2* noncarriers during the clopidogrel phase. The *CYP2C19* genotype did not significantly affect the AUC_{0-13h} values of clopidogrel *cis*-5-thiol, carboxylic acid, or acyl-β-D-glucuronide.

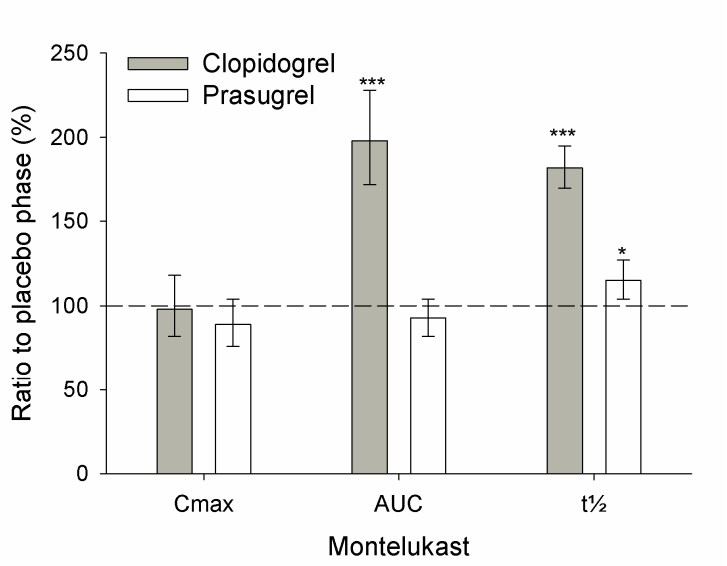

Figure 8. Effect of clopidogrel and prasugrel on the C_{max}, $AUC_{0-\infty}$, and $t_{½}$ of montelukast. Bars represent geometric mean ratios to the placebo phase (%) with 90% confidence intervals. * $P < 0.05$, *** $P < 0.001$ compared with the placebo phase.

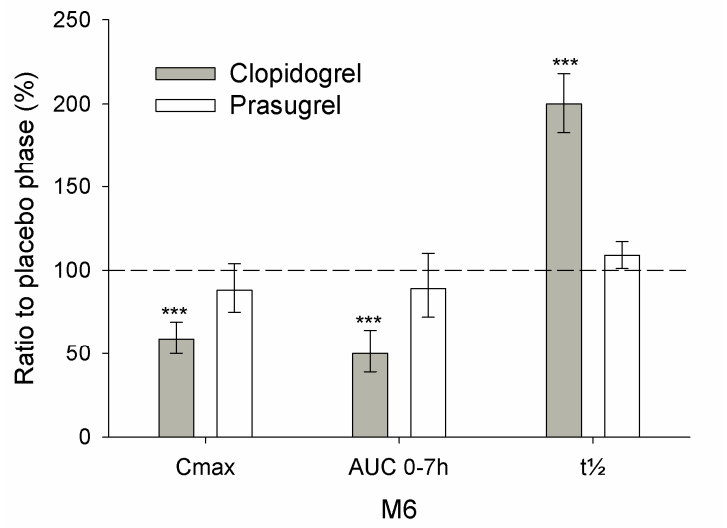

Figure 9. Effect of clopidogrel and prasugrel on the C_{max}, $AUC_{0-\infty}$, and $t_{½}$ of montelukast 1,2 diol (M6). Bars represent geometric mean ratios to the placebo phase (%) with 90% confidence intervals. *** $P < 0.001$ compared with the placebo phase.

4. Effect of clopidogrel, ritonavir, and their combination on dasabuvir pharmacokinetics, and the impact of ritonavir on the pharmacokinetics and antiplatelet effect of clopidogrel (Study IV)

In the clopidogrel phase, the $AUC_{0-\infty}$ of dasabuvir was 467% (P = $8 \cdot 10^{-7}$, 90% CI 323%–674%) of that during placebo phase, whereas the $AUC_{0-\infty}$ of its *tert*-butyl hydroxyl metabolite (M1) remained unchanged (**Figure 10**). Accordingly, clopidogrel decreased the M1:dasabuvir $AUC_{0-\infty}$ ratio to 17% (P = $2 \cdot 10^{-14}$, 90% CI 16–19%) of that when placebo was coadministered. Moreover, clopidogrel prolonged dasabuvir $t_{½}$ from 8.4 to 15.4 hours (P = $1 \cdot 10^{-6}$, geometric mean ratio 90% CI 159–213%). The 1.6-fold increase in dasabuvir C_{max} by clopidogrel did not reach the level of significance (P = 0.07, 90% CI 1.03–2.57-fold). Furthermore, clopidogrel diminished M1 C_{max} to 20% (P = $6 \cdot 10^{-5}$, 90% CI 11–36%) and prolonged the $t_{½}$ of M1 from 7.9 to 17.3 hours (P = $1 \cdot 10^{-5}$, geometric mean ratio 90% CI 172–281%) of those in the placebo phase.

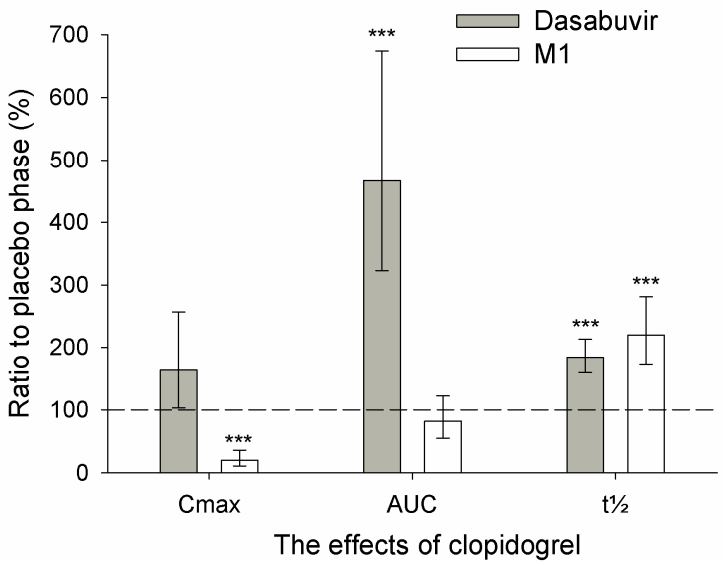

Figure 10. Effect of clopidogrel on the C_{max}, $AUC_{0-\infty}$, and $t_{½}$ of dasabuvir and M1. Bars represent geometric mean ratios to the placebo phase (%) with 90% confidence intervals. *** P < 0.001 compared with the placebo phase.

Compared with ritonavir only, the combination of ritonavir and clopidogrel increased dasabuvir C_{max} and $AUC_{0-\infty}$ to 182% (P = 0.004, 90% CI 127%–261%) and 389% (P = $2 \cdot 10^{-6}$, 90% CI 275%–551%), respectively (**Figure 11**). Furthermore, the combination did not significantly affect the $AUC_{0-\infty}$ of M1 and thereby decreased the M1:dasabuvir $AUC_{0-\infty}$ ratio to 20% (P = $5 \cdot 10^{-10}$, 90% CI 17%–25%) of that during the ritonavir phase. In addition, the concurrent administration of ritonavir and clopidogrel prolonged the $t_{½}$ of dasabuvir and M1 from 8.2 to 11.5 hours (P = 0.005, geometric mean ratio 90% CI 114–173%) and from 7.3 to 14.1 hours (P = $1 \cdot 10^{-5}$, geometric mean ratio 90% CI 157–234%) of those in the ritonavir phase, respectively. When compared with placebo, ritonavir did not demonstrate significant alterations in the pharmacokinetics of dasabuvir or M1. Neither with nor without ritonavir, significant correlations did not exist between the $AUC_{0-\infty}$ of clopidogrel acyl-β-D-glucuronide and the fold-changes in dasabuvir $AUC_{0-\infty}$ or M1:dasabuvir $AUC_{0-\infty}$ ratio.

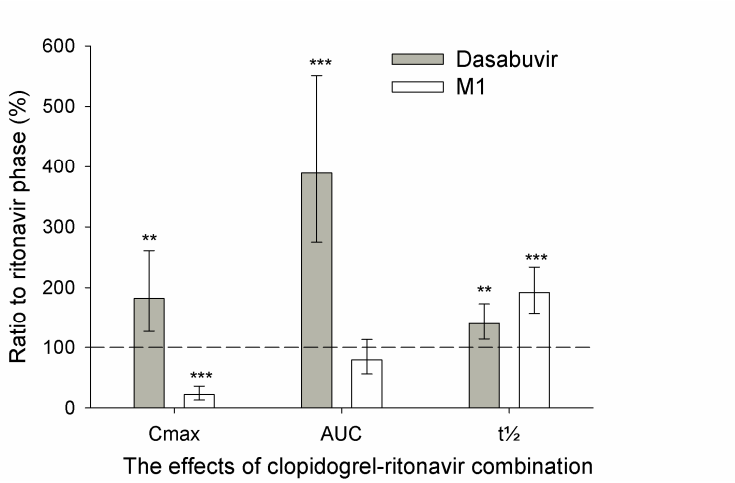

Figure 11. Effect of clopidogrel-ritonavir combination on the C_{max}, $AUC_{0-\infty}$, and $t_{½}$ of dasabuvir and M1. Bars represent geometric mean ratios to the placebo phase (%) with 90% confidence intervals. *** P < 0.001 compared with the ritonavir phase.

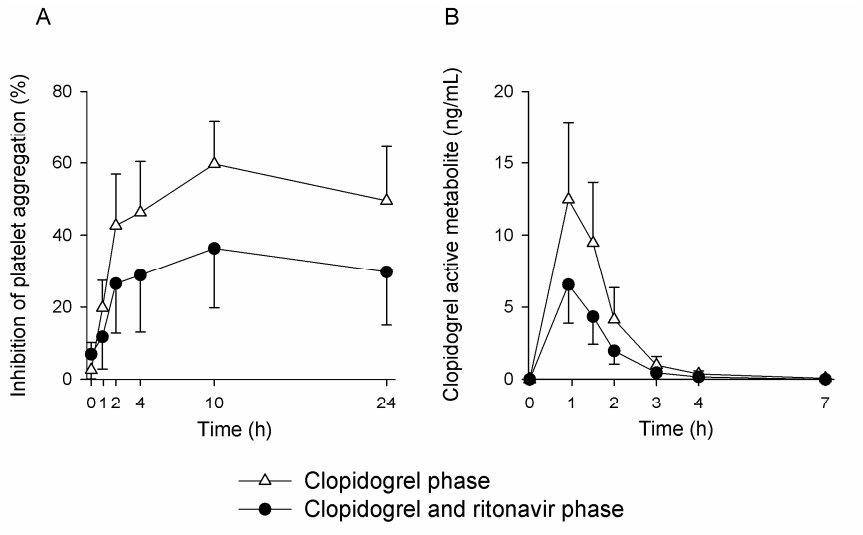

Figure 12. The mean percent inhibition of platelet aggregation (**A**) and plasma concentrations of clopidogrel active *cis*-5-thiol metabolite (**B**) after clopidogrel 300 mg. Data are presented as geometric means (except for the mean percent inhibition of platelet aggregation, which are given as arithmetic means) with 90% CI.

Ritonavir decreased the C_{max} and AUC_{0-4h} of clopidogrel active metabolite to 52% (P = 0.0003, 90% CI 41–65%) and 49% (P = 0.0001, 90% CI 39–61%) of those during placebo phase, respectively (**Figure 12A**). In addition, the average and maximal platelet inhibition decreased from 51% and 60% in the clopidogrel phase to 31% (P = 0.0007, SD -27 to -12); and 40% (P = 0.002, SD -29 to -11), respectively, by combining ritonavir to clopidogrel treatment.

5. Effect of clopidogrel and gemfibrozil on desloratadine pharmacokinetics (Study V)

In Study V, clopidogrel augmented the $AUC_{0-\infty}$ and C_{max} of parent desloratadine to 280% ($P = 3 \cdot 10^{-7}$, 90% CI 232–338%) and 165% (P = 0.0006; 90% CI 133–204%) of those during placebo phase, respectively (**Figure 13**). The corresponding increases by gemfibrozil were to 462% ($P = 4 \cdot 10^{-7}$; 90% CI 346–616%) and 174% (P = 0.0006; 90% CI 137–221%), respectively. Furthermore, the 3-hydroxydesloratadine:desloratadine AUC_{0-71h} ratios were 21% ($P = 7 \cdot 10^{-10}$, 90% CI 18–24%) and 1.7% ($P = 8 \cdot 10^{-11}$, 90% CI 1.3–2.4%) of placebo during the clopidogrel and gemfibrozil phases, respectively (**Figure 14**). The $t_{½}$ of desloratadine was prolonged from 17 hours to 26 hours (P = 0.0003, geometric mean ratio 90% CI 130–180%) and to 39 hours ($P = 3 \cdot 10^{-8}$, geometric mean ratio 90% CI 200–250%) by clopidogrel and gemfibrozil, respectively. In addition, gemfibrozil delayed the 3-hydroxydesloratadine t_{max} from 4.0 hours in the placebo phase to 11.0 hours (P = 0.009). Clopidogrel and gemfibrozil decreased the 3-hydroxydesloratadine-O-glucuronide C_{max} to 37% ($P = 3 \cdot 10^{-7}$, 90% CI 31–45%) and to 4% ($P = 3 \cdot 10^{-8}$, 90% CI 2–6%), and diminished its AUC_{0-71h} to 55% ($P = 2 \cdot 10^{-5}$, 90% CI 46–65%) and 6% ($P = 7 \cdot 10^{-8}$, 90% CI 4–10%) of those in the placebo phase, respectively. In addition, the t_{max} of 3-hydroxydesloratadine-O-glucuronide was delayed from 9.0 to 11.0 hours (P = 0.03). In the gemfibrozil phase, the plasma concentrations of 3-hydroxydesloratadine and 3-hydroxydesloratadine-O-glucuronide were too low for reliable determination of their $t_{½}$ and $AUC_{0-\infty}$.

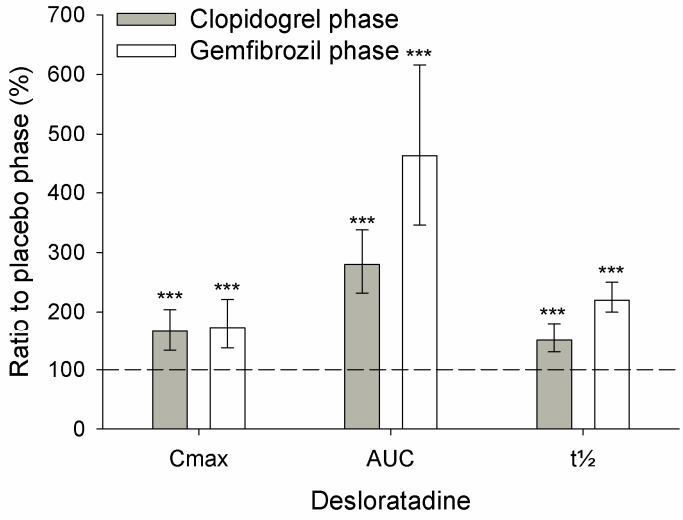

Figure 13. Effect of clopidogrel and gemfibrozil on the C_{max}, $AUC_{0-\infty}$, and $t_{½}$ of desloratadine. Bars represent geometric mean ratios to the placebo phase (%) with 90% confidence intervals. *** P < 0.001 compared with placebo the phase.

Compared to clopidogrel phase, the $AUC_{0-\infty}$ of desloratadine was 165% ($P = 2 \cdot 10^{-5}$, 90% CI 142–191%) in the gemfibrozil phase. Furthermore, in the gemfibrozil phase, 3-hydroxydesloratadine AUC_{0-71h} was 12% ($P = 1 \cdot 10^{-8}$, 90% CI 9–16%) and the 3-hydroxydesloratadine:desloratadine AUC_{0-71h} ratio was 8% ($P = 7 \cdot 10^{-10}$, 90% CI 7–11%), when compared with those in the clopidogrel phase. Moreover, in the gemfibrozil phase, the AUC_{0-71h} of 3-hydroxydesloratadine-O-glucuronide was 11% ($P = 1 \cdot 10^{-8}$, 90% CI 9–15%) of that in the clopidogrel phase. A significant (Pearson two-tailed P = 0.002, $R^2 = 0.80$) correlation existed between the fold-change in the $AUC_{0-\infty}$ of desloratadine caused by clopidogrel and that by

gemfibrozil. There were no significant correlations between the plasma concentrations of clopidogrel acyl-β-D-glucuronide or gemfibrozil 1-O-glucuronide, and alterations in desloratadine pharmacokinetics. Furthermore, regarding the pharmacokinetics of desloratadine or its metabolites, none of the subjects was an outlier that could have been considered to exhibit desloratadine "poor metabolizer" phenotype.

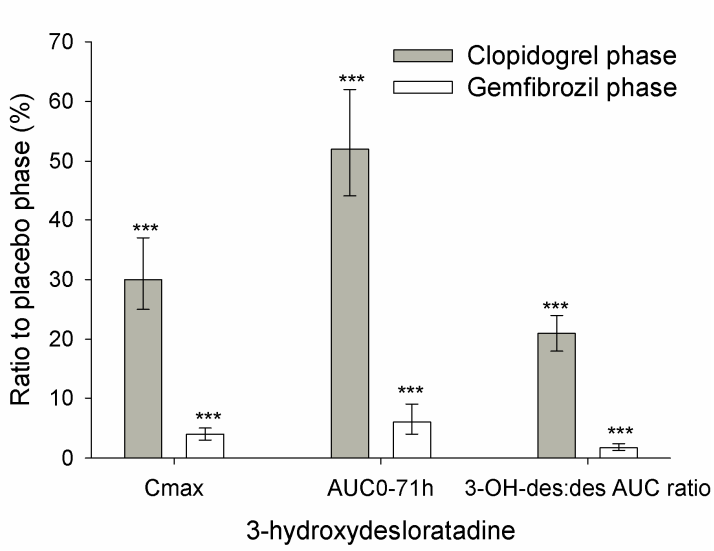

Figure 14. Effect of clopidogrel and gemfibrozil on the C_{max}, $AUC_{0\text{-}71h}$, and 3-hydroxydesloratadine:desloratadine $AUC_{0\text{-}71h}$ that depicts the CYP2C8 activity. Bars represent geometric mean ratios to the placebo phase (%) with 90% confidence intervals. *** $P < 0.001$ compared with the placebo phase.

DISCUSSION

1. Methodological considerations

This work sought to provide data that is applicable in clinical DDI studies and patient care, and therefore, the studies were exclusively conducted *in vivo* in humans. The five pharmacokinetic studies included in this work were randomized, prospective, and placebo-controlled in nature. As subjects' or researchers' expectations have no meaningful effect on pharmacokinetics, blinding was not considered necessary, especially when the safety of the subjects was improved with this practice. Furthermore, carry-over effects due to other reasons were also considered mechanistically unconvincing. Every study used a crossover design, where the subjects acted as their own controls, which decreased variance in the pharmacokinetic parameters. To ensure the elimination of the investigated drugs and the recovery of enzyme activity after inhibitor exposure, study phases were separated by a wash-out period of two to three weeks. The confounding factors were kept to a minimum by recruiting young adults who were healthy, nonsmoking, and unmedicated. Depending on the drug, comorbid patients from relevant populations might demonstrate pharmacokinetics that differ from those of healthy individuals. These differences are modest for the drugs investigated, however, and thus the results can be applied in real-world circumstances. To ensure compliance, the majority of the pretreatments and all the victim drugs were administered under supervised conditions, and the perpetrator concentrations were determined from plasma samples. Furthermore, when the researchers did not administer ritonavir or gemfibrozil in Studies IV or V, respectively, the subjects documented the exact time of ingestion for these pretreatments.

The dosing regimens of the examined drugs were selected to match clinical settings. In situations where rapid $P2Y_{12}$ antagonism and antiplatelet effect are required, clopidogrel is routinely commenced with a 300 mg (or even 600 mg) loading dose, and the treatment is most often continued with a 75 mg maintenance dose (FDA 2010), and this dosing protocol was also applied in the studies. Although the dose of clopidogrel affects its potential to alter the metabolism of victim drugs, the difference between the effect of the loading and maintenance dose is not drastic. For example, the 300 mg dose increased repaglinide AUC 5.1-fold, whereas the 75 mg dose augmented it 3.9-fold (Tornio et al. 2014). As concentration-dependent adverse effects can occur rapidly after initiating a perpetrator drug to a patient already using a victim a drug, the dosing protocol of clopidogrel applied in the studies was appropriate. Accordingly, prasugrel, ritonavir, and gemfibrozil, as well as all the victim drugs, were administered in clinically used doses.

To control the effect of food on pharmacokinetics, the subjects were administered standard meals after an overnight fast during the study days. During the studies, use of other medications, nutritional supplements, and grapefruit products was prohibited to avoid pharmacodynamic and pharmacokinetic interactions. The sampling schedules of each study were designed to match the pharmacokinetic profile of the drugs under investigation. Whenever the terminal $t_{½}$ of the drugs or their metabolites could be reliably determined, the AUC was extrapolated to infinity in order to reflect potential changes in dose interval AUC in steady-state conditions. In Study I, the part of the AUC extrapolated to infinity exceeded 20% in certain study phases, and therefore, $AUC_{0\text{-}12h}$ was the main reported variable to reflect drug exposure. However, when the exposure of the drugs and their metabolites were reported in the other studies, the percentage of AUC extrapolated to infinity was lower than 20% or even 5%. The statistical parameters and methods were applied in the studies by following the general guidelines recommended for pharmacokinetic research (Clinical Pharmacology and Therapeutics Editorial Team 2010, FDA 2017a).

2. Interpretations of the study results

2.1. Implications for pharmacokinetic and drug-drug interaction studies

2.1.1. Study I

Preceding Study I, an epidemiological paper associated clopidogrel use with increased risk of myotoxicity from cerivastatin, which is a CYP2C8 and OATP1B1 substrate (Floyd et al. 2012). Clopidogrel was also observed to increase the exposure of BCRP/OATP1B1 and CYP2C8/CYP3A4/OATP1B1 substrates rosuvastatin and repaglinide, respectively (**Table 10**) (Pinheiro et al. 2012, Remsberg et al. 2013, Tornio et al. 2014). Furthermore, *in vitro* studies and PBPK simulations suggested that in addition to inhibiting CYP2C8, clopidogrel could also inhibit OATP1B1 and CYP3A4 (Floyd et al. 2012, Tamraz et al. 2013, Tornio et al. 2014). Accordingly, Study I was performed in order to clarify the DDI mechanisms of clopidogrel, especially regarding its potential to inhibit OATP1B1 and CYP3A4, which play crucial roles in simvastatin pharmacokinetics (Backman et al. 2000, Neuvonen et al. 1998, Niemi et al. 2011, Prueksaritanont et al. 1997, Prueksaritanont et al. 2003).

In Study I, neither the 300 mg loading dose, nor the 75 mg maintenance dose of clopidogrel affected simvastatin pharmacokinetics to a meaningful extent. The modest increases in the $AUC_{0\text{-}2h}$ of simvastatin and simvastatin acid caused by the higher clopidogrel dose were the only conspicuous alterations observed. *In vitro* experiments have reported that the parent clopidogrel is a weak time-dependent inhibitor of CYP3A4 (Tornio et al. 2014), and the unsubstantially augmented $AUC_{0\text{-}2h}$ by the higher clopidogrel dose could be caused by weak inhibition of intestinal CYP3A4. However, since potent CYP3A4 inhibitors can raise simvastatin exposure up to 20-fold (Neuvonen et al. 1998, Neuvonen et al. 2006), clopidogrel is not suspected to markedly influence the pharmacokinetics of other CYP3A4 substrates, either. Furthermore, the results from Study I indicate that OATP1B1 inhibition is very unlikely to have a meaningful role in the DDIs caused by clopidogrel. This notion is corroborated by a clinical report published after Studies I and II, which observed no changes in the OATP1B1 substrate pitavastatin exposure by clopidogrel (Kim et al. 2016). These findings lead to the conclusion that CYP2C8 inhibition by clopidogrel acyl-β-D-glucuronide is the principal mechanism in the clopidogrel-cerivastatin and clopidogrel-repaglinide DDIs. In addition, the previously observed clopidogrel-rosuvastatin DDI is implausibly due to OATP1B1 inhibition. Because BCRP and NTCP also contribute to the disposition of rosuvastatin (Ho et al. 2006, Huang et al. 2006, Kitamura et al. 2008), their altered activity could hypothetically explain the augmented rosuvastatin exposure by clopidogrel.

2.1.2. Study II

After Study I had concluded that CYP2C8 inhibition is the predominant DDI mechanism of clopidogrel, its effect on pharmacokinetics of specific CYP2C8 substrates required exploration. Pioglitazone metabolism is mediated mainly by CYP2C8, whereas CYP3A or other individual enzymes have only a minor role in it. Therefore, Study II continued investigating clopidogrel's CYP2C8 inhibitory effect with pioglitazone as a victim drug.

Clopidogrel increased the mean pioglitazone exposure 2.1-fold while the $AUC_{0\text{-}\infty}$ of its primary M-IV and secondary M-III metabolites remained unaffected. Accordingly, the M-IV:pioglitazone $AUC_{0\text{-}\infty}$ ratio, which reflects the CYP2C8-mediated metabolism of pioglitazone, was decreased to 49%, when

compared with placebo. The concentration of pioglitazone at 24 hours after its ingestion, which corresponds to its trough concentration, was 450% of that in the placebo phase. The carriers of the *CYP2C8*3* allele exhibited the highest fold-increases in pioglitazone exposure, but the statistical power of Study II was insufficient to draw firm conclusions about genotype effects.

A small-dose study recently corroborated the results from Study II by observing a 2.0-fold increase in pioglitazone exposure by clopidogrel 300 mg (Kim et al. 2016). In three previous studies, gemfibrozil increased mean pioglitazone exposure 3.2–4.3-fold (**Table 10**) (Aquilante et al. 2013, Deng et al. 2005, Jaakkola et al. 2005). Furthermore, one of the studies observed a mean 5.2-fold increase in pioglitazone AUC in 15 subjects carrying the *CYP2C8*3* allele, compared with a mean 3.3-fold increase in 15 *CYP2C8*1* homozygotes (Aquilante et al. 2013). When the observations from the clinical studies with pioglitazone and clopidogrel or gemfibrozil are combined, they suggest an average $f_{mCYP2C8}$ of approximately 60–80% for pioglitazone, which is consistent with the estimation by the *in vivo-in vitro* prediction model of Study II. Somewhat surprisingly, inhibiting CYP3A4, the secondary enzyme in pioglitazone metabolism, does not lead to markedly increased pioglitazone exposure, even if CYP2C8 is already almost completely inactivated by gemfibrozil (Jaakkola et al. 2005). As the vast majority of ingested pioglitazone is metabolized and no other enzymes are recognized to be clinically important in its metabolism (Eckland and Danhof 2000, Jaakkola et al. 2006a), inhibition of CYP2C8 has a smaller effect on pioglitazone exposure than could be expected. Despite these ambiguities in the pharmacokinetics of pioglitazone, its relatively benign adverse-effect profile and CYP2C8-selectivity make it a potential index substrate. Pioglitazone's mediocre sensitivity to alterations in CYP2C8 activity limits its usefulness for the purpose, however, especially in DDIs where the perpetrator is a weak to moderate inhibitor or inducer of CYP2C8 (**Table 9**).

2.1.3. Study III

Previous DDI studies have suggested montelukast as a sensitive CYP2C8 substrate owing to the marked increase in its exposure by gemfibrozil. Due to the complexities of montelukast pharmacokinetics and DDI mechanisms of gemfibrozil, further studies were required. Accordingly, Study III was conducted to clarify the role of CYP2C8 in montelukast pharmacokinetics, and to compare the CYP2C8 inhibition potential of clopidogrel to that of prasugrel.

In Study III, clopidogrel increased the exposure of montelukast 2.0-fold, and reduced the M6:montelukast $AUC_{0\text{-}7h}$ ratio, which reflects the CYP2C8-mediated metabolism of montelukast, to 45% of those in the placebo phase. Based on previous clinical data and the PBPK simulations of Study III, OATP1B1 inhibition is unlikely to contribute to the observed effect of clopidogrel on montelukast pharmacokinetics. Prasugrel lacked any clinically important effects on montelukast pharmacokinetics, which makes it an unlikely perpetrator to cause marked changes in CYP2C8 activity. The minor (from 4.5 to 5.2 hours) prolongation of montelukast $t_{½}$ caused by prasugrel might be a random finding, considering that the difference barely reached the level of significance while other pharmacokinetic variables of montelukast remained unaffected.

In Study III, the observed 2.0-fold increase in montelukast AUC by clopidogrel is smaller than could be predicted based on previous DDI studies, where gemfibrozil has increased montelukast exposure 4.3–4.5-fold (**Table 10**) (Karonen et al. 2010, Karonen et al. 2012). CYP2C8 inhibition has been mainly accounted for the effect of gemfibrozil on montelukast disposition, but a PBPK modeling study also suggested a role for the OATP1B1-inhibitory property of gemfibrozil (Varma et al. 2017). As clopidogrel

inhibits CYP2C8 but not OATP1B1 *in vivo* in humans, the results from Study III imply that the $f_{mCYP2C8}$ of montelukast averages approximately 0.55–0.70, which is smaller than previously thought. Therefore, one or several mechanisms, such as membrane transporter inhibition, plausibly complement CYP2C8 inhibition in the gemfibrozil-montelukast DDI. As gemfibrozil has decreased repaglinide glucuronidation via UGT1A1 and UGT1A3 inhibition *in vitro* (Gan et al. 2010), and as a GWAS has implied that UGT1A3 significantly participates in montelukast metabolism (Hirvensalo et al. 2018), UGT1A3 inhibition by gemfibrozil could theoretically complement its DDI with montelukast. In any case, montelukast's modest specificity and sensitivity to alterations in CYP2C8 activity make it a sub-optimal CYP2C8 probe substrate.

2.1.4. Study IV

In 2015, dasabuvir received marketing approval as a part of ritonavir-containing four-drug regimen indicated for the treatment of HCV genotype 1 infection. The pre-marketing clinical studies found that in the presence of ritonavir, gemfibrozil increases the AUC and $t_{½}$ of dasabuvir approximately 11- and 18-fold, respectively (King et al. 2017, Menon et al. 2015). Without concurrently administered ritonavir, however, the effect of gemfibrozil on dasabuvir metabolism was not investigated, nor was the $f_{mCYP2C8}$ of dasabuvir. Furthermore, CYP3A4 was considered to participate significantly in the metabolism of dasabuvir without concomitantly administered ritonavir. Moreover, the potential of ritonavir to modify the pharmacokinetics and antiplatelet action of clopidogrel was also uninvestigated. Accordingly, Study IV was conducted to examine the effect of clopidogrel on dasabuvir pharmacokinetics with and without ritonavir, and to elucidate the impact of ritonavir on clopidogrel metabolism and antiplatelet efficacy.

In the clopidogrel phase, the exposure of dasabuvir and the M1:dasabuvir AUC ratio, which reflects the CYP2C8-mediated metabolism of dasabuvir, were 470% and 17% of those in the placebo phase, respectively. Compared with the ritonavir phase, the corresponding changes produced by the clopidogrel-ritonavir combination were 390% and 20%, respectively. When compared to the placebo phase, an insignificant trend of decreased dasabuvir concentrations was observed in the ritonavir phase. Furthermore, in the clopidogrel-ritonavir phase, the C_{max} and $AUC_{0\text{-}4h}$ of clopidogrel active metabolite were 52% and 49% of those in the clopidogrel phase, respectively. In addition, ritonavir markedly diminished the antiplatelet effect of clopidogrel.

The previous DDI studies with gemfibrozil and dasabuvir combined with the results from Study IV indicate that dasabuvir is among the most sensitive known CYP2C8 substrates with an $f_{mCYP2C8}$ of > 90% (**Table 10**). According to previous data, dasabuvir 500 mg (twice the dose applied in Study IV) failed to cause significant QTc prolongation (EMA 2014), and its C_{max} was comparable to that by concurrently administered gemfibrozil with dasabuvir 250 mg (Menon et al. 2015). While supratherapeutic dasabuvir exposure is still suggested to possess potential to prolong the QTc interval (FDA 2014b), these implications are not based on publicly available data. Accordingly, dasabuvir could be applied as a CYP2C8 index substrate in clinical DDI studies, if the dosage is appropriately adjusted to ensure the safety of studied subjects, for example, regarding the risk of cardiac adverse events (**Table 9**). Based on current knowledge, it is challenging to give an exact estimate for the aforementioned dosage reduction of dasabuvir for the purposes of DDI studies, because its $f_{mCYP2C8}$ in subtherapeutic doses should first be determined. However, examining the impact of strong CYP2C8 inhibitors on dasabuvir 50 mg (20% of the clinical dose) could be a reasonable starting point.

The role of CYP3A4 in dasabuvir metabolism seems to be of minor importance, because if CYP3A4 would significantly contribute to dasabuvir pharmacokinetics, coadministering ritonavir would accentuate the

effect of CYP2C8 inhibitors on dasabuvir metabolism. As this was not the case in Study IV where ritonavir slightly reduced the impact of clopidogrel-mediated CYP2C8 inhibition on dasabuvir pharmacokinetics, CYP3A4 seems to metabolize dasabuvir only to a negligible extent, even without coadministered ritonavir. The decreasing effect of ritonavir on dasabuvir concentrations lacks an unequivocal mechanistic explanation. A PBPK modeling study explained the increase in dasabuvir clearance observed with concurrent administration of ritonavir by hypothetical CYP2C8 induction (Shebley et al. 2017a), but this claim is unsupported by available clinical data. Moreover, the theoretical CYP2C8 induction by ritonavir should make the pharmacokinetics of dasabuvir more susceptible to the effects of CYP2C8 inhibitors, which was not observed in Study IV. Alternatively, induction of P-gp by ritonavir has been suggested to account for the decrease in dasabuvir exposure (Varma et al. 2019), but the concurrent administration of ritonavir and dasabuvir in 3D regimen would likely result in inhibition, not induction, of P-gp. The approximately 5-fold increase in dasabuvir exposure by clopidogrel support classifying the latter as strong CYP2C8 inhibitor (FDA 2017a). Furthermore, the exposure of clopidogrel active metabolite and its thrombocyte aggregation inhibition were significantly reduced by ritonavir, which corroborates the importance of CYP3A4 in clopidogrel bioactivation.

2.1.5. Study V

In 2015, an *in vitro* study proposed an obligatory role for CYP2C8 in the formation of the 3-hydroxy metabolite of desloratadine (Kazmi et al. 2015), which possesses a good safety profile and could therefore act as a CYP2C8 index substrate. Furthermore, the CYP2C8 inhibition potency of clopidogrel had not been compared to that of gemfibrozil. Therefore, Study V was performed in order to examine the role of CYP2C8 in desloratadine metabolism *in vivo* in humans, and to compare head-to-head the magnitude of CYP2C8 inhibition produced by clopidogrel and gemfibrozil.

Clopidogrel increased desloratadine exposure to 280% and decreased the 3-hydroxydesloratadine:desloratadine $AUC_{0\text{-}71h}$ ratio to 21%, when compared with those in the placebo phase. Gemfibrozil altered the same parameters to 460% and 2% of those in the placebo phase, respectively. Furthermore, in the gemfibrozil phase, desloratadine exposure and the 3-hydroxydesloratadine:desloratadine $AUC_{0\text{-}71h}$ ratio were 160% and 8% of those in the clopidogrel phase, respectively (**Table 10**). Consistently with Studies II–IV and previous reports on gemfibrozil-mediated DDIs (Backman et al. 2016), these findings indicate that clinical doses of clopidogrel cause about 80% inhibition of CYP2C8 activity, while those of gemfibrozil almost completely inactivate the enzyme.

The biotransformation of desloratadine to its 3-hydroxy metabolite involves three steps, as suggested by Kazmi and colleagues: First, UGT2B10 glucuronidizes desloratadine forming an *N*-glucuronide metabolite, which is then rapidly 3-hydroxylated by CYP2C8, and finally the glucuronide moiety is deconjugated during, or right after, the CYP2C8-mediated 3-hydroxylation (**Figure 4**) (Kazmi et al. 2015). The proposed metabolism route seems convincing, since desloratadine was not 3-hydroxylated by CYP2C8 unless coincubated with UGT2B10 *in vitro*. The results from Study V corroborate the crucial role of CYP2C8 in 3-hydroxydesloratadine formation, as the exposure of this metabolite was significantly decreased by clopidogrel and almost abolished by gemfibrozil, and gemfibrozil or clopidogrel are not known to inhibit UGT2B10. As the formation of 3-hydroxydesloratadine is also dependent on UGT2B10 activity, subjects with low UGT2B10 function may be relatively insensitive to changes in desloratadine pharmacokinetics by CYP2C8 inhibitors. Low UGT2B10 activity could mechanistically, and consistently, explain the desloratadine “poor metabolizer” phenotype, which has a prevalence range from 2% in Caucasians and Hispanics to 17% in African Americans (Prenner et al. 2006). As genotypes that result in

lowered UGT2B10 activity and the desloratadine "poor metabolizer" phenotype are observed in similar frequencies in corresponding ethnic populations (Berg et al. 2010, Chen et al. 2010, Prenner et al. 2006), this unconfirmed hypothesis seems plausible. For these reasons, the applicability of desloratadine as a CYP2C8 index substrate may be compromised in populations with high prevalence of *UGT2B10* loss-of-function alleles. Furthermore, if a hypothetical perpetrator drug would inhibit UGT2B10 instead of CYP2C8, using desloratadine as a CYP2C8 probe substrate could lead to false conclusions on the DDI profile of the perpetrator. On the other hand, the benign safety profile and lack of CYP or transporter inhibition could support its use for the purpose (**Table 9**).

In the DDIs observed in Study V, both the perpetrator and victim compounds are glucuronide metabolites. Therefore, these results highlight the importance of screening glucuronides as potential interacting molecules in DDI studies, especially when CYP2C8-mediated DDIs are examined. According to the findings from Study V and previous research, gemfibrozil is a more potent CYP2C8 inhibitor than clopidogrel in clinically relevant doses. However, clopidogrel could be applied as an optional CYP2C8 index inhibitor in situations where examining the CYP2C8-mediated metabolism of a dual CYP2C8/OATP1B1 substrate is of special interest, in which gemfibrozil is a suboptimal CYP2C8 index inhibitor. It should be noted that if clopidogrel is employed as a CYP2C8 index inhibitor, its CYP2B6-inhibitory properties should be taken into account when the DDI data is being interpreted.

Table 9. Benefits, disadvantages, and clinically most important risks related to supratherapeutic exposure of potential CYP2C8 index substrates.

	Benefits as a CYP2C8 index substrate	Disadvantages as a CYP2C8 index substrate	Most important risks of DDIs with CYP2C8 inhibitors in clinical practise
Dasabuvir	High $f_{mCYP2C8}$ and sensitivity to CYP2C8 inhibition	Adverse event characteristics that requires subtherapeutic dosage	Decreased hemoglobin levels; potential for QTc prolongation
Desloratadine	High sensitivity to CYP2C8 inhibition in most populations; benign adverse effect profile	UGT2B10-mediated glucuronidation preceding oxidation by CYP2C8; may not be suited in populations where *UGT2B10* loss-of-function alleles are prevalent	Dry mouth, fatigue
Montelukast	Good adverse effect profile in single doses	Metabolism by UGT1A3; intermediate $f_{mCYP2C8}$	Headache; neuropsychiatric adverse effects
Pioglitazone	Average-high $f_{mCYP2C8}$; well-tolerated in single doses	Mediocre sensitivity to CYP2C8 inhibition	Fluid retention that can aggravate illnesses
Repaglinide	High sensitivity to CYP2C8 inhibition	OATP1B1- and CYP3A4-mediated disposition; adverse event profile that requires subtherapeutic dosage	Hypoglycemia; GI adverse events

2.2. Clinical implications

This work has several implications of clinical importance. The specific findings concern the investigated drug combinations and the safety of their use, and the general ones concern all drugs that have similar DDI profile than the drugs examined. Studies II and IV had the most relevant findings concerning patient safety, whereas the data from other studies are informative mainly for clinical DDI research.

In Study IV, the clopidogrel-ritonavir combination increased dasabuvir exposure approximately 4-fold on average, while the largest increase was about 8-fold, when compared with ritonavir alone. Furthermore, the combination raised dasabuvir C_{max} about 2-fold; however, the increase could be plausibly more pronounced in continuous administration. Phase II trials, which investigated dasabuvir safety, implied that its supratherapeutic exposures are associated with hemoglobin decrease and potential to prolong the QTc interval, and therefore, concurrent administration of gemfibrozil and dasabuvir is contraindicated (FDA 2014b). In contrast, after PBPK simulations suggested a 2–3-fold increase in dasabuvir exposure by clopidogrel (Shebley et al. 2017a), the United States Food and Drug Administration (FDA) did not contraindicate their concomitant use (Arya et al. 2017). A PBPK analysis criticized the findings of Study IV by arguing that there were two major limitations in the study design, and thereby concluded that coadministration of clopidogrel and dasabuvir should not be contraindicated (Shebley 2019). First, the 300 mg loading dose of clopidogrel was considered to markedly affect the extent of the clopidogrel-dasabuvir DDI observed in Study IV, and second, the two-day pretreatment was regarded to be insufficient to achieve full CYP2C8 induction by ritonavir, which would hypothetically attenuate the effect of clopidogrel on dasabuvir pharmacokinetics. The rationale, however, has several major inconsistencies (Itkonen et al. 2019). The CYP2C8-inductive effect of ritonavir, which is unsupported by clinical data published in scientific journals, should make dasabuvir more susceptible to CYP2C8 inhibition, not the opposite as observed in Study IV. In addition, according to the referenced PBPK model by Shebley and colleagues, the increase in dasabuvir hepatic clearance by ritonavir reaches about 80% of the steady state effect at 48 hours after commencing ritonavir (the time when dasabuvir was administered in Study IV) (Shebley et al. 2017a). Furthermore, the difference in CYP2C8 inhibition caused by the 300 mg clopidogrel does not radically differ from that caused by clopidogrel 75 mg, as demonstrated by the approximately 20% difference in the increase in repaglinide AUC (5.1-fold vs. 3.9-fold) by the two dosages (Tornio et al. 2014). Therefore, the results from the aforementioned PBPK simulations cannot be used to refute the clinical observations from Study IV. Study IV suggests contraindicating the clopidogrel-dasabuvir combination, or at least, recommends caution when they are coadministered, especially if the patient possesses additional risk factors for the adverse events of dasabuvir, such as electrolyte imbalances, polypharmacy with QTc prolonging drugs, or disturbances in bone marrow function.

Study IV observed a significantly attenuated antiplatelet effect of clopidogrel and a halving of the C_{max} and $AUC_{0\text{-}4h}$ of its active metabolite caused by ritonavir. These findings imply that ritonavir can markedly diminish the antithrombotic efficacy of clopidogrel, which is consistent with previous reports on patients using ritonavir and other strong CYP3A4 inhibitors concurrently with clopidogrel (Farid et al. 2007, Holmberg et al. 2014, Marsousi et al. 2018, Metzger and Momary 2014). Therefore, clopidogrel should not be the primarily prescribed $P2Y_{12}$ receptor antagonist for patients on ritonavir therapy.

The concentration-dependent adverse effects of pioglitazone include fluid retention that can lead to worsening of congestive heart failure and even pulmonary edema (Waugh et al. 2006), and therefore, European Medicines Agency (EMA) warns about coadministration of pioglitazone with gemfibrozil. Accordingly, the mean 2.1-fold and 4.5-fold increases in pioglitazone exposure and trough concentration, respectively, caused by clopidogrel also warrant caution if the drugs are used

concomitantly. Similar to clopidogrel-dasabuvir combination, the risks of coadministering clopidogrel with pioglitazone are higher in patients who are predisposed to adverse effects of the victim drug by other factors.

Study I showed that concurrent use of clopidogrel is unlikely to lead to increased risk for adverse effects of simvastatin due to pharmacokinetic reasons. Furthermore, despite the reported adverse effects of montelukast, most notably neuropsychiatric symptoms, the 2-fold increase in its AUC by clopidogrel observed in Study III is unlikely to substantially worsen the safety profile of montelukast. In addition, prasugrel seems to be a safe alternative to clopidogrel concerning its CYP2C8-inhibitory DDI potential. Moreover, the DDIs of desloratadine with clopidogrel and gemfibrozil reported in Study V are unlikely to cause relevant adverse effects. This interpretation is further supported by the fact that 3-hydoxydesloratadine also possesses H1 receptor antagonistic properties (Murdoch et al. 2003), which implies that CYP2C8 inhibition implausibly compromises the clinical efficacy of desloratadine.

Table 10. A comparison of the fold-changes (R) in pharmacokinetic variables of CYP2C8 substrates (given in single doses) caused by gemfibrozil and clopidogrel in Studies I–V and previous reports. Data are expressed as either arithmetic or geometric mean ratios with 90% or 95% confidence intervals (CI), or ranges.

Victim drug and its dose	Clopidogrel 300 mg followed by 75 mg q.d. ratio (R) to placebo (90% or 95% CI, or range)			Gemfibrozil 600 mg b.i.d. ratio (R) to placebo (90% or 95% CI, or range)		
	$AUC_{0\text{-}\infty}R$	$C_{max}R$	Reference	$AUC_{0\text{-}\infty}R$	$C_{max}R$	Reference
Dasabuvir						
250 mg	4.7 (90% CI 3.2–6.7)	1.6 (90% CI 1.0–2.6)	Study IV	11.3 (90% CI 9.1–14)	2.0 (90% CI 1.7–2.4)	(Menon et al. 2015)
Desloratadine						
5 mg	2.8 (90% CI 2.3–3.4)	1.7 (90% CI 1.3–2.0)	Study V	4.6 (90% CI 3.5–6.2)	1.7 (90% CI 1.4–2.2)	Study V
Montelukast						
10 mg	2.0 (90% CI 1.7–2.3)	0.98 (90% CI 0.8–1.2)	Study III	4.4 (95% CI 3.8–5.0)	1.5 (95% CI 1.3–1.8)	(Karonen et al. 2010)
				4.3 (95% CI 2.9–6.3)	1.5 (95% CI 0.9–2.3)	(Karonen et al. 2012)
Pioglitazone						
1 mg	2.0 (95% CI 1.6–2.5)[a]	not reported	Kim et al. (2016)			
15 mg	2.1 (90% CI 1.8–2.6)	1.0 (90% CI 0.8–1.4)	Study II	3.2 (range 2.3–6.5)	1.1 (range 0.5–2.6)	(Jaakkola et al. 2005)
				4.3 (95% CI 3.5–5.1)	1.3 (95% CI 1.0–1.6)	(Aquilante et al. 2013)
30 mg				3.4 (range 2.3–6.2)	not reported	(Deng et al. 2005)
Repaglinide						
0.1 mg	3.1 (95% CI 2.1–4.1)[b]	not reported	(Kim et al. 2016)			
0.25 mg	5.1 (90% CI 3.9–6.6)	2.5 (90% CI 1.8–3.5)	(Tornio et al. 2014)	8.1 (range 5.5–15)	2.4 (range 1.7–6.1)	(Niemi et al. 2003)
	3.9 (90% CI 2.9–5.3)[c]	2.0 (90% CI 1.3–3.1)[c]	(Tornio et al. 2014)	7.0 (range 2.9–14)	2.2 (range 1.4–2.9)	(Tornio et al. 2008)
				7.6 (range 4.2–12)	2.7 (range 1.5–3.7)	(Backman et al. 2009)
				7.0 (90% CI 6.0–8.1)	2.0 (90% CI 1.6–2.5)	(Honkalammi et al. 2012)

[a]$AUC_{0\text{-}24h}$ ratio. [b]$AUC_{0\text{-}8h}$ ratio. [c]Effect on day 3 of treatment with clopidogrel 300 mg on day 1 followed by 75 mg q.d. AUC_{0-9h}, area under the plasma concentration-time curve from 0 to 9 h; AUC_{0-24h}, area under the plasma concentration-time curve from 0 to 24 h; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 to infinity; C_{max}, peak plasma concentration.

CONCLUSIONS

Based on the results of this work, the following conclusions have been made

1. Clinical doses of clopidogrel do not decrease OATP1B1 or CYP3A4 activity, but instead cause strong CYP2C8 inhibition, which can lead to clinically relevant DDIs with CYP2C8 substrates.

2. Gemfibrozil is a stronger CYP2C8 inhibitor than clopidogrel. However, the latter may be used as a CYP2C8 index inhibitor in situations where the investigated victim drug is also a substrate of OATP1B1, which gemfibrozil, but not clopidogrel, inhibits.

3. Coadministering clopidogrel in clinical practise may lead to hazardous adverse events of dasabuvir and pioglitazone.

4. As the metabolism of dasabuvir is CYP2C8-mediated with high specificity and sensitivity, it can be applied as a CYP2C8 index substrate, but its dosage should be reduced from the therapeutic level to guarantee safety of subjects.

5. Montelukast, desloratadine, and pioglitazone are suboptimal CYP2C8 probe substrates due to their mediocre $f_{mCYP2C8}$, multiple metabolism routes, or both.

6. The concurrent use of strong CYP3A4 inhibitors and clopidogrel is not recommended, because they impair its antiplatelet effect, as demonstrated by ritonavir-clopidogrel interaction.

7. Prasugrel is unlikely to cause clinically relevant DDIs due to CYP2C8 inhibition.

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REFERENCES

Aarnoutse RE, Kleinnijenhuis J, Koopmans PP, Touw DJ, Wieling J, Hekster YA, and Burger DM. Effect of low-dose ritonavir (100 mg twice daily) on the activity of cytochrome P450 2D6 in healthy volunteers. *Clin Pharmacol Ther* (2005) **78**:664-674.

AbbVie (2018) Norvir Product Monograph https://www.abbvie.ca/content/dam/abbviecorp/ca/en/docs/NORVIR_PM_EN.pdf (Accessed February 27, 2019).

Accurso FJ, Rowe SM, Clancy JP, Boyle MP, Dunitz JM, Durie PR, Sagel SD, Hornick DB, Konstan MW, Donaldson SH, Moss RB, Pilewski JM, Rubenstein RC, Uluer AZ, Aitken ML, Freedman SD, Rose LM, Mayer-Hamblett N, Dong Q, Zha J, et al. Ramsey BW. Effect of VX-770 in persons with cystic fibrosis and the G551D-CFTR mutation. *N Engl J Med* (2010) **363**:1991-2003.

Amstutz U, Henricks LM, Offer SM, Barbarino J, Schellens JHM, Swen JJ, Klein TE, McLeod HL, Caudle KE, Diasio RB, and Schwab M. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing: 2017 Update. *Clin Pharmacol Ther* (2018) **103**:210-216.

Ancrenaz V, Deglon J, Samer C, Staub C, Dayer P, Daali Y, and Desmeules J. Pharmacokinetic interaction between prasugrel and ritonavir in healthy volunteers. *Basic Clin Pharmacol Toxicol* (2013) **112**:132-137.

Andersen O and Aaseth J. A review of pitfalls and progress in chelation treatment of metal poisonings. *J Trace Elem Med Biol* (2016) **38**:74-80.

Angiolillo DJ, Rollini F, Storey RF, Bhatt DL, James S, Schneider DJ, Sibbing D, So DYF, Trenk D, Alexopoulos D, Gurbel PA, Hochholzer W, De Luca L, Bonello L, Aradi D, Cuisset T, Tantry US, Wang TY, Valgimigli M, Waksman R, et al. Price MJ. International Expert Consensus on Switching Platelet P2Y12 Receptor-Inhibiting Therapies. *Circulation* (2017) **136**:1955-1975.

Aquilante CL, Bushman LR, Knutsen SD, Burt LE, Rome LC, and Kosmiski LA. Influence of SLCO1B1 and CYP2C8 gene polymorphisms on rosiglitazone pharmacokinetics in healthy volunteers. *Hum Genomics* (2008) **3**:7-16.

Aquilante CL, Kosmiski LA, Bourne DW, Bushman LR, Daily EB, Hammond KP, Hopley CW, Kadam RS, Kanack AT, Kompella UB, Le M, Predhomme JA, Rower JE, and Sidhom MS. Impact of the CYP2C8 *3 polymorphism on the drug-drug interaction between gemfibrozil and pioglitazone. *Br J Clin Pharmacol* (2013) **75**:217-226.

Arya V, Zhao P, Reynolds KS, Mishra P, and Younis IR. Utilizing PBPK modeling to evaluate the potential of a significant drug-drug interaction between clopidogrel and dasabuvir: A scientific perspective. *Clin Pharmacol Ther* (2017) **102**:578-580.

Backes JM, Gibson CA, Ruisinger JF, and Moriarty PM. Fibrates: what have we learned in the past 40 years? *Pharmacotherapy* (2007) **27**:412-424.

Backman JT, Filppula AM, Niemi M, and Neuvonen PJ. Role of cytochrome P450 2C8 in drug metabolism and interactions. *Pharmacol Rev* (2016) **68**:168-241.

Backman JT, Kyrklund C, Neuvonen M, and Neuvonen PJ. Gemfibrozil greatly increases plasma concentrations of cerivastatin. *Clin Pharmacol Ther* (2002) **72**:685-691.

Backman JT, Kyrklund C, Kivistö KT, Wang JS, and Neuvonen PJ. Plasma concentrations of active simvastatin acid are increased by gemfibrozil. *Clin Pharmacol Ther* (2000) **68**:122-129.

Backman JT, Honkalammi J, Neuvonen M, Kurkinen KJ, Tornio A, Niemi M, and Neuvonen PJ. CYP2C8 activity recovers within 96 hours after gemfibrozil dosing: estimation of CYP2C8 half-life using repaglinide as an in vivo probe. *Drug Metab Dispos* (2009) **37**:2359-2366.

Balani SK, Xu X, Pratha V, Koss MA, Amin RD, Dufresne C, Miller RR, Arison BH, Doss GA, Chiba M, Freeman A, Holland SD, Schwartz JI, Lasseter KC, Gertz BJ, Isenberg JI, Rogers JD, Lin JH, and Baillie TA. Metabolic profiles of montelukast sodium (Singulair), a potent cysteinyl leukotriene1 receptor antagonist, in human plasma and bile. *Drug Metab Dispos* (1997) **25**:1282-1287.

Barecki ME, Casciano CN, Johnson WW, and Clement RP. In vitro characterization of the inhibition profile of loratadine, desloratadine, and 3-OH-desloratadine for five human cytochrome P-450 enzymes. *Drug Metab Dispos* (2001) **29**:1173-1175.

Bean GW and Camras CB. Commercially available prostaglandin analogs for the reduction of intraocular pressure: similarities and differences. *Surv Ophthalmol* (2008) **53 (Suppl1)**:S69-84.

Benard B, Bastien V, Vinet B, Yang R, Krajinovic M, and Ducharme FM. Neuropsychiatric adverse drug reactions in children initiated on montelukast in real-life practice. *Eur Respir J* (2017) **50**:1700148.

Berg JZ, Mason J, Boettcher AJ, Hatsukami DK, and Murphy SE. Nicotine metabolism in African Americans and European Americans: variation in glucuronidation by ethnicity and UGT2B10 haplotype. *J Pharmacol Exp Ther* (2010) **332**:202-209.

Beutler E. Drug-induced hemolytic anemia. *Pharmacol Rev* (1969) **21**:73-103.

Bouman HJ, Schomig E, van Werkum JW, Velder J, Hackeng CM, Hirschhauser C, Waldmann C, Schmalz HG, ten Berg JM, and Taubert D. Paraoxonase-1 is a major determinant of clopidogrel efficacy. *Nat Med* (2011) **17**:110-116.

Bristol-Myers Squibb/ Sanofi Pharmaceuticals (2018) Highlights of Plavix prescribing information. http://products.sanofi.us/plavix/plavix.pdf (Accessed February 13, 2019).

Brown SM, Holtzman M, Kim T, and Kharasch ED. Buprenorphine metabolites, buprenorphine-3-glucuronide and norbuprenorphine-3-glucuronide, are biologically active. *Anesthesiology* (2011) **115**:1251-1260.

Bruderer S, Petersen-Sylla M, Boehler M, Remenova T, Halabi A, and Dingemanse J. Effect of gemfibrozil and rifampicin on the pharmacokinetics of selexipag and its active metabolite in healthy subjects. *Br J Clin Pharmacol* (2017) **83**:2778-2788.

Brunton L, Lazo J, and Parker K eds. (2006) *Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 13th Edition*, New York, NY.The McGraw-Hill companies.

Burchell B, Nebert DW, Nelson DR, Bock KW, Iyanagi T, Jansen PL, Lancet D, Mulder GJ, Chowdhury JR, and Siest G. The UDP glucuronosyltransferase gene superfamily: suggested nomenclature based on evolutionary divergence. *DNA Cell Biol* (1991) **10**:487-494.

CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. *Lancet* (1996) **348**:1329-1339.

Cardoso Jde O, Oliveira RV, Lu JB, and Desta Z. In vitro metabolism of montelukast by cytochrome P450s and UDP-glucuronosyltransferases. *Drug Metab Dispos* (2015) **43**:1905-1916.

Cavalli-Sforza LL and Bodmer WF. (1971) *The Genetics of Human Populations, Pp. 41 and 118.* WH Freeman & Co., San Francisco.

Chen G, Giambrone NE,Jr, Dluzen DF, Muscat JE, Berg A, Gallagher CJ, and Lazarus P. Glucuronidation genotypes and nicotine metabolic phenotypes: importance of functional UGT2B10 and UGT2B17 polymorphisms. *Cancer Res* (2010) **70**:7543-7552.

Chen Y and Goldstein JA. The transcriptional regulation of the human CYP2C genes. *Curr Drug Metab* (2009) **10**:567-578.

Cheng H, Leff JA, Amin R, Gertz BJ, De Smet M, Noonan N, Rogers JD, Malbecq W, Meisner D, and Somers G. Pharmacokinetics, bioavailability, and safety of montelukast sodium (MK-0476) in healthy males and females. *Pharm Res* (1996) **13**:445-448.

Chiba M, Xu X, Nishime JA, Balani SK, and Lin JH. Hepatic microsomal metabolism of montelukast, a potent leukotriene D4 receptor antagonist, in humans. *Drug Metab Dispos* (1997) **25**:1022-1031.

Clinical Pharmacology and Therapeutics Editorial Team. Statistical guide for Clinical Pharmacology & Therapeutics. *Clin Pharmacol Ther* (2010) **88**:150-152.

Coleman JA, Green EM, and Gouaux E. X-ray structures and mechanism of the human serotonin transporter. *Nature* (2016) **532**:334-339.

Congiu M, Mashford ML, Slavin JL, and Desmond PV. Coordinate regulation of metabolic enzymes and transporters by nuclear transcription factors in human liver disease. *J Gastroenterol Hepatol* (2009) **24**:1038-1044.

Cribb AE, Peyrou M, Muruganandan S, and Schneider L. The endoplasmic reticulum in xenobiotic toxicity. *Drug Metab Rev* (2005) **37**:405-442.

de Jong M, van der Worp HB, van der Graaf Y, Visseren FLJ, and Westerink J. Pioglitazone and the secondary prevention of cardiovascular disease. A meta-analysis of randomized-controlled trials. *Cardiovasc Diabetol* (2017) **16**:134-017-0617-4.

Deeks ED. Ombitasvir/paritaprevir/ritonavir plus dasabuvir: A review in chronic HCV genotype 1 infection. *Drugs* (2015) **75**:1027-1038.

Delaforge M, Pruvost A, Perrin L, and Andre F. Cytochrome P450-mediated oxidation of glucuronide derivatives: example of estradiol-17beta-glucuronide oxidation to 2-hydroxy-estradiol-17beta-glucuronide by CYP 2C8. *Drug Metab Dispos* (2005) **33**:466-473.

Delavenne X, Basset T, Zufferey P, Malouk N, Laporte S, and Mismetti P. Ultra-performance LC MS/MS method for quantification of clopidogrel active metabolite. *J Sep Sci* (2010) **33**:1968-1972.

Deng LJ, Wang F, and Li HD. Effect of gemfibrozil on the pharmacokinetics of pioglitazone. *Eur J Clin Pharmacol* (2005) **61**:831-836.

Ding X and Kaminsky LS. Human extrahepatic cytochromes P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annu Rev Pharmacol Toxicol* (2003) **43**:149-173.

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

Edwards DJ, Bellevue FH,3rd, and Woster PM. Identification of 6',7'-dihydroxybergamottin, a cytochrome P450 inhibitor, in grapefruit juice. *Drug Metab Dispos* (1996) **24**:1287-1290.

Eichelbaum M, Ingelman-Sundberg M, and Evans WE. Pharmacogenomics and individualized drug therapy. *Annu Rev Med* (2006) **57**:119-137.

Ekroos M and Sjogren T. Structural basis for ligand promiscuity in cytochrome P450 3A4. *Proc Natl Acad Sci U S A* (2006) **103**:13682-13687.

EMA (2014) Exviera: Public assessment report. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/003837/WC500182235.pdf (Accessed October 13, 2019).

EMA (2012) Guideline on the investigation of drug interactions. https://www.ema.europa.eu/documents/scientific-guideline/guideline-investigation-drug-interactions_en.pdf (Accessed January 15, 2019).

EMA (2010) Summary of product characteristics for Actos. https://www.ema.europa.eu/en/documents/product-information/actos-epar-product-information_en.pdf (Accessed February 28, 2019).

EMA (2009) Assessment Report for Efient. https://www.ema.europa.eu/documents/assessment-report/efient-epar-public-assessment-report_en.pdf (Accessed February 26, 2019).

EMA (2008) Summary of product characteristics for Plavix. https://www.ema.europa.eu/documents/product-information/plavix-epar-product-information_en.pdf (Accessed February 15, 2019).

EMA (2007) ICH Topic E15: Definitions for genomic biomarkers, pharmacogenomics, pharmacogenetics, genomic data and sample coding categories. Available at http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002880.pdf (Accessed February 15, 2019).

Evans DA, Manley KA, and McKusick VA. Genetic control of isoniazid metabolism in man. *Br Med J* (1960) **2**:485-491.

Evans WE and McLeod HL. Pharmacogenomics--drug disposition, drug targets, and side effects. *N Engl J Med* (2003) **348**:538-549.

Evans WE and Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* (1999) **286**:487-491.

Farid NA, Kurihara A, and Wrighton SA. Metabolism and disposition of the thienopyridine antiplatelet drugs ticlopidine, clopidogrel, and prasugrel in humans. *J Clin Pharmacol* (2010) **50**:126-142.

Farid NA, Smith RL, Gillespie TA, Rash TJ, Blair PE, Kurihara A, and Goldberg MJ. The disposition of prasugrel, a novel thienopyridine, in humans. *Drug Metab Dispos* (2007) **35**:1096-1104.

Farid NA, Payne CD, Small DS, Winters KJ, Ernest CS,2nd, Brandt JT, Darstein C, Jakubowski JA, and Salazar DE. Cytochrome P450 3A inhibition by ketoconazole affects prasugrel and clopidogrel pharmacokinetics and pharmacodynamics differently. *Clin Pharmacol Ther* (2007) **81**:735-741.

Farid NA, Jakubowski JA, Payne CD, Li YG, Jin Y, Ernest II CS, Winters KJ, Brandt JT, Salazar DE, and Small DS. Effect of rifampin on the pharmacokinetics and pharmacodynamics of prasugrel in healthy male subjects. *Curr Med Res Opin* (2009) **25**:1821-1829.

Farmer JA. Learning from the cerivastatin experience. *Lancet* (2001) **358**:1383-1385.

FDA (2017b) Center for Drug Evaluation and Research. In vitro metabolism and transporter mediated drug-drug interaction studies guidance for industry. https://www.fda.gov/downloads/Drugs/Guidances/UCM581965.pdf (Accessed January 15, 2019).

FDA (2017a) Center for Drug Evaluation and Research. Clinical drug interaction studies— Study design, data analysis, and clinical implications guidance for industry. https://www.fda.gov/downloads/drugs/guidances/ucm292362.pdf (Accessed January 15, 2019).

FDA (2016) Center for Drug Evaluation and Research. Product label for Lopid. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/018422s055lbl.pdf (Accessed February 25, 2019).

FDA (2014c) Center for Drug Evaluation and Research. Prescribing information for Clarinex. https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/021165s017,021300s014,021312s015,021563s003lbl.pdf (Accessed March 19, 2019).

FDA (2014b) Center for Drug Evaluation and Research. Clinical pharmacology and biopharmaceutics review(s) for Viekira Pak. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/206619Orig1s000ClinPharmR.pdf (Accessed March 12, 2019).

FDA (2014a) Center for Drug Evaluation and Research. Prescribing highlights for Viekira Pak. https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/206619lbl.pdf (Accessed March 12, 2019).

FDA (2013) Center for Drug Evaluation and Research. Effient approval package. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/022307Orig1s008.pdf (Accessed February 26, 2019).

FDA (2011) Center for Drug Evaluation and Research. Rilpivirine clinical pharmacology and biopharmaceutics review(s). https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202022Orig1s000ClinPharmR.pdf (Accessed February 6, 2019).

FDA (2010) Center for Drug Evaluation and Research. Plavix approval package. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2010/020839Orig1s042.pdf (Accessed February 12, 2019).

FDA (1998) Center for Drug Evaluation and Research. Pharmacology review(s) for Singulair. https://www.accessdata.fda.gov/drugsatfda_docs/nda/98/020829s000_Singular_Pharma.pdf (Accessed March 1, 2019).

Ferguson SS, Chen Y, LeCluyse EL, Negishi M, and Goldstein JA. Human CYP2C8 is transcriptionally regulated by the nuclear receptors constitutive androstane receptor, pregnane X receptor, glucocorticoid receptor, and hepatic nuclear factor 4alpha. *Mol Pharmacol* (2005) **68**:747-757.

Feuk L, Carson AR, and Scherer SW. Structural variation in the human genome. *Nat Rev Genet* (2006) **7**:85-97.

Fichtenbaum CJ, Gerber JG, Rosenkranz SL, Segal Y, Aberg JA, Blaschke T, Alston B, Fang F, Kosel B, Aweeka F, and NIAID AIDS Clinical Trials Group. Pharmacokinetic interactions between protease inhibitors and statins in HIV seronegative volunteers: ACTG Study A5047. *AIDS* (2002) **16**:569-577.

Filppula AM, Laitila J, Neuvonen PJ, and Backman JT. Reevaluation of the microsomal metabolism of montelukast: major contribution by CYP2C8 at clinically relevant concentrations. *Drug Metab Dispos* (2011) **39**:904-911.

Floyd JS, Kaspera R, Marciante KD, Weiss NS, Heckbert SR, Lumley T, Wiggins KL, Tamraz B, Kwok PY, Totah RA, and Psaty BM. A screening study of drug-drug interactions in cerivastatin users: an adverse effect of clopidogrel. *Clin Pharmacol Ther* (2012) **91**:896-904.

Foisy MM, Yakiwchuk EM, and Hughes CA. Induction effects of ritonavir: implications for drug interactions. *Ann Pharmacother* (2008) **42**:1048-1059.

Fontana E, Dansette PM, and Poli SM. Cytochrome p450 enzymes mechanism based inhibitors: common sub-structures and reactivity. *Curr Drug Metab* (2005) **6**:413-454.

Frotschl R, Chichmanov L, Kleeberg U, Hildebrandt AG, Roots I, and Brockmoller J. Prediction of aryl hydrocarbon receptor-mediated enzyme induction of drugs and chemicals by mRNA quantification. *Chem Res Toxicol* (1998) **11**:1447-1452.

Fujiwara R, Yokoi T, and Nakajima M. Structure and protein-protein interactions of human UDP-glucuronosyltransferases. *Front Pharmacol* (2016) **7**:388.

Fukuda K, Ohta T, Oshima Y, Ohashi N, Yoshikawa M, and Yamazoe Y. Specific CYP3A4 inhibitors in grapefruit juice: furocoumarin dimers as components of drug interaction. *Pharmacogenetics* (1997) **7**:391-396.

Gachet C, Stierle A, Cazenave JP, Ohlmann P, Lanza F, Bouloux C, and Maffrand JP. The thienopyridine PCR 4099 selectively inhibits ADP-induced platelet aggregation and fibrinogen binding without modifying the membrane glycoprotein IIb-IIIa complex in rat and in man. *Biochem Pharmacol* (1990) **40**:229-238.

Gammal RS, Court MH, Haidar CE, Iwuchukwu OF, Gaur AH, Alvarellos M, Guillemette C, Lennox JL, Whirl-Carrillo M, Brummel SS, Ratain MJ, Klein TE, Schackman BR, Caudle KE, Haas DW, and Clinical Pharmacogenetics Implementation Consortium. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for UGT1A1 and Atazanavir Prescribing. *Clin Pharmacol Ther* (2016) **99**:363-369.

Gan J, Chen W, Shen H, Gao L, Hong Y, Tian Y, Li W, Zhang Y, Tang Y, Zhang H, Humphreys WG, and Rodrigues AD. Repaglinide-gemfibrozil drug interaction: inhibition of repaglinide glucuronidation as a potential additional contributing mechanism. *Br J Clin Pharmacol* (2010) **70**:870-880.

Garcia-Martin E, Martinez C, Tabares B, Frias J, and Agundez JA. Interindividual variability in ibuprofen pharmacokinetics is related to interaction of cytochrome P450 2C8 and 2C9 amino acid polymorphisms. *Clin Pharmacol Ther* (2004) **76**:119-127.

Gessner A, Konig J, and Fromm MF. Clinical aspects of transporter-mediated drug-drug interactions. *Clin Pharmacol Ther* (2019) .

Ghosal A, Yuan Y, Hapangama N, Su AD, Alvarez N, Chowdhury SK, Alton KB, Patrick JE, and Zbaida S. Identification of human UDP-glucuronosyltransferase enzyme(s) responsible for the glucuronidation of 3-hydroxydesloratadine. *Biopharm Drug Dispos* (2004) **25**:243-252.

Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, Dahlin A, Evers R, Fischer V, Hillgren KM, Hoffmaster KA, Ishikawa T, Keppler D, Kim RB, Lee CA, Niemi M, Polli JW, Sugiyama Y, Swaan PW, Ware JA, et al. Zhang L. Membrane transporters in drug development. *Nat Rev Drug Discov* (2010) **9**:215-236.

Gilard M, Arnaud B, Cornily JC, Le Gal G, Lacut K, Le Calvez G, Mansourati J, Mottier D, Abgrall JF, and Boschat J. Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: the randomized, double-blind OCLA (Omeprazole CLopidogrel Aspirin) study. *J Am Coll Cardiol* (2008) **51**:256-260.

Gillies PS and Dunn CJ. Pioglitazone. *Drugs* (2000) **60**:333-43; discussion 344-5.

Graham DJ, Staffa JA, Shatin D, Andrade SE, Schech SD, La Grenade L, Gurwitz JH, Chan KA, Goodman MJ, and Platt R. Incidence of hospitalized rhabdomyolysis in patients treated with lipid-lowering drugs. *JAMA* (2004) **292**:2585-2590.

Green H, Khan MS, Jakobsen-Falk I, Avall-Lundqvist E, and Peterson C. Impact of CYP3A5*3 and CYP2C8-HapC on paclitaxel/carboplatin-induced myelosuppression in patients with ovarian cancer. *J Pharm Sci* (2011) **100**:4205-4209.

Gu X and Manautou JE. Regulation of hepatic ABCC transporters by xenobiotics and in disease states. *Drug Metab Rev* (2010) **42**:482-538.

Guengerich FP. Cytochrome p450 and chemical toxicology. *Chem Res Toxicol* (2008) **21**:70-83.

Guengerich FP. Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity. *Chem Res Toxicol* (2001) **14**:611-650.

Guillemette C. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. *Pharmacogenomics J* (2003) **3**:136-158.

Guillemette C, Levesque E, and Rouleau M. Pharmacogenomics of human uridine diphospho-glucuronosyltransferases and clinical implications. *Clin Pharmacol Ther* (2014) **96**:324-339.

Gupta A, Mugundu GM, Desai PB, Thummel KE, and Unadkat JD. Intestinal human colon adenocarcinoma cell line LS180 is an excellent model to study pregnane X receptor, but not constitutive androstane receptor, mediated CYP3A4 and multidrug resistance transporter 1 induction: studies with anti-human immunodeficiency virus protease inhibitors. *Drug Metab Dispos* (2008) **36**:1172-1180.

Haarman MG, van Hunsel F, and de Vries TW. Adverse drug reactions of montelukast in children and adults. *Pharmacol Res Perspect* (2017) **5**:10.1002/prp2.341.

Hagenbuch B and Meier PJ. Organic anion transporting polypeptides of the OATP/ SLC21 family: phylogenetic classification as OATP/ SLCO superfamily, new nomenclature and molecular/functional properties. *Pflugers Arch* (2004) **447**:653-665.

Hagenbuch B and Meier PJ. The superfamily of organic anion transporting polypeptides. *Biochim Biophys Acta* (2003) **1609**:1-18.

Hagihara K, Kazui M, Kurihara A, Yoshiike M, Honda K, Okazaki O, Farid NA, and Ikeda T. A possible mechanism for the differences in efficiency and variability of active metabolite formation from thienopyridine antiplatelet agents, prasugrel and clopidogrel. *Drug Metab Dispos* (2009) **37**:2145-2152.

Hannemann F, Bichet A, Ewen KM, and Bernhardt R. Cytochrome P450 systems--biological variations of electron transport chains. *Biochim Biophys Acta* (2007) **1770**:330-344.

He L, Vasiliou K, and Nebert DW. Analysis and update of the human solute carrier (SLC) gene superfamily. *Hum Genomics* (2009) **3**:195-206.

Hediger MA, Clemencon B, Burrier RE, and Bruford EA. The ABCs of membrane transporters in health and disease (SLC series): introduction. *Mol Aspects Med* (2013) **34**:95-107.

Hegazy SK, Mabrouk MM, Elsisi AE, and Mansour NO. Effect of clarithromycin and fluconazole on the pharmacokinetics of montelukast in human volunteers. *Eur J Clin Pharmacol* (2012) **68**:1275-1280.

Henricks LM, Lunenburg CATC, de Man FM, Meulendijks D, Frederix GWJ, Kienhuis E, Creemers GJ, Baars A, Dezentje VO, Imholz ALT, Jeurissen FJF, Portielje JEA, Jansen RLH, Hamberg P, Ten Tije AJ, Droogendijk HJ, Koopman M, Nieboer P, van de Poel MHW, Mandigers CMPW, et al. Schellens JHM.

DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. *Lancet Oncol* (2018) **19**:1459-1467.

Henz BM. The pharmacologic profile of desloratadine: a review. *Allergy* (2001) **56 (Suppl 65)**:7-13.

Hertz DL, Roy S, Motsinger-Reif AA, Drobish A, Clark LS, McLeod HL, Carey LA, and Dees EC. CYP2C8*3 increases risk of neuropathy in breast cancer patients treated with paclitaxel. *Ann Oncol* (2013) **24**:1472-1478.

Hirsh-Rokach B, Spectre G, Shai E, Lotan A, Ritter A, Al-Aieshy F, Malmstrom RE, Varon D, and Alcalai R. Differential impact of selective serotonin reuptake inhibitors on platelet response to clopidogrel: a randomized, double-blind, crossover trial. *Pharmacotherapy* (2015) **35**:140-147.

Hirvensalo P, Tornio A, Neuvonen M, Tapaninen T, Paile-Hyvarinen M, Karja V, Mannisto VT, Pihlajamaki J, Backman JT, and Niemi M. Comprehensive pharmacogenomic study reveals an important role of UGT1A3 in montelukast pharmacokinetics. *Clin Pharmacol Ther* (2018) **104**:158-168.

Ho RH, Tirona RG, Leake BF, Glaeser H, Lee W, Lemke CJ, Wang Y, and Kim RB. Drug and bile acid transporters in rosuvastatin hepatic uptake: function, expression, and pharmacogenetics. *Gastroenterology* (2006) **130**:1793-1806.

Hollenberg PF, Kent UM, and Bumpus NN. Mechanism-based inactivation of human cytochromes p450s: experimental characterization, reactive intermediates, and clinical implications. *Chem Res Toxicol* (2008) **21**:189-205.

Hollopeter G, Jantzen HM, Vincent D, Li G, England L, Ramakrishnan V, Yang RB, Nurden P, Nurden A, Julius D, and Conley PB. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* (2001) **409**:202-207.

Holmberg MT, Tornio A, Neuvonen M, Neuvonen PJ, Backman JT, and Niemi M. Grapefruit juice inhibits the metabolic activation of clopidogrel. *Clin Pharmacol Ther* (2014) **95**:307-313.

Holmberg MT, Tornio A, Hyvarinen H, Neuvonen M, Neuvonen PJ, Backman JT, and Niemi M. Effect of grapefruit juice on the bioactivation of prasugrel. *Br J Clin Pharmacol* (2015) **80**:139-145.

Holmberg MT, Tornio A, Paile-Hyvarinen M, Tarkiainen EK, Neuvonen M, Neuvonen PJ, Backman JT, and Niemi M. CYP3A4*22 impairs the elimination of ticagrelor, but has no significant effect on the bioactivation of clopidogrel or prasugrel. *Clin Pharmacol Ther* (2019) **105**:448-457.

Honkalammi J, Niemi M, Neuvonen PJ, and Backman JT. Gemfibrozil is a strong inactivator of CYP2C8 in very small multiple doses. *Clin Pharmacol Ther* (2012) **91**:846-855.

Hsu A, Granneman GR, and Bertz RJ. Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. *Clin Pharmacokinet* (1998) **35**:275-291.

Hu DG, Meech R, McKinnon RA, and Mackenzie PI. Transcriptional regulation of human UDP-glucuronosyltransferase genes. *Drug Metab Rev* (2014) **46**:421-458.

Huang B, Jiang C, and Zhang R. Epigenetics: the language of the cell? *Epigenomics* (2014) **6**:73-88.

Huang L, Wang Y, and Grimm S. ATP-dependent transport of rosuvastatin in membrane vesicles expressing breast cancer resistance protein. *Drug Metab Dispos* (2006) **34**:738-742.

Hughes D and Andersson DI. Evolutionary consequences of drug resistance: shared principles across diverse targets and organisms. *Nat Rev Genet* (2015) **16**:459-471.

Hukkanen J, Pelkonen O, Hakkola J, and Raunio H. Expression and regulation of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human lung. *Crit Rev Toxicol* (2002) **32**:391-411.

Ingelman-Sundberg M, Rane A, and Gustafasson JA. Properties of hydroxylase systems in the human fetal liver active on free and sulfoconjugated steroids. *Biochemistry* (1975) **14**:429-437.

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

Isin EM and Guengerich FP. Complex reactions catalyzed by cytochrome P450 enzymes. *Biochim Biophys Acta* (2007) **1770**:314-329.

Itkonen MK, Tornio A, Lapatto-Reiniluoto O, Neuvonen M, Neuvonen PJ, Niemi M, and Backman JT. Response to "Interaction of dasabuvir with clopidogrel: did predictions by physiologically based pharmacokinetics modeling pass the test?". *Clin Pharmacol Ther* (2019) **105**:322.

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

Jaakkola T, Backman JT, Neuvonen M, and Neuvonen PJ. Effects of gemfibrozil, itraconazole, and their combination on the pharmacokinetics of pioglitazone. *Clin Pharmacol Ther* (2005) **77**:404-414.

Jaakkola T, Backman JT, Neuvonen M, Laitila J, and Neuvonen PJ. Effect of rifampicin on the pharmacokinetics of pioglitazone. *Br J Clin Pharmacol* (2006b) **61**:70-78.

Jaakkola T, Backman JT, Neuvonen M, Niemi M, and Neuvonen PJ. Montelukast and zafirlukast do not affect the pharmacokinetics of the CYP2C8 substrate pioglitazone. *Eur J Clin Pharmacol* (2006c) **62**:503-509.

Jeong YH, Bliden KP, Antonino MJ, Park KS, Tantry US, and Gurbel PA. Usefulness of the VerifyNow P2Y12 assay to evaluate the antiplatelet effects of ticagrelor and clopidogrel therapies. *Am Heart J* (2012) **164**:35-42.

Ji JZ, Huang BB, Gu TT, Tai T, Zhou H, Jia YM, Mi QY, Zhang MR, and Xie HG. Human UGT2B7 is the major isoform responsible for the glucuronidation of clopidogrel carboxylate. *Biopharm Drug Dispos* (2018) **39**:88-98.

Jigorel E, Le Vee M, Boursier-Neyret C, Parmentier Y, and Fardel O. Differential regulation of sinusoidal and canalicular hepatic drug transporter expression by xenobiotics activating drug-sensing receptors in primary human hepatocytes. *Drug Metab Dispos* (2006) **34**:1756-1763.

Johansson M, Strahm E, Rane A, and Ekstrom L. CYP2C8 and CYP2C9 mRNA expression profile in the human fetus. *Front Genet* (2014) **5**:58.

Johnson EF and Stout CD. Structural diversity of human xenobiotic-metabolizing cytochrome P450 monooxygenases. *Biochem Biophys Res Commun* (2005) **338**:331-336.

Jones PH and Davidson MH. Reporting rate of rhabdomyolysis with fenofibrate + statin versus gemfibrozil + any statin. *Am J Cardiol* (2005) **95**:120-122.

Jones PM and George AM. The ABC transporter structure and mechanism: perspectives on recent research. *Cell Mol Life Sci* (2004) **61**:682-699.

Josephy P, Guengerich F, and Miners J. "Phase I and Phase II" drug metabolism: terminology that we should phase out? *Drug Metab Rev* (2005) **37**:575-580.

Jung D, Hagenbuch B, Gresh L, Pontoglio M, Meier PJ, and Kullak-Ublick GA. Characterization of the human OATP-C (SLC21A6) gene promoter and regulation of liver-specific OATP genes by hepatocyte nuclear factor 1 alpha. *J Biol Chem* (2001) **276**:37206-37214.

Kadam R, Bourne D, Kompella U, and Aquilante C. Effect of cytochrome P450 2C8*3 on the population pharmacokinetics of pioglitazone in healthy caucasian volunteers. *Biol Pharm Bull* (2013) **36**:245-251.

Kahma H, Filppula AM, Neuvonen M, Tarkiainen EK, Tornio A, Holmberg MT, Itkonen MK, Finel M, Neuvonen PJ, Niemi M, and Backman JT. Clopidogrel carboxylic acid glucuronidation is mediated mainly by UGT2B7, UGT2B4, and UGT2B17: Implications for pharmacogenetics and drug-drug interactions. *Drug Metab Dispos* (2018) **46**:141-150.

Kajosaari LI, Niemi M, Backman JT, and Neuvonen PJ. Telithromycin, but not montelukast, increases the plasma concentrations and effects of the cytochrome P450 3A4 and 2C8 substrate repaglinide. *Clin Pharmacol Ther* (2006) **79**:231-242.

Kakadiya PP, Higginson RT, and Fulco PP. Ritonavir-boosted protease inhibitors but not cobicistat appear safe in HIV-positive patients ingesting dabigatran. *Antimicrob Agents Chemother* (2018) **62**:10.1128/AAC.02275-17. Print 2018 Feb.

Kalgutkar AS, Obach RS, and Maurer TS. Mechanism-based inactivation of cytochrome P450 enzymes: chemical mechanisms, structure-activity relationships and relationship to clinical drug-drug interactions and idiosyncratic adverse drug reactions. *Curr Drug Metab* (2007) **8**:407-447.

Kalliokoski A, Neuvonen M, Neuvonen PJ, and Niemi M. No significant effect of SLCO1B1 polymorphism on the pharmacokinetics of rosiglitazone and pioglitazone. *Br J Clin Pharmacol* (2008) **65**:78-86.

Kanai N, Lu R, Bao Y, Wolkoff AW, Vore M, and Schuster VL. Estradiol 17 beta-D-glucuronide is a high-affinity substrate for oatp organic anion transporter. *Am J Physiol* (1996) **270**:F326-331.

Karonen T, Neuvonen PJ, and Backman JT. CYP2C8 but not CYP3A4 is important in the pharmacokinetics of montelukast. *Br J Clin Pharmacol* (2012) **73**:257-267.

Karonen T, Filppula A, Laitila J, Niemi M, Neuvonen PJ, and Backman JT. Gemfibrozil markedly increases the plasma concentrations of montelukast: a previously unrecognized role for CYP2C8 in the metabolism of montelukast. *Clin Pharmacol Ther* (2010) **88**:223-230.

Kazmi F, Barbara JE, Yerino P, and Parkinson A. A long-standing mystery solved: the formation of 3-hydroxydesloratadine is catalyzed by CYP2C8 but prior glucuronidation of desloratadine by UDP-glucuronosyltransferase 2B10 is an obligatory requirement. *Drug Metab Dispos* (2015) **43**:523-533.

Kazui M, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O, Ikeda T, and Kurihara A. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. *Drug Metab Dispos* (2010) **38**:92-99.

Kharasch ED, Bedynek PS, Walker A, Whittington D, and Hoffer C. Mechanism of ritonavir changes in methadone pharmacokinetics and pharmacodynamics: II. Ritonavir effects on CYP3A and P-glycoprotein activities. *Clin Pharmacol Ther* (2008) **84**:506-512.

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

Kim KA, Park PW, Kim KR, and Park JY. Effect of multiple doses of montelukast on the pharmacokinetics of rosiglitazone, a CYP2C8 substrate, in humans. *Br J Clin Pharmacol* (2007) **63**:339-345.

Kim SJ, Yoshikado T, Ieiri I, Maeda K, Kimura M, Irie S, Kusuhara H, and Sugiyama Y. Clarification of the mechanism of clopidogrel-mediated drug-drug interaction in a clinical cassette small-dose study and its prediction based on in vitro information. *Drug Metab Dispos* (2016) **44**:1622-1632.

King JR, Zha J, Khatri A, Dutta S, and Menon RM. Clinical pharmacokinetics of dasabuvir. *Clin Pharmacokinet* (2017) **56**:1115-1124.

Kirby BJ, Collier AC, Kharasch ED, Whittington D, Thummel KE, and Unadkat JD. Complex drug interactions of the HIV protease inhibitors 3: effect of simultaneous or staggered dosing of digoxin and ritonavir, nelfinavir, rifampin, or bupropion. *Drug Metab Dispos* (2012) **40**:610-616.

Kitamura S, Maeda K, Wang Y, and Sugiyama Y. Involvement of multiple transporters in the hepatobiliary transport of rosuvastatin. *Drug Metab Dispos* (2008) **36**:2014-2023.

Klibanov OM, Gale SE, and Santevecchi B. Ombitasvir/paritaprevir/ritonavir and dasabuvir tablets for hepatitis C virus genotype 1 infection. *Ann Pharmacother* (2015) **49**:566-581.

Klingenberg M. Pigments of rat liver microsomes. *Arch Biochem Biophys* (1958) **75**:376-386.

Klose TS, Blaisdell JA, and Goldstein JA. Gene structure of CYP2C8 and extrahepatic distribution of the human CYP2Cs. *J Biochem Mol Toxicol* (1999) **13**:289-295.

Knights KM, Rowland A, and Miners JO. Renal drug metabolism in humans: the potential for drug-endobiotic interactions involving cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT). *Br J Clin Pharmacol* (2013) **76**:587-602.

König J, Cui Y, Nies AT, and Keppler D. A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. *Am J Physiol Gastrointest Liver Physiol* (2000) **278**:G156-164.

Koudriakova T, Iatsimirskaia E, Utkin I, Gangl E, Vouros P, Storozhuk E, Orza D, Marinina J, and Gerber N. Metabolism of the human immunodeficiency virus protease inhibitors indinavir and ritonavir by human intestinal microsomes and expressed cytochrome P4503A4/3A5: mechanism-based inactivation of cytochrome P4503A by ritonavir. *Drug Metab Dispos* (1998) **26**:552-561.

Kumar P, Gordon LA, Brooks KM, George JM, Kellogg A, McManus M, Alfaro RM, Nghiem K, Lozier J, Hadigan C, and Penzak SR. Differential influence of the antiretroviral pharmacokinetic enhancers ritonavir and cobicistat on intestinal P-glycoprotein transport and the pharmacokinetic/pharmacodynamic disposition of dabigatran. *Antimicrob Agents Chemother* (2017) **61**:e01201-17.

Kumar S, Samuel K, Subramanian R, Braun MP, Stearns RA, Chiu SH, Evans DC, and Baillie TA. Extrapolation of diclofenac clearance from in vitro microsomal metabolism data: role of acyl glucuronidation and sequential oxidative metabolism of the acyl glucuronide. *J Pharmacol Exp Ther* (2002) **303**:969-978.

Kyrklund C, Backman JT, Kivistö KT, Neuvonen M, Laitila J, and Neuvonen PJ. Rifampin greatly reduces plasma simvastatin and simvastatin acid concentrations. *Clin Pharmacol Ther* (2000) **68**:592-597.

Lamb DC, Lei L, Warrilow AG, Lepesheva GI, Mullins JG, Waterman MR, and Kelly SL. The first virally encoded cytochrome p450. *J Virol* (2009) **83**:8266-8269.

Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, et al. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* (2001) **409**:860-921.

Lapple F, von Richter O, Fromm MF, Richter T, Thon KP, Wisser H, Griese EU, Eichelbaum M, and Kivisto KT. Differential expression and function of CYP2C isoforms in human intestine and liver. *Pharmacogenetics* (2003) **13**:565-575.

Laslett LJ, Alagona P,Jr, Clark BA,3rd, Drozda JP,Jr, Saldivar F, Wilson SR, Poe C, and Hart M. The worldwide environment of cardiovascular disease: prevalence, diagnosis, therapy, and policy issues: a report from the American College of Cardiology. *J Am Coll Cardiol* (2012) **60 (Suppl)**:S1-49.

Lee HH and Ho RH. Interindividual and interethnic variability in drug disposition: polymorphisms in organic anion transporting polypeptide 1B1 (OATP1B1; SLCO1B1). *Br J Clin Pharmacol* (2017) **83**:1176-1184.

Lehmann H and Ryan E. The familial incidence of low pseudocholinesterase level. *Lancet* (1956) **271**:124.

Lilja JJ, Kivistö KT, and Neuvonen PJ. Grapefruit juice-simvastatin interaction: effect on serum concentrations of simvastatin, simvastatin acid, and HMG-CoA reductase inhibitors. *Clin Pharmacol Ther* (1998) **64**:477-483.

Lin JH. CYP induction-mediated drug interactions: in vitro assessment and clinical implications. *Pharm Res* (2006) **23**:1089-1116.

Lin JH and Lu AY. Inhibition and induction of cytochrome P450 and the clinical implications. *Clin Pharmacokinet* (1998) **35**:361-390.

Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, Gut I, Lathrop M, and Collins R. SLCO1B1 variants and statin-induced myopathy--a genomewide study. *N Engl J Med* (2008) **359**:789-799.

Lipworth BJ. Leukotriene-receptor antagonists. *Lancet* (1999) **353**:57-62.

Liu X, Cheng D, Kuang Q, Liu G, and Xu W. Association of UGT1A1*28 polymorphisms with irinotecan-induced toxicities in colorectal cancer: a meta-analysis in Caucasians. *Pharmacogenomics J* (2014) **14**:120-129.

Lordkipanidze M, Pharand C, Nguyen TA, Schampaert E, and Diodati JG. Assessment of VerifyNow P2Y12 assay accuracy in evaluating clopidogrel-induced platelet inhibition. *Ther Drug Monit* (2008) **30**:372-378.

Mackenzie P, Little JM, and Radominska-Pandya A. Glucosidation of hyodeoxycholic acid by UDP-glucuronosyltransferase 2B7. *Biochem Pharmacol* (2003) **65**:417-421.

Mackenzie PI, Gregory PA, Lewinsky RH, Yasmin SN, Height T, McKinnon RA, and Gardner-Stephen DA. Polymorphic variations in the expression of the chemical detoxifying UDP glucuronosyltransferases. *Toxicol Appl Pharmacol* (2005) **207**:77-83.

Mackenzie PI, Bock KW, Burchell B, Guillemette C, Ikushiro S, Iyanagi T, Miners JO, Owens IS, and Nebert DW. Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. *Pharmacogenet Genomics* (2005) **15**:677-685.

Madan A, Graham RA, Carroll KM, Mudra DR, Burton LA, Krueger LA, Downey AD, Czerwinski M, Forster J, Ribadeneira MD, Gan LS, LeCluyse EL, Zech K, Robertson P,Jr, Koch P, Antonian L, Wagner G, Yu L, and Parkinson A. Effects of prototypical microsomal enzyme inducers on cytochrome P450 expression in cultured human hepatocytes. *Drug Metab Dispos* (2003) **31**:421-431.

Mano Y, Usui T, and Kamimura H. The UDP-glucuronosyltransferase 2B7 isozyme is responsible for gemfibrozil glucuronidation in the human liver. *Drug Metab Dispos* (2007) **35**:2040-2044.

Marsousi N, Daali Y, Fontana P, Reny JL, Ancrenaz-Sirot V, Calmy A, Rudaz S, Desmeules JA, and Samer CF. Impact of boosted antiretroviral therapy on the pharmacokinetics and efficacy of clopidogrel and prasugrel active metabolites. *Clin Pharmacokinet* (2018) **57**:1347-1354.

Masubuchi Y and Horie T. Toxicological significance of mechanism-based inactivation of cytochrome p450 enzymes by drugs. *Crit Rev Toxicol* (2007) **37**:389-412.

Mayhew BS, Jones DR, and Hall SD. An in vitro model for predicting in vivo inhibition of cytochrome P450 3A4 by metabolic intermediate complex formation. *Drug Metab Dispos* (2000) **28**:1031-1037.

Meech R and Mackenzie PI. UDP-glucuronosyltransferase, the role of the amino terminus in dimerization. *J Biol Chem* (1997a) **272**:26913-26917.

Meech R and Mackenzie PI. Structure and function of uridine diphosphate glucuronosyltransferases. *Clin Exp Pharmacol Physiol* (1997b) **24**:907-915.

Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias WL, Braunwald E, and Sabatine MS. Cytochrome P450 genetic polymorphisms and the response to prasugrel: relationship to pharmacokinetic, pharmacodynamic, and clinical outcomes. *Circulation* (2009) **119**:2553-2560.

Mega JL, Close SL, Wiviott SD, Man M, Duvvuru S, Walker JR, Sundseth SS, Collet JP, Delaney JT, Hulot JS, Murphy SA, Pare G, Price MJ, Sibbing D, Simon T, Trenk D, Antman EM, and Sabatine MS. PON1 Q192R genetic variant and response to clopidogrel and prasugrel: pharmacokinetics, pharmacodynamics, and a meta-analysis of clinical outcomes. *J Thromb Thrombolysis* (2016) **41**:374-383.

Menon RM, Polepally AR, Khatri A, Awni WM, and Dutta S. Clinical Pharmacokinetics of Paritaprevir. *Clin Pharmacokinet* (2017) **56**:1125-1137.

Menon RM, Badri PS, Wang T, Polepally AR, Zha J, Khatri A, Wang H, Hu B, Coakley EP, Podsadecki TJ, Awni WM, and Dutta S. Drug-drug interaction profile of the all-oral anti-hepatitis C virus regimen of paritaprevir/ritonavir, ombitasvir, and dasabuvir. *J Hepatol* (2015) **63**:20-29.

Merck (2018) Zocor Product Monograph. https://www.merck.ca/static/pdf/ZOCOR-PM_E.pdf (Accessed February 28, 2019).

Metzger NL and Momary KM. A patient with HIV and tuberculosis with diminished clopidogrel response. *Int J STD AIDS* (2014) **25**:532-534.

Meulendijks D, Henricks LM, Sonke GS, Deenen MJ, Froehlich TK, Amstutz U, Largiader CR, Jennings BA, Marinaki AM, Sanderson JD, Kleibl Z, Kleiblova P, Schwab M, Zanger UM, Palles C, Tomlinson I, Gross E, van Kuilenburg AB, Punt CJ, Koopman M, et al. Schellens JH. Clinical relevance of DPYD variants c.1679T>G, c.1236G>A/HapB3, and c.1601G>A as predictors of severe fluoropyrimidine-associated toxicity: a systematic review and meta-analysis of individual patient data. *Lancet Oncol* (2015) **16**:1639-1650.

Meunier B, de Visser SP, and Shaik S. Mechanism of oxidation reactions catalyzed by cytochrome p450 enzymes. *Chem Rev* (2004) **104**:3947-3980.

Miley MJ, Zielinska AK, Keenan JE, Bratton SM, Radominska-Pandya A, and Redinbo MR. Crystal structure of the cofactor-binding domain of the human phase II drug-metabolism enzyme UDP-glucuronosyltransferase 2B7. *J Mol Biol* (2007) **369**:498-511.

Molimard M, Diquet B, and Benedetti MS. Comparison of pharmacokinetics and metabolism of desloratadine, fexofenadine, levocetirizine and mizolastine in humans. *Fundam Clin Pharmacol* (2004) **18**:399-411.

Muck W. Rational assessment of the interaction profile of cerivastatin supports its low propensity for drug interactions. *Drugs* (1998) **56 (Suppl 1)**:15-23.

Murdoch D, Goa KL, and Keam SJ. Desloratadine: an update of its efficacy in the management of allergic disorders. *Drugs* (2003) **63**:2051-2077.

Naraharisetti SB, Lin YS, Rieder MJ, Marciante KD, Psaty BM, Thummel KE, and Totah RA. Human liver expression of CYP2C8: gender, age, and genotype effects. *Drug Metab Dispos* (2010) **38**:889-893.

Nebert DW and Russell DW. Clinical importance of the cytochromes P450. *Lancet* (2002) **360**:1155-1162.

Nelson DR, Zeldin DC, Hoffman SM, Maltais LJ, Wain HM, and Nebert DW. Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. *Pharmacogenetics* (2004) **14**:1-18.

Neuvonen PJ, Niemi M, and Backman JT. Drug interactions with lipid-lowering drugs: mechanisms and clinical relevance. *Clin Pharmacol Ther* (2006) **80**:565-581.

Neuvonen PJ, Kantola T, and Kivistö KT. Simvastatin but not pravastatin is very susceptible to interaction with the CYP3A4 inhibitor itraconazole. *Clin Pharmacol Ther* (1998) **63**:332-341.

Niemi M, Pasanen MK, and Neuvonen PJ. Organic anion transporting polypeptide 1B1: a genetically polymorphic transporter of major importance for hepatic drug uptake. *Pharmacol Rev* (2011) **63**:157-181.

Niemi M, Backman JT, and Neuvonen PJ. Effects of trimethoprim and rifampin on the pharmacokinetics of the cytochrome P450 2C8 substrate rosiglitazone. *Clin Pharmacol Ther* (2004) **76**:239-249.

Niemi M, Backman JT, Neuvonen M, and Neuvonen PJ. Effects of gemfibrozil, itraconazole, and their combination on the pharmacokinetics and pharmacodynamics of repaglinide: potentially hazardous interaction between gemfibrozil and repaglinide. *Diabetologia* (2003) **46**:347-351.

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

Niemi M, Backman JT, Kajosaari LI, Leathart JB, Neuvonen M, Daly AK, Eichelbaum M, Kivisto KT, and Neuvonen PJ. Polymorphic organic anion transporting polypeptide 1B1 is a major determinant of repaglinide pharmacokinetics. *Clin Pharmacol Ther* (2005) **77**:468-478.

Nishiya Y, Hagihara K, Ito T, Tajima M, Miura S, Kurihara A, Farid NA, and Ikeda T. Mechanism-based inhibition of human cytochrome P450 2B6 by ticlopidine, clopidogrel, and the thiolactone metabolite of prasugrel. *Drug Metab Dispos* (2009) **37**:589-593.

Ogilvie BW, Zhang D, Li W, Rodrigues AD, Gipson AE, Holsapple J, Toren P, and Parkinson A. Glucuronidation converts gemfibrozil to a potent, metabolism-dependent inhibitor of CYP2C8: implications for drug-drug interactions. *Drug Metab Dispos* (2006) **34**:191-197.

Omura T and Sato R. A new cytochrome in liver microsomes. *J Biol Chem* (1962) **237**:1375-1376.

Oshiro C, Mangravite L, Klein T, and Altman R. PharmGKB very important pharmacogene: SLCO1B1. *Pharmacogenet Genomics* (2010) **20**:211-216.

Osnabrugge RL, Head SJ, Zijlstra F, ten Berg JM, Hunink MG, Kappetein AP, and Janssens AC. A systematic review and critical assessment of 11 discordant meta-analyses on reduced-function

CYP2C19 genotype and risk of adverse clinical outcomes in clopidogrel users. *Genet Med* (2015) **17**:3-11.

Pasanen MK, Neuvonen PJ, and Niemi M. Global analysis of genetic variation in SLCO1B1. *Pharmacogenomics* (2008) **9**:19-33.

Pasanen MK, Neuvonen M, Neuvonen PJ, and Niemi M. SLCO1B1 polymorphism markedly affects the pharmacokinetics of simvastatin acid. *Pharmacogenet Genomics* (2006) **16**:873-879.

Paul D, Standifer KM, Inturrisi CE, and Pasternak GW. Pharmacological characterization of morphine-6 beta-glucuronide, a very potent morphine metabolite. *J Pharmacol Exp Ther* (1989) **251**:477-483.

Perucca E. Clinically relevant drug interactions with antiepileptic drugs. *Br J Clin Pharmacol* (2006) **61**:246-255.

Pinheiro LF, Franca CN, Izar MC, Barbosa SP, Bianco HT, Kasmas SH, Mendes GD, Povoa RM, and Fonseca FA. Pharmacokinetic interactions between clopidogrel and rosuvastatin: effects on vascular protection in subjects with coronary heart disease. *Int J Cardiol* (2012) **158**:125-129.

Polepally AR, King JR, Ding B, Shuster DL, Dumas EO, Khatri A, Chiu YL, Podsadecki TJ, and Menon RM. Drug-drug interactions between the anti-hepatitis C virus 3D regimen of ombitasvir, paritaprevir/ritonavir, and dasabuvir and eight commonly used medications in healthy volunteers. *Clin Pharmacokinet* (2016) **55**:1003-1014.

Prenner B, Kim K, Gupta S, Khalilieh S, Kantesaria B, Manitpisitkul P, Lorber R, Wang Z, and Lutsky B. Adult and paediatric poor metabolisers of desloratadine: an assessment of pharmacokinetics and safety. *Expert Opin Drug Saf* (2006) **5**:211-223.

Price MJ, Endemann S, Gollapudi RR, Valencia R, Stinis CT, Levisay JP, Ernst A, Sawhney NS, Schatz RA, and Teirstein PS. Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events after drug-eluting stent implantation. *Eur Heart J* (2008) **29**:992-1000.

Prueksaritanont T, Ma B, and Yu N. The human hepatic metabolism of simvastatin hydroxy acid is mediated primarily by CYP3A, and not CYP2D6. *Br J Clin Pharmacol* (2003) **56**:120-124.

Prueksaritanont T, Gorham LM, Ma B, Liu L, Yu X, Zhao JJ, Slaughter DE, Arison BH, and Vyas KP. In vitro metabolism of simvastatin in humans [SBT]identification of metabolizing enzymes and effect of the drug on hepatic P450s. *Drug Metab Dispos* (1997) **25**:1191-1199.

Prueksaritanont T, Chu X, Evers R, Klopfer SO, Caro L, Kothare PA, Dempsey C, Rasmussen S, Houle R, Chan G, Cai X, Valesky R, Fraser IP, and Stoch SA. Pitavastatin is a more sensitive and selective organic anion-transporting polypeptide 1B clinical probe than rosuvastatin. *Br J Clin Pharmacol* (2014) **78**:587-598.

Radominska-Pandya A, Bratton SM, Redinbo MR, and Miley MJ. The crystal structure of human UDP-glucuronosyltransferase 2B7 C-terminal end is the first mammalian UGT target to be revealed: the significance for human UGTs from both the 1A and 2B families. *Drug Metab Rev* (2010) **42**:133-144.

Radominska-Pandya A, Ouzzine M, Fournel-Gigleux S, and Magdalou J. Structure of UDP-glucuronosyltransferases in membranes. *Methods Enzymol* (2005) **400**:116-147.

Radominska-Pandya A, Czernik PJ, Little JM, Battaglia E, and Mackenzie PI. Structural and functional studies of UDP-glucuronosyltransferases. *Drug Metab Rev* (1999) **31**:817-899.

Ramsey LB, Johnson SG, Caudle KE, Haidar CE, Voora D, Wilke RA, Maxwell WD, McLeod HL, Krauss RM, Roden DM, Feng Q, Cooper-DeHoff RM, Gong L, Klein TE, Wadelius M, and Niemi M. The clinical pharmacogenetics implementation consortium guideline for SLCO1B1 and simvastatin-induced myopathy: 2014 update. *Clin Pharmacol Ther* (2014) **96**:423-428.

Rathbun RC and Rossi DR. Low-dose ritonavir for protease inhibitor pharmacokinetic enhancement. *Ann Pharmacother* (2002) **36**:702-706.

Raunio H, Kuusisto M, Juvonen RO, and Pentikainen OT. Modeling of interactions between xenobiotics and cytochrome P450 (CYP) enzymes. *Front Pharmacol* (2015) **6**:123.

Redwood AJ, Pavlos RK, White KD, and Phillips EJ. HLAs: Key regulators of T-cell-mediated drug hypersensitivity. *HLA* (2018) **91**:3-16.

Regan SL, Maggs JL, Hammond TG, Lambert C, Williams DP, and Park BK. Acyl glucuronides: the good, the bad and the ugly. *Biopharm Drug Dispos* (2010) **31**:367-395.

Rehmel JL, Eckstein JA, Farid NA, Heim JB, Kasper SC, Kurihara A, Wrighton SA, and Ring BJ. Interactions of two major metabolites of prasugrel, a thienopyridine antiplatelet agent, with the cytochromes P450. *Drug Metab Dispos* (2006) **34**:600-607.

Reiss TF, Chervinsky P, Dockhorn RJ, Shingo S, Seidenberg B, and Edwards TB. Montelukast, a once-daily leukotriene receptor antagonist, in the treatment of chronic asthma: a multicenter, randomized, double-blind trial. Montelukast Clinical Research Study Group. *Arch Intern Med* (1998) **158**:1213-1220.

Relling MV and Evans WE. Pharmacogenomics in the clinic. *Nature* (2015) **526**:343-350.

Relling MV and Klein TE. CPIC: Clinical Pharmacogenetics Implementation Consortium of the Pharmacogenomics Research Network. *Clin Pharmacol Ther* (2011) **89**:464-467.

Remsberg CM, Frassetto LA, Okochi H, and Benet LZ. Identification of a new drug-drug interaction between clopidogrel and rosuvastatin mediated through OATP1B1. *Clin Pharmacol Ther* (2013) **93 (Suppl 1)**:S8.

Rendic S and Di Carlo FJ. Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. *Drug Metab Rev* (1997) **29**:413-580.

Richter T, Murdter TE, Heinkele G, Pleiss J, Tatzel S, Schwab M, Eichelbaum M, and Zanger UM. Potent mechanism-based inhibition of human CYP2B6 by clopidogrel and ticlopidine. *J Pharmacol Exp Ther* (2004) **308**:189-197.

Ridderström M, Zamora I, Fjellström O, and Andersson TB. Analysis of selective regions in the active sites of human cytochromes P450, 2C8, 2C9, 2C18, and 2C19 homology models using GRID/CPCA. *J Med Chem* (2001) **44**:4072-4081.

Ritter JK. Roles of glucuronidation and UDP-glucuronosyltransferases in xenobiotic bioactivation reactions. *Chem Biol Interact* (2000) **129**:171-193.

Rost S, Fregin A, Ivaskevicius V, Conzelmann E, Hortnagel K, Pelz HJ, Lappegard K, Seifried E, Scharrer I, Tuddenham EG, Muller CR, Strom TM, and Oldenburg J. Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. *Nature* (2004) **427**:537-541.

Rostami-Hodjegan A and Tucker GT. Simulation and prediction of in vivo drug metabolism in human populations from in vitro data. *Nat Rev Drug Discov* (2007) **6**:140-148.

Rowland A, Miners JO, and Mackenzie PI. The UDP-glucuronosyltransferases: their role in drug metabolism and detoxification. *Int J Biochem Cell Biol* (2013) **45**:1121-1132.

Rowland M and Tozer TN. (2011) *Clinical Pharmacokinetics and Pharmacodynamics: Concepts and Applications.*, 4th edition ed. Lippincott Williams & Wilkins, Baltimore.

Sadee W and Dai Z. Pharmacogenetics/genomics and personalized medicine. *Hum Mol Genet* (2005) **14 Spec No. 2**:R207-14.

Satoh T and Hosokawa M. Structure, function and regulation of carboxylesterases. *Chem Biol Interact* (2006) **162**:195-211.

Schmiedlin-Ren P, Edwards DJ, Fitzsimmons ME, He K, Lown KS, Woster PM, Rahman A, Thummel KE, Fisher JM, Hollenberg PF, and Watkins PB. Mechanisms of enhanced oral availability of CYP3A4 substrates by grapefruit constituents. Decreased enterocyte CYP3A4 concentration and mechanism-based inactivation by furanocoumarins. *Drug Metab Dispos* (1997) **25**:1228-1233.

Schoch GA, Yano JK, Sansen S, Dansette PM, Stout CD, and Johnson EF. Determinants of cytochrome P450 2C8 substrate binding: structures of complexes with montelukast, troglitazone, felodipine, and 9-cis-retinoic acid. *J Biol Chem* (2008) **283**:17227-17237.

Schoch GA, Yano JK, Wester MR, Griffin KJ, Stout CD, and Johnson EF. Structure of human microsomal cytochrome P450 2C8. Evidence for a peripheral fatty acid binding site. *J Biol Chem* (2004) **279**:9497-9503.

Schrör K. Clinical pharmacology of the adenosine diphosphate (ADP) receptor antagonist, clopidogrel. *Vasc Med* (1998) **3**:247-251.

Senafi SB, Clarke DJ, and Burchell B. Investigation of the substrate specificity of a cloned expressed human bilirubin UDP-glucuronosyltransferase: UDP-sugar specificity and involvement in steroid and xenobiotic glucuronidation. *Biochem J* (1994) **303**:233-240.

Shebley M. Interaction of dasabuvir with clopidogrel: did predictions by physiologically based pharmacokinetics modeling pass the test? *Clin Pharmacol Ther* (2019) **105**:320-321.

Shebley M, Fu W, Badri P, Bow D, and Fischer V. Physiologically based pharmacokinetic modeling suggests limited drug-drug interaction between clopidogrel and dasabuvir. *Clin Pharmacol Ther* (2017a) **102**:679-687.

Shebley M, Liu J, Kavetskaia O, Sydor J, de Morais SM, Fischer V, Nijsen MJMA, and Bow DAJ. Mechanisms and predictions of drug-drug interactions of the hepatitis C virus three direct-acting antiviral regimen: Paritaprevir/ritonavir, ombitasvir, and dasabuvir. *Drug Metab Dispos* (2017b) **45**:755-764.

Shen J, Serby M, Reed A, Lee AJ, Menon R, Zhang X, Marsh K, Wan X, Kavetskaia O, and Fischer V. Metabolism and disposition of hepatitis C polymerase inhibitor dasabuvir in humans. *Drug Metab Dispos* (2016) **44**:1139-1147.

Shitara Y. Clinical importance of OATP1B1 and OATP1B3 in drug-drug interactions. *Drug Metab Pharmacokinet* (2011) **26**:220-227.

Shitara Y, Horie T, and Sugiyama Y. Transporters as a determinant of drug clearance and tissue distribution. *Eur J Pharm Sci* (2006) **27**:425-446.

Shitara Y, Hirano M, Sato H, and Sugiyama Y. Gemfibrozil and its glucuronide inhibit the organic anion transporting polypeptide 2 (OATP2/OATP1B1:SLC21A6)-mediated hepatic uptake and CYP2C8-mediated metabolism of cerivastatin: analysis of the mechanism of the clinically relevant drug-drug interaction between cerivastatin and gemfibrozil. *J Pharmacol Exp Ther* (2004) **311**:228-236.

Silverman RB. Mechanism-based enzyme inactivators. *Methods Enzymol* (1995) **249**:240-283.

Silverman RB and Hoffman SJ. The organic chemistry of mechanism-based enzyme inhibition: a chemical approach to drug design. *Med Res Rev* (1984) **4**:415-447.

Sim SC, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, and Ingelman-Sundberg M. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther* (2006) **79**:103-113.

Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Meneveau N, Steg PG, Ferrieres J, Danchin N, Becquemont L, and French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) Investigators. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med* (2009) **360**:363-375.

Spahn-Langguth H and Benet LZ. Acyl glucuronides revisited: is the glucuronidation process a toxification as well as a detoxification mechanism? *Drug Metab Rev* (1992) **24**:5-47.

Stark JE. Potential for a significant interaction between clopidogrel and dasabuvir. *Clin Infect Dis* (2015) **61**:134-135.

Stingl JC, Bartels H, Viviani R, Lehmann ML, and Brockmoller J. Relevance of UDP-glucuronosyltransferase polymorphisms for drug dosing: a quantitative systematic review. *Pharmacol Ther* (2014) **141**:92-116.

Tamraz B, Fukushima H, Wolfe AR, Kaspera R, Totah RA, Floyd JS, Ma B, Chu C, Marciante KD, Heckbert SR, Psaty BM, Kroetz DL, and Kwok PY. OATP1B1-related drug-drug and drug-gene interactions as potential risk factors for cerivastatin-induced rhabdomyolysis. *Pharmacogenet Genomics* (2013) **23**:355-364.

Tang M, Mukundan M, Yang J, Charpentier N, LeCluyse EL, Black C, Yang D, Shi D, and Yan B. Antiplatelet agents aspirin and clopidogrel are hydrolyzed by distinct carboxylesterases, and clopidogrel is transesterificated in the presence of ethyl alcohol. *J Pharmacol Exp Ther* (2006) **319**:1467-1476.

Tapaninen T, Karonen T, Backman JT, Neuvonen PJ, and Niemi M. SLCO2B1 c.935G>A single nucleotide polymorphism has no effect on the pharmacokinetics of montelukast and aliskiren. *Pharmacogenet Genomics* (2013) **23**:19-24.

Tarkiainen EK, Holmberg MT, Tornio A, Neuvonen M, Neuvonen PJ, Backman JT, and Niemi M. Carboxylesterase 1 c.428G>A single nucleotide variation increases the antiplatelet effects of clopidogrel by reducing its hydrolysis in humans. *Clin Pharmacol Ther* (2015) **97**:650-658.

Tirona RG, Leake BF, Merino G, and Kim RB. Polymorphisms in OATP-C: identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. *J Biol Chem* (2001) **276**:35669-35675.

Tornio A and Backman JT. Cytochrome P450 in pharmacogenetics: an update. *Adv Pharmacol* (2018) **83**:3-32.

Tornio A, Filppula AM, Niemi M, and Backman JT. Clinical Studies on Drug-Drug Interactions Involving Metabolism and Transport: Methodology, Pitfalls, and Interpretation. *Clin Pharmacol Ther* (2019) **105**:1345-1361.

Tornio A, Neuvonen PJ, Niemi M, and Backman JT. Role of gemfibrozil as an inhibitor of CYP2C8 and membrane transporters. *Expert Opin Drug Metab Toxicol* (2017) **13**:83-95.

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

Tornio A, Filppula AM, Kailari O, Neuvonen M, Nyronen TH, Tapaninen T, Neuvonen PJ, Niemi M, and Backman JT. Glucuronidation converts clopidogrel to a strong time-dependent inhibitor of CYP2C8: a phase II metabolite as a perpetrator of drug-drug interactions. *Clin Pharmacol Ther* (2014) **96**:498-507.

Totah RA and Rettie AE. Cytochrome P450 2C8: substrates, inhibitors, pharmacogenetics, and clinical relevance. *Clin Pharmacol Ther* (2005) **77**:341-352.

Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, and Nichols M. Cardiovascular disease in Europe: epidemiological update 2016. *Eur Heart J* (2016) **37**:3232-3245.

Tracy TS, Chaudhry AS, Prasad B, Thummel KE, Schuetz EG, Zhong XB, Tien YC, Jeong H, Pan X, Shireman LM, Tay-Sontheimer J, and Lin YS. Interindividual variability in cytochrome P450-mediated drug metabolism. *Drug Metab Dispos* (2016) **44**:343-351.

Turpeinen M, Tolonen A, Uusitalo J, Jalonen J, Pelkonen O, and Laine K. Effect of clopidogrel and ticlopidine on cytochrome P450 2B6 activity as measured by bupropion hydroxylation. *Clin Pharmacol Ther* (2005) **77**:553-559.

van de Steeg E, Stranecky V, Hartmannova H, Noskova L, Hrebicek M, Wagenaar E, van Esch A, de Waart DR, Oude Elferink RP, Kenworthy KE, Sticova E, al-Edreesi M, Knisely AS, Kmoch S, Jirsa M, and

Schinkel AH. Complete OATP1B1 and OATP1B3 deficiency causes human Rotor syndrome by interrupting conjugated bilirubin reuptake into the liver. *J Clin Invest* (2012) **122**:519-528.

Varma MV, Kimoto E, Scialis R, Bi Y, Lin J, Eng H, Kalgutkar AS, El-Kattan AF, Rodrigues AD, and Tremaine LM. Transporter-mediated hepatic uptake plays an important role in the pharmacokinetics and drug-drug interactions of montelukast. *Clin Pharmacol Ther* (2017) **101**:406-415.

Varma MVS, Bi YA, Lazzaro S, and West M. Clopidogrel as a perpetrator of drug-drug interactions: A challenge for quantitative predictions? *Clin Pharmacol Ther* (2019) **105**:1295-1299.

Vickers S, Duncan CA, Chen IW, Rosegay A, and Duggan DE. Metabolic disposition studies on simvastatin, a cholesterol-lowering prodrug. *Drug Metab Dispos* (1990a) **18**:138-145.

Vickers S, Duncan CA, Vyas KP, Kari PH, Arison B, Prakash SR, Ramjit HG, Pitzenberger SM, Stokker G, and Duggan DE. In vitro and in vivo biotransformation of simvastatin, an inhibitor of HMG CoA reductase. *Drug Metab Dispos* (1990b) **18**:476-483.

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

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

Wells PG, Mackenzie PI, Chowdhury JR, Guillemette C, Gregory PA, Ishii Y, Hansen AJ, Kessler FK, Kim PM, Chowdhury NR, and Ritter JK. Glucuronidation and the UDP-glucuronosyltransferases in health and disease. *Drug Metab Dispos* (2004) **32**:281-290.

Wilkinson GR. Drug metabolism and variability among patients in drug response. *N Engl J Med* (2005) **352**:2211-2221.

Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, Neumann FJ, Ardissino D, De Servi S, Murphy SA, Riesmeyer J, Weerakkody G, Gibson CM, Antman EM, and TRITON-TIMI 38 Investigators. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* (2007) **357**:2001-2015.

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

Xiang X, Han Y, Neuvonen M, Pasanen MK, Kalliokoski A, Backman JT, Laitila J, Neuvonen PJ, and Niemi M. Effect of SLCO1B1 polymorphism on the plasma concentrations of bile acids and bile acid synthesis marker in humans. *Pharmacogenet Genomics* (2009) **19**:447-457.

Yang N, Sun R, Liao X, Aa J, and Wang G. UDP-glucuronosyltransferases (UGTs) and their related metabolic cross-talk with internal homeostasis: A systematic review of UGT isoforms for precision medicine. *Pharmacol Res* (2017) **121**:169-183.

Yuen AW, Land G, Weatherley BC, and Peck AW. Sodium valproate acutely inhibits lamotrigine metabolism. *Br J Clin Pharmacol* (1992) **33**:511-513.

Zanger UM and Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* (2013) **138**:103-141.

Zhao Y, White MA, Muralidhara BK, Sun L, Halpert JR, and Stout CD. Structure of microsomal cytochrome P450 2B4 complexed with the antifungal drug bifonazole: insight into P450 conformational plasticity and membrane interaction. *J Biol Chem* (2006) **281**:5973-5981.

Zhou Y, Ingelman-Sundberg M, and Lauschke VM. Worldwide distribution of cytochrome P450 alleles: a meta-analysis of population-scale sequencing projects. *Clin Pharmacol Ther* (2017) **102**:688-700.

Zolk O and Fromm MF. Transporter-mediated drug uptake and efflux: important determinants of adverse drug reactions. *Clin Pharmacol Ther* (2011) **89**:798-805.