PHARMACOKINETIC INTERACTIONS AND PHARMACOGENETICS OF ALISKIREN

Tuija Tapaninen

ACADEMIC DISSERTATION

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABC</td>
<td>ATP-binding cassette</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin-converting enzyme</td>
</tr>
<tr>
<td>Ae</td>
<td>Amount excreted into urine</td>
</tr>
<tr>
<td>AhR</td>
<td>Aryl hydrocarbon receptor</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the plasma concentration-time curve</td>
</tr>
<tr>
<td>BCRP</td>
<td>Breast cancer resistance protein</td>
</tr>
<tr>
<td>BSEP</td>
<td>Bile salt export pump</td>
</tr>
<tr>
<td>c</td>
<td>Nucleotide position in the coding deoxyribonucleic acid</td>
</tr>
<tr>
<td>CAR</td>
<td>Constitutive androstane receptor</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CI/F</td>
<td>Oral clearance</td>
</tr>
<tr>
<td>Cl&lt;sub&gt;renal&lt;/sub&gt;</td>
<td>Renal clearance</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Peak plasma concentration</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EM</td>
<td>Extensive metabolizer</td>
</tr>
<tr>
<td>EMA</td>
<td>European Medicines Agency</td>
</tr>
<tr>
<td>f</td>
<td>Female</td>
</tr>
<tr>
<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
</tr>
<tr>
<td>g</td>
<td>Nucleotide position in the genomic deoxyribonucleic acid</td>
</tr>
<tr>
<td>GR</td>
<td>Glucocorticoid receptor</td>
</tr>
<tr>
<td>HEK293</td>
<td>Human embryonic kidney 293 cells</td>
</tr>
<tr>
<td>HeLa</td>
<td>Human cervical carcinoma cells (named after Henrietta Lacks)</td>
</tr>
<tr>
<td>HMG-CoA</td>
<td>3-hydroxy-3-methylglutaryl-coenzyme A</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Inhibitor concentration producing 50% inhibition</td>
</tr>
<tr>
<td>IM</td>
<td>Intermediate metabolizer</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>k&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Elimination rate constant</td>
</tr>
<tr>
<td>K&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Inhibition constant</td>
</tr>
<tr>
<td>K&lt;sub&gt;m&lt;/sub&gt;</td>
<td>Michaelis-Menten kinetic constant</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>LC/MS/MS</td>
<td>Liquid chromatography-tandem mass spectrometry</td>
</tr>
<tr>
<td>m</td>
<td>Male</td>
</tr>
<tr>
<td>MATE</td>
<td>Multidrug and toxin extrusion protein</td>
</tr>
<tr>
<td>MCF-7</td>
<td>Michigan Cancer Foundation breast cancer cell line</td>
</tr>
<tr>
<td>MDR</td>
<td>Multidrug resistance protein</td>
</tr>
<tr>
<td>MRP</td>
<td>Multidrug resistance-associated protein</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>OAT</td>
<td>Organic anion transporter</td>
</tr>
<tr>
<td>OATP</td>
<td>Organic anion-transporting polypeptide</td>
</tr>
<tr>
<td>OCT</td>
<td>Organic cation transporter</td>
</tr>
<tr>
<td>p.</td>
<td>Amino acid position in the protein sequence</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PEPT</td>
<td>Peptide transporter</td>
</tr>
<tr>
<td>P-gp</td>
<td>P-glycoprotein</td>
</tr>
<tr>
<td>PM</td>
<td>Poor metabolizer</td>
</tr>
<tr>
<td>PXR</td>
<td>Pregnane X receptor</td>
</tr>
<tr>
<td>RAAS</td>
<td>Renin-angiotensin-aldosterone system</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SLCO</td>
<td>Solute carrier organic anion transporter</td>
</tr>
<tr>
<td>SN-38</td>
<td>Active metabolite of irinotecan</td>
</tr>
<tr>
<td>SNP</td>
<td>Single-nucleotide polymorphism</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>Elimination half-life</td>
</tr>
<tr>
<td>$t_{\text{max}}$</td>
<td>Time to peak concentration</td>
</tr>
<tr>
<td>UM</td>
<td>Ultrarapid metabolizer</td>
</tr>
<tr>
<td>$V_d$</td>
<td>Volume of distribution</td>
</tr>
</tbody>
</table>
LIST OF ORIGINAL PUBLICATIONS

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


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ABSTRACT

Aliskiren is an antihypertensive drug approved for clinical use in 2007. It acts by inhibiting renin, the first enzyme in the renin-angiotensin-aldosterone system. Marked interindividual variability exists in the pharmacokinetics of aliskiren. Interestingly, the pharmacokinetic properties of aliskiren suggest an important role for drug transporters in its pharmacokinetics. Aliskiren is poorly absorbed, and therefore, its oral bioavailability is only 2-3%. The elimination of aliskiren occurs mainly as an unchanged drug by biliary and renal excretion, and only a small proportion is metabolized by cytochrome P450 (CYP) 3A4. Organic anion-transporting polypeptide 2B1 (OATP2B1) influx transporter is thought to facilitate the intestinal absorption and hepatic uptake of aliskiren. Based on a more recent finding, OATP1A2 may also contribute to aliskiren absorption. Moreover, aliskiren is a substrate of P-glycoprotein (P-gp) efflux transporter, which can reduce the intestinal absorption of its substrates and enhance their elimination into bile, urine, and intestine. Furthermore, P-gp limits the passage of its substrates across many blood-tissue barriers such as the blood-brain barrier. In previous studies, cyclosporine (an inhibitor of P-gp, OATP2B1, and CYP3A4) as well as ketoconazole and atorvastatin (inhibitors of P-gp and CYP3A4) have raised the area under the plasma aliskiren concentration-time curve (AUC) 5-fold, 1.8-fold, and 1.5-fold, respectively. Considering the interindividual differences in aliskiren pharmacokinetics, information on related pharmacokinetic interactions and genetic variations may improve the safety of aliskiren therapy.

This thesis comprises four randomized, placebo-controlled, cross-over pharmacokinetic interaction studies and two prospective genotype panel studies in healthy volunteers to assess the potential pharmacokinetic interactions and genetic variations affecting the pharmacokinetics and pharmacodynamics of aliskiren. The effects of induction and inhibition of P-gp and CYP3A4 were investigated by using rifampicin and itraconazole as a model inducer and inhibitor, respectively. Furthermore, the effects of grapefruit juice, orange juice, and apple juice, all of which have inhibited OATP1A2 and OATP2B1 in vitro, were also examined. Genetic variations of P-gp and OATP2B1 for the pharmacogenetic studies were selected on the basis of previous studies reporting their associations with altered plasma concentrations of the substrates of respective drug transporters, and on the basis of their frequencies in the Finnish population. Therefore, the effects of common haplotypes of the ABCB1 gene encoding P-gp, c.1236C-c.2677G-c.3435C and c.1236T-c.2677T-c.3435T, as well as the effects of c.935G>A
single-nucleotide polymorphism (SNP) in the \textit{SLCO2B1} gene encoding OATP2B1 were evaluated. In all studies, aliskiren was administered as a single dose. Furthermore, in pharmacokinetic interaction studies, the potentially interacting substances were administered according to relevant dosing schemes. Blood and urine samples were collected for the determination of drug concentrations and plasma renin activity, in addition to which blood pressure was measured.

Rifampicin, grapefruit juice, orange juice, and apple juice markedly reduced the plasma concentrations of aliskiren, and the reductions in the AUC values of aliskiren were 56%, 61%, 62%, and 63%, respectively ($P < 0.001$). In addition, the reduced exposure to aliskiren by rifampicin, orange juice, and apple juice led to the attenuation of the renin-inhibiting effect of aliskiren. During the rifampicin, orange juice, and apple juice phases plasma renin activity 24 hours after aliskiren ingestion was 61% ($P = 0.008$), 87% ($P = 0.037$), and 67% ($P = 0.036$) higher, respectively, than during the placebo or water phases. Itraconazole raised the AUC of aliskiren considerably, 6.5-fold ($P < 0.001$), and also enhanced the renin-inhibiting effect of aliskiren. Plasma renin activity 24 hours after aliskiren ingestion was 68% lower during the itraconazole phase, than during the placebo phase ($P = 0.011$). The \textit{ABCB1} c.1236C-c.2677G-c.3435C and c.1236T-c.2677T-c.3435T haplotypes and the \textit{SLCO2B1} c.935G>A SNP were not significantly associated with the pharmacokinetics or pharmacodynamics of aliskiren.

In conclusion, aliskiren was found to be susceptible to transporter-mediated pharmacokinetic interactions of clinical significance. The interactions of rifampicin and itraconazole with aliskiren probably resulted from induction and inhibition of P-gp in the small intestine, respectively, with a minor contribution from a parallel effect on CYP3A4. Grapefruit, orange, and apple juices reduced the absorption of aliskiren from the gastrointestinal tract, possibly by inhibiting intestinal OATP transporters. The genetic variations of P-gp and OATP2B1 examined did not explain the large interindividual differences in aliskiren pharmacokinetics. Clinicians should be aware of the possibility that rifampicin may reduce the antihypertensive efficacy of aliskiren. Itraconazole can markedly raise the plasma concentrations of aliskiren and enhance its renin-inhibiting efficacy, and thus, should not be used with aliskiren. In addition, the inhibition of P-gp by itraconazole may alter the tissue distribution of aliskiren and potentially produce adverse reactions not observed with higher doses of aliskiren alone. Moreover, the concomitant use of aliskiren with grapefruit, orange, or apple juices is best avoided because of the risk of therapeutic failure due to reduced aliskiren exposure.
INTRODUCTION

Hypertension is a major healthcare issue worldwide due to its high frequency and the concomitant risk of cardiovascular diseases (Kearney et al. 2005). When the cut-off values of 140 mmHg and 90 mmHg are used, about half of the Finnish men aged 25-64 years and one-third of the women in the same age group have hypertension (Kastarinen et al. 2009). Lifestyle modification is the first-line treatment of hypertension, but drug therapy is also often necessary to achieve treatment goals (Mancia et al. 2007; Mancia et al. 2009). The treatment of hypertension according to Finnish Current Care Guidelines is considered cost-effective (Booth et al. 2007). In 2010, over 500 000 patients received the special reimbursement for antihypertensive drugs from the Social Insurance Institution of Finland (KELA 2011).

Patients with essential hypertension differ markedly in their response to different antihypertensive drugs (Materson et al. 1993; Dickerson et al. 1999). Such variability may partly be accounted for by interindividual differences in pharmacokinetics due to genetic variation in drug transporters and metabolizing enzymes or due to pharmacokinetic interactions (Wilkinson 2005; Eichelbaum et al. 2006). Knowledge of the crucial role of cytochrome P450 (CYP) enzymes in drug metabolism and interactions has improved enormously during the past two decades, whereas the significance of the various drug transporters in drug pharmacokinetics has not been recognized until more recently. Identification of unrecognized interactions and genetic factors affecting drug pharmacokinetics will improve the safety and predictability of drug therapies and are important steps towards personalized medicine.

Aliskiren is a renin-inhibiting antihypertensive drug with considerable interindividual variability in its pharmacokinetics (Wood et al. 2003; Vaidyanathan et al. 2008c). Interestingly, drug transporters appear to be important determinants of its absorption and disposition. Aliskiren has a low oral bioavailability and passive membrane permeability, and it is only slightly metabolized by CYP3A4 (FDA 2007; Waldmeier et al. 2007; Vaidyanathan et al. 2008c). The absorption of aliskiren from the small intestine is thought to be facilitated by organic-anion transporting polypeptide 2B1 (OATP2B1) influx transporter, and, based on a more recent finding, also by OATP1A2 (Vaidyanathan et al. 2008b; Rebello et al. 2011c). P-glycoprotein (P-gp) efflux transporter transports aliskiren back into the intestinal lumen and mediates its biliary and renal excretion (Vaidyanathan et al. 2008b).
Given the pharmacokinetic properties of aliskiren, we decided to investigate its susceptibility to transporter-mediated interactions in more detail. In previous studies, P-gp, OATP2B1, and CYP3A4 inhibitor cyclosporine raised exposure to aliskiren 5-fold (Rebello et al. 2011a), and the P-gp and CYP3A4 inhibitors ketoconazole and atorvastatin 1.8-fold and 1.5-fold, respectively (Vaidyanathan et al. 2008b). The effects of inducers of P-gp and CYP3A4 on aliskiren pharmacokinetics have not been reported. In this thesis work, the effects of induction and inhibition of P-gp and CYP3A4 on aliskiren pharmacokinetics and pharmacodynamics were investigated by using rifampicin and itraconazole, respectively.

To date, no published studies have demonstrated clinically significant OATP2B1-mediated drug interactions. However, certain constituents of grapefruit, orange, and apple juice have been found to inhibit OATP1A2 and OATP2B1 in vitro (Dresser et al. 2002a; Satoh et al. 2005; Fuchikami et al. 2006; Bailey et al. 2007; Mandery et al. 2010; Imanaga et al. 2011), and these juices have also reduced the exposure to orally administered substrates of these transporters, such as fexofenadine (Dresser et al. 2002a; Bailey 2010). Grapefruit, orange, and apple juices were thus interesting substances for interaction studies with the intestinal OATP-substrate aliskiren. Moreover, grapefruit juice is a mechanism-based inhibitor of intestinal CYP3A4 that enhances the oral bioavailabilities of many CYP3A4 substrates (Bailey et al. 1991; Lown et al. 1997; Schmiedlin-Ren et al. 1997; Bailey and Dresser 2004), complicating prediction of its effects on aliskiren pharmacokinetics. By contrast, orange and apple juices have not been reported to affect CYP3A4 activity (Bailey et al. 1991; Yee et al. 1995; Edwards et al. 1996; Kakar et al. 2004; Imanaga et al. 2011).

Considering the marked interindividual variability in aliskiren pharmacokinetics, the impact of the common genetic variations of P-gp and OATP2B1 on aliskiren pharmacokinetics was examined. The common single-nucleotide polymorphisms (SNPs) in the \textit{ABCB1} gene encoding P-gp, c.1236C>T (synonymous), c.2677G>T/A (p.Ala893Ser/Thr), and c.3435C>T (synonymous), have been variably associated with altered expression and function of P-gp in vitro and plasma concentrations of P-gp substrate drugs in vivo, especially when occurring as the c.1236T-c.2677T-c.3435T (TTT) and c.1236C-c.2677G-c.3435C (CGC) haplotypes (Hoffmeyer et al. 2000; Kim et al. 2001; Kroetz et al. 2003; Kimchi-Sarfaty et al. 2007; Keskitalo et al. 2008). For instance, the \textit{ABCB1} TTT haplotype has been associated with increased plasma concentrations of and enhanced lipid-lowering response to simvastatin (acid) and atorvastatin, compared with the \textit{ABCB1} CGC haplotype (Kajinami et al. 2004;
Fiegenbaum et al. 2005; Keskitalo et al. 2008). In the Finnish population, the frequencies of the ABCB1 TTT and CGC haplotypes are 42.7% and 34.4%, respectively, and the corresponding frequencies of the TTT/TTT and CGC/CGC genotypes are 17.6% and 12.4% (Keskitalo et al. 2008). Moreover, a non-synonymous SNP in the SLC02B1 gene encoding OATP2B1, c.935G>A (p.Arg312Gln), has been associated with reduced plasma concentrations of and impaired response to the leukotriene receptor antagonist montelukast in patients with asthma (Mougey et al. 2009). In the Finnish population, the frequency of the c.935A variant allele is 13.6%, and the frequencies of the c.935GA and c.935AA genotypes 23.6% and 1.8%, respectively (Laitinen and Niemi 2011).

To summarize, the purpose of this thesis was to investigate the effects of rifampicin and itraconazole as well as of grapefruit, orange, and apple juices on the pharmacokinetics and pharmacodynamics of the antihypertensive drug aliskiren in healthy volunteers. Furthermore, the roles of the ABCB1 c.1236T-c.2677T-c.3435T and c.1236C-c.2677G-c.3435C haplotypes and the SLC02B1 c.935G>A SNP in the pharmacokinetics of aliskiren were evaluated in prospective genotype panel studies.
1 Aliskiren

Aliskiren is an antihypertensive drug that acts by inhibiting renin, the first enzyme in the renin-angiotensin-aldosterone system (RAAS) (Skeggs et al. 1957; Wood et al. 2003; Vaidyanathan et al. 2008c). It was approved by the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) in 2007 and is the first direct renin inhibitor in clinical use (FDA 2007; EMA 2012).

1.1 Physicochemical properties

Aliskiren has a molecular formula of $C_{30}H_{53}N_{3}O_{6}$ and a molecular mass of 551.8 g/mol as its free base (609.8 g/mol as hemi-fumarate salt) (Figure 1) (Wood et al. 2003). It is a rather hydrophilic molecule (logarithm of the octanol/water partition coefficient is 2.45 and solubility in water >350 mg/ml at pH 7.4), and, as a base with pKa of 9.49, it is mostly ionized at physiological pH. Aliskiren has a low passive permeability through biological membranes due to its physicochemical properties (FDA 2007).

![Chemical structure of aliskiren](image)

*Figure 1. Chemical structure of aliskiren (IUPAC name (2S,4S,5S,7S)-5-amino-N-(2-carbamoyl-2,2-dimethylethyl)-4-hydroxy-7-[[4-methoxy-3-(3-methoxypropoxy)-phenyl]methyl]-8-methyl-2-(propan-2-yl)nonanamide).*
1.2 Pharmacokinetics

Aliskiren has a low oral bioavailability of 2-3% (Waldmeier et al. 2007), meaning that only a small fraction of the drug administered orally reaches the systemic circulation. The peak plasma concentration (C_{max}) is reached within 1-3 hours (Vaidyanathan et al. 2008c). Aliskiren is moderately bound to plasma proteins (47-51%), and the volume of distribution (V_d) averages 1.9 l/kg. The elimination occurs primarily as an unchanged drug by biliary excretion, and enterohepatic circulation ensues (Waldmeier et al. 2007). A small amount of aliskiren is also excreted unchanged into urine (0.4% of the oral dose). About 1.4% of the oral dose undergoes oxidative biotransformation, principally via the CYP3A4 enzyme. Minor amounts of nine different metabolites have been identified in plasma, urine, and faeces. At the time of the C_{max}, the most abundant metabolites in plasma have been M3 (alcohol, O-demethylated; 1-5% of aliskiren C_{max}), M2 (carboxylic acid, oxidized side chain; ≤1% of aliskiren C_{max}), and M1 (phenol, O-demethylated; trace amounts). The M3 metabolite (<1% of the dose) and trace amounts of M2, M4 (phenol, O-dealkylated), M6 (O-glucuronide conjugate of M4), and M9 (lactone) have been detected in urine. In addition, faecal extracts have contained M3 (0.6% of the dose), M2 (0.5% of the dose), M1 (0.1% of the dose), M4 (trace amounts), and M9 (trace amounts) metabolites, as well as M12 (N-acetylated), M13, and M14 metabolites (structural isomers containing an additional C_3H_4O_2 moiety), which have not been found elsewhere (M12, M13, and M14 have together accounted for 1% of the dose). Aliskiren has a low hepatic extraction ratio of 0.10 (Azizi et al. 2006). The elimination half-life (t_{1/2}) is approximately 30-40 hours (Vaidyanathan et al. 2008c).

According to the Biopharmaceutics Classification System, based on its high solubility and low permeability, aliskiren is categorized as a class three compound with typical pharmacokinetic characteristics, with metabolism having a minor role and drug transporters having a large impact on aliskiren absorption and disposition (FDA 2007; Shugarts and Benet 2009).

Aliskiren is a substrate of P-gp, with a Michaelis-Menten kinetic constant (K_m) of 2.1-3 μmol/l, but not of breast cancer resistance protein (BCRP), multidrug resistance-associated protein 2 (MRP2), OATP1B1, or OATP1B3 (Vaidyanathan et al. 2008b). In the initial studies, aliskiren was also shown to be transported by OATP2B1 in human embryonic kidney 293 (HEK293) cells, with a K_m of 72 μmol/l (Vaidyanathan et al. 2008b). Aliskiren has no inhibitory effect on CYP enzymes or P-gp, but it has inhibited OATP2B1, with an inhibitor concentration producing 50% inhibition (IC_{50}) of 95 μmol/l. Subsequently, in a study in HEK293 cells, aliskiren was found to be a substrate
of OATP1A2 ($K_m$ value could not be estimated in the concentration range of 0.8-50 μmol/l), and transport by OATP2B1 could not be demonstrated (Rebello et al. 2011c).

The pharmacokinetics of aliskiren is non-linear (Vaidyanathan et al. 2008c). In the range of 75-600 mg as a single oral dose, a 2-fold increase in dose has resulted in about 2.6- and 2.3-fold increases in the $C_{\text{max}}$ and the area under the plasma concentration-time curve (AUC), respectively. Mechanisms responsible for non-linearity have not been identified. A possible mechanism is saturation of transporters at the absorption site or in the hepatobiliary clearance route. A high degree of interindividual variability has been reported in the pharmacokinetics of aliskiren, and the between-subjects coefficients of variation (CV) have ranged between 50% and 100% for the $C_{\text{max}}$ and between 30% and 70% for the AUC (Vaidyanathan et al. 2006a; Vaidyanathan et al. 2007b; Waldmeier et al. 2007). The AUC of aliskiren has been shown to negatively correlate with body weight, and the $C_{\text{max}}$ and AUC of aliskiren to be 24% and 22% lower in men than in women, attributable to differences in body weight (Jarugula et al. 2010).

### 1.3 Pharmacokinetic interactions

Cyclosporine is a potent inhibitor of P-gp, OATP2B1, and CYP3A4 (Rao and Scarborough 1994; Kajosaari et al. 2005b; Ho et al. 2006b), and it has raised the $C_{\text{max}}$ and AUC of aliskiren 2.5-fold and 5-fold, respectively, when single oral doses of cyclosporine 600 mg and aliskiren 75 mg have been taken concomitantly (Rebello et al. 2011a). Accordingly, the concomitant use of aliskiren with potent P-gp inhibitors, such as cyclosporine or quinidine, has been contraindicated by EMA (EMA 2012). The exposure to aliskiren has also been increased by the dual inhibitors of CYP3A4 and P-gp verapamil (2-fold increases in $C_{\text{max}}$ and AUC of aliskiren) (Lemma et al. 2006; Rebello et al. 2011b), ketoconazole ($C_{\text{max}}$ and AUC of aliskiren increased by 81% and 76%, respectively) (Jurima-Romet et al. 1994; Olkkola et al. 1994; Wang et al. 2002; Vaidyanathan et al. 2008b), and atorvastatin ($C_{\text{max}}$ and AUC of aliskiren increased by 50% and 47%, respectively) (Neuvonen et al. 2006; Vaidyanathan et al. 2008b). High-fat meals have substantially decreased the absorption of aliskiren ($C_{\text{max}}$ and AUC of aliskiren decreased by 85% and 71%, respectively) (Vaidyanathan et al. 2008c).

Co-administration of aliskiren with the following drugs has resulted in 20-30% changes in the AUC of aliskiren: amlodipine (aliskiren AUC increased by 29%) (Vaidyanathan et al. 2006b), metformin (aliskiren AUC decreased by 27%) (Vaidyanathan et al. 2006b), and...
cimetidine (aliskiren AUC increased by 20%) (Dieterle et al. 2005; Ayalasomayajula et al. 2008), and valsartan (aliskiren AUC decreased by 26%) (Vaidyanathan et al. 2006b). In addition, irbesartan has reduced the $C_{\text{max}}$ of aliskiren by 50% (Vaidyanathan et al. 2007a). Aliskiren has reduced the $C_{\text{max}}$ and AUC of furosemide by 49% and 28%, respectively (Vaidyanathan et al. 2008a). No clinically relevant interactions have been found between aliskiren and acenocoumarol, allopurinol, atenolol, celecoxib, digoxin, fenofibrate, hydrochlorothiazide, isosorbide-5-mononitrate, lovastatin, pioglitazone, ramipril, or single-dose warfarin (Dieterle et al. 2004; Dieterle et al. 2005; Vaidyanathan et al. 2006b; Ayalasomayajula et al. 2008; Huang et al. 2008; Vaidyanathan et al. 2008a; Vaidyanathan et al. 2008b; Vaidyanathan et al. 2008d).

1.4 Renin-angiotensin-aldosterone system and pharmacodynamics of aliskiren

The history of the discovery of the renin-angiotensin-aldosterone system (RAAS) began in 1898, when the Finnish physiologist Robert Tigerstedt and his Swedish student Per Bergman demonstrated that an extract from the renal cortex of rabbits (later named renin) increased blood pressure when injected intravenously (Tigerstedt and Bergman 1898). Nowadays, RAAS is known to have an important role in the regulation of blood pressure, fluid volume, and vascular response to injury and inflammation (Figure 2) (Peach 1977; Fyhrquist and Saijonmaa 2008; Ma et al. 2010; Ruggenenti et al. 2010).

The RAAS cascade begins with the release of renin from the juxtaglomerular cells of the kidneys in response to reduction in blood pressure, blood volume, renal perfusion, or plasma sodium levels, or when the β-adrenergic receptors are stimulated (Peach 1977; Fyhrquist and Saijonmaa 2008; Ma et al. 2010). The excretion of renin is further reduced by negative feedback action of angiotensin II. Renin catalyses the first and the rate-limiting step of the cascade where angiotensinogen, mainly produced by the liver, is converted to biologically inactive angiotensin I. Angiotensin I is further activated in the circulation and tissues by angiotensin-converting enzyme (ACE) to form angiotensin II, the main effector of the system. Angiotensin II may also be formed by non-ACE pathways involving chymases.

Angiotensin II has a number of rapid effects, including vasoconstriction, increased aldosterone secretion, increased thirst and release of antidiuretic hormone, increased myocardial contractility, and increased activity of the sympathetic nervous system,
which are all intended to increase the effective circulating volume (Fyhrquist and Saijonmaa 2008; Ma et al. 2010; Ruggenenti et al. 2010). However, in the long run, angiotensin II can cause remodelling of the cardiovascular system, characterized by hypertrophy and fibrosis. Angiotensin II exerts its actions via AT₁ and AT₂ receptors, and the foregoing effects are mainly consequences of the activation of AT₁ receptor. By contrast, AT₂ receptor mediates in principle the opposite actions, such as vasodilatation and inhibition of growth, although it may also contribute to some potentially harmful effects of angiotensin II. The inappropriate activation of RAAS may result in hypertension. The drugs affecting RAAS include ACE inhibitors, angiotensin II type 1 receptor blockers, aldosterone antagonists, and direct renin inhibitors.

Figure 2. Renin-angiotensin-aldosterone system. Adapted from Fyhrquist and Saijonmaa 2008, Ma et al. 2010, and Ruggenenti et al. 2010.
ACE, angiotensin-converting enzyme.
Aliskiren is a competitive inhibitor of human renin with an IC\textsubscript{50} of 0.6 nmol/l (Wood et al. 2003). In clinical trials, aliskiren has reduced plasma renin activity by 50-80% (FDA 2007; EMA 2012). The renin-inhibiting effect of aliskiren is detectable already 30 minutes after the first aliskiren dose (Nussberger et al. 2002), but the maximal blood pressure-lowering effect is not achieved until about two weeks (EMA 2012; Novartis 2012). Aliskiren has had no effect on blood pressure or heart rate in healthy volunteers in dosages up to 640 mg once daily for eight days (Nussberger et al. 2002). In a pooled analysis of placebo-controlled trials, including altogether 7045 patients with mild to moderate hypertension and treatment durations of 6-8 weeks, aliskiren has reduced mean sitting systolic/diastolic blood pressures from baseline by 8.6-12.1/7.2-10.3 mmHg (75 mg), 8.7-13.0/7.8-10.3 mmHg (150 mg), 14.1-15.8/10.3-12.3 mmHg (300 mg), and 15.7-15.8/11.5-12.5 mmHg (600 mg), compared with 2.9-10.0/3.3-8.6 mmHg for placebo (Weir et al. 2007). In clinical studies ranging in duration from four to 52 weeks, the antihypertensive efficacy of aliskiren has been essentially similar to that of the diuretic hydrochlorothiazide, beta blocker atenolol, calcium-channel blocker amlodipine, ACE inhibitor ramipril, and angiotensin-receptor blockers irbesartan, losartan, and valsartan (Stanton et al. 2003; Gradman et al. 2005; Oparil et al. 2007; Dietz et al. 2008; Schmieder et al. 2009; Brown et al. 2011). The efficacy of aliskiren in the prevention of hypertension-related end-organ damage, such as left ventricular hypertrophy or kidney failure, or in the reduction of cardiovascular morbidity and mortality has not been proven. Instead, at the end of 2011, the independent Data Monitoring Committee recommended early termination of the ALTITUDE study (Aliskiren trial in type 2 diabetes using cardio-renal disease endpoints), in which aliskiren was used in addition to an ACE inhibitor or angiotensin-receptor blocker in high-risk patients with diabetes and renal impairment (EMA 2012). Patients treated with aliskiren experienced a significantly higher number of cardiovascular and renal problems than patients given placebo.

1.5 Clinical use and adverse effects

At the moment, the role of aliskiren in the treatment of hypertension has not been established. Aliskiren is approved for the treatment of essential hypertension at once-daily doses of 150 mg and 300 mg as monotherapy or in combination with other antihypertensive agents (FDA 2007; EMA 2012). However, the combination of aliskiren with ACE inhibitors or angiotensin-receptor blockers is not recommended in any patient groups and contraindicated in patients with diabetes or moderate or severe
kidney impairment because of the risk of adverse outcomes such as hyperkalaemia, hypotension, stroke, or kidney complications (EMA 2012). As high-fat meals reduce aliskiren absorption (Vaidyanathan et al. 2008c), aliskiren is recommended to be taken with a light meal or in a routine pattern with regard to meals (FDA 2007; EMA 2012).

The most common adverse effect with aliskiren is diarrhoea (FDA 2007; Parving et al. 2008; EMA 2012; Novartis 2012). Other adverse reactions include rash, arthralgia, cough, small decreases in haemoglobin and haematocrit, hyperkalaemia, and an increase in blood creatinine. In addition, rare cases of hypersensitivity reactions, severe cutaneous adverse reactions, angioedema, and renal failure have been reported.
2. Individual differences in drug response

Substantial differences in response to drugs commonly exist among patients (Wilkinson 2005; Eichelbaum et al. 2006). Such variability arises from differences in both intrinsic and extrinsic factors, which further affect the pharmacokinetics and pharmacodynamics of drugs (Figure 3).

![Diagram of drug response and factors](image)

**Figure 3.** Factors modifying drug response.

2.1 Pharmacokinetic aspects

Pharmacokinetics is defined as quantification of the time course of a drug and its metabolites in the body (Rowland and Tozer 2011). The concentration of the drug in plasma and peripheral tissues depends on the extent of its absorption, distribution, and elimination (i.e. metabolism and excretion). Drugs may pass through biological membranes by either a paracellular or transcellular pathway, the latter comprising passive diffusion and passage involving facilitated mechanisms, such as channels and
drug transporters. Important factors affecting transmembrane passage of drugs are physicochemical properties of the drug (molecular size, charge, and lipophilicity), membrane characteristics (thickness, surface area, existence of tight junctions or fenestrations), and activity of drug transporters.

Pharmacokinetic drug interactions (i.e. the delivery of a drug to its site of action is altered) are important causes of variability in drug response, having consequences ranging from lack of efficacy to toxicity (Pirmohamed et al. 2004; Wilkinson 2005). The main mechanisms of these interactions are physicochemical interactions, plasma protein binding-related interactions, and interactions affecting the activity of drug transporters and metabolizing enzymes (Bjornsson et al. 2003; Neuvonen 2012). In general, changes in plasma drug concentrations of 2-fold or more are considered potentially relevant clinically (Dresser et al. 2000). However, less pronounced pharmacokinetic interactions may be clinically important, especially for drugs with a narrow therapeutic index. Coefficient of variation (CV) is commonly used to express the variability in pharmacokinetics (Rowland and Tozer 2011). In general, a CV of 10% or less is considered low variability, 25% moderate variability, and above 40% high variability.

2.2 Pharmacodynamic aspects

By definition, pharmacodynamics is a study of the mechanisms of drug action and the relationship between the effects produced and the systemic exposure to the drug over time (Rowland and Tozer 2011). Most drugs interact with biological molecules, such as receptors, ion channels, enzymes, transporters, or deoxyribonucleic acid (DNA), to produce a response by blocking or triggering biochemical and physiological events (Mager et al. 2003). Pharmacodynamic drug interactions may be caused by a large variety of mechanisms, which may lead to antagonistic, synergistic, or additive effects (Danhof et al. 2007).

2.3 Pharmacogenetic aspects

By definition, pharmacogenomics is a study of variations of DNA and ribonucleic acid (RNA) characteristics as related to drug response (EMA 2007). Pharmacogenetics, its subset, is defined as the study of variations in DNA sequence as related to drug
response. Nucleotide sequence variation (polymorphism) in drug transporters, drug metabolizing enzymes, and drug targets can significantly contribute to interindividual variability in drug response (Eichelbaum et al. 2006). Polymorphisms include nucleotide substitutions (single-nucleotide polymorphism, SNP), insertions, deletions, and duplications of one or more nucleotides, short sequence repeats, and gene copy-number variations (Nebert and Vesell 2004; Eichelbaum et al. 2006; Ingelman-Sundberg et al. 2007).

Polymorphisms can cause phenotypic variation by many mechanisms (Sadée and Dai 2005; Pastinen et al. 2006; Cheung and Spielman 2009). Polymorphisms in the coding regions of the gene may alter the amino acid sequence of the translated protein (non-synonymous variants), thus affecting protein structure and function. Moreover, both synonymous and non-synonymous coding region variants can alter the secondary structure of mRNA, causing differences in mRNA stability or translation efficiency and altered protein expression levels. In addition, polymorphisms in splice sites or splicing regulatory sites can alter RNA splicing, altering the mRNA produced. Polymorphisms in the gene regulatory regions can influence gene expression, and consequently, the amount of protein. Cis-acting polymorphisms are found close to the target genes, whereas trans-acting polymorphisms are located far from the target genes, often on another chromosome.

Phenotypes are not solely determined by genotypes, but are also influenced by environmental factors. Pharmacoepigenetics is a study of heritable changes in gene expression or phenotype caused by mechanisms other than variations in underlying DNA sequence, such as DNA methylation or histone modification (Ivanov et al. 2012).
3. Drug transporters

Drug transporters are important determinants of absorption, tissue distribution, and elimination of drugs (Giacomini et al. 2010). They may be classified as influx (uptake into the cell) or efflux (out of the cell) transporters, which typically are localized in either the apical or basolateral membrane of polarized cells. Different drug transporters can be expressed in the same cell, share the same substrates, and act in concert with each other and with drug-metabolizing enzymes (Wacher et al. 1995; Glaeser et al. 2007; Giacomini et al. 2010). It is thus this complex interplay that ultimately determines the absorption and disposition as well as the clinical effects of the drug (Giacomini et al. 2010). Drug transporters also have important physiological functions, as they transport many endogenous substrates, including amino acids, lipids, sugars, bile acids, and hormones (Ho and Kim 2005).

The genetic variability in genes encoding drug transporters can partly explain the interindividual variability in drug pharmacokinetics and drug response (Giacomini et al. 2010). It has also become evident that clinically significant drug interactions can result from the inhibition or induction of drug transporters (Müller and Fromm 2011). For instance, inhibition of intestinal influx or efflux transporters can lead to decreased or increased systemic exposure to the parent compound. Modification of the activity of drug transporters can also affect the distribution of drugs into organs where these transporters are expressed (e.g. liver, central nervous system) as well as alter the transporter-mediated excretion of drugs and metabolites. Altered activity of drug transporters may also contribute to adverse drug reactions (Zolk and Fromm 2011). The estimation of the role of a single transporter in drug pharmacokinetics and pharmacokinetic interactions is challenging because drugs are usually substrates of several drug transporters and often subject to metabolism by CYP enzymes. The human genome contains numerous genes encoding membrane transporters; the two major superfamilies, adenosine triphosphate (ATP)-binding cassette (ABC) and solute carrier (SLC), alone comprise more than 400 members (Giacomini et al. 2010). The International Transporter Consortium has compiled a list of drug transporters that have evidence from published studies demonstrating their role in pharmacokinetics and pharmacokinetic interactions (Figure 4, Tables 1 and 2).
Figure 4. Selected human transporters for drugs and endogenous compounds, expressed in the apical or basolateral membrane of intestinal enterocyte (A), hepatocyte (B), renal proximal tubular cell (C), or brain capillary endothelial cell (D).

3.1 Influx transporters

3.1.1 Organic-anion transporting polypeptides

Organic anion-transporting polypeptides (OATPs) are membrane influx transporters that regulate the cellular uptake of a number of endogenous compounds and therapeutic drugs in various tissues important for pharmacokinetics (Niemi 2007). They are encoded by genes of the solute carrier organic anion transporter (SLCO) superfamily.
OATPs are classified based on amino acid sequence homology into families designated by Arabic numbering (≥ 40% amino acid sequence identity), e.g. OATP1, subfamilies designated by letters (≥ 60% amino acid sequence identity), e.g. OATP1A, and individual proteins designated by additional continuous Arabic numbering based on the chronology of identification, e.g. OATP1A2. Since the first Oatp, rat Oatp1a1, was identified and isolated by expression cloning in 1994 from rat liver (Jacquemin et al. 1994), 12 OATPs have been identified in humans: OATP1A2, OATP1B1, OATP1B3, OATP1B7, OATP1C1, OATP2A1, OATP2B1, OATP3A1, OATP4A1, OATP4C1, OATP5A1, and OATP6A1 (Hagenbuch and Meier 2004; NCBI 2012).

The general predicted OATP structure consists of glycoproteins with 12 transmembrane domains with 643-722 amino acids and apparent molecular masses between 70 and 90 kDa (Hagenbuch and Gui 2008). Transport has been suggested to occur through a central positively charged pore in a so-called rocker-switch type of mechanism (Meier-Abt et al. 2005). This mechanism consists of anion exchange, in which the cellular uptake of substrate is coupled with the efflux of endogenous intracellular substances, such as bicarbonate, glutathione, or glutathione-S-conjugates, in a process that seems to be electroneutral, but may be driven by the pH gradient (Satlin et al. 1997; Li et al. 1998; Li et al. 2000; Kobayashi et al. 2003; Leuthold et al. 2009).

OATP1A2, the first cloned human OATP, facilitates the absorption of its substrates in the small intestine, in addition to which it probably mediates the influx across the blood-brain barrier (Kullak-Ublick et al. 1995; Gao et al. 2000; Steckelbroeck et al. 2004; Lee et al. 2005; Glaser et al. 2007). The transporter is also found in several other tissues, including cholangiocytes in the liver and the apical membrane of cells in distal nephrons in the kidney (Kullak-Ublick et al. 1995; Lee et al. 2005). OATP1A2 transports a wide range of endogenous as well as exogenous substrates, including bile acids, steroid hormones and their conjugates, thyroid hormones, and numerous drugs, such as beta blockers and fexofenadine (Cvetkovic et al. 1999; Maeda et al. 2007; Kalliokoski and Niemi 2009; Kato et al. 2009; Shirasaka et al. 2010). Rifampicin, certain fruit juices, and some flavonoids have been shown to inhibit OATP1A2 in vitro (Dresser et al. 2002a; Vavricka et al. 2002; Bailey et al. 2007; Mandery et al. 2010; Rebello et al. 2011c). Grapefruit juice, orange juice, and apple juice have also reduced the oral bioavailabilities of some OATP1A2 substrate drugs in vivo (Dresser et al. 2002a; Bailey 2010; Jeon et al. 2012). Several SNPs in the SLCO1A2 gene have been found of which the c.516A>C (p.Glu172Asp), c.382A>T (p.Asn128Tyr), c.404A>T (p.Asn135Ile), and
c.2003C>G (p.Thr668Ser) SNPs have been associated with decreased OATP1A2 transport activity in vitro (Lee et al. 2005; Eechoute et al. 2011). However, they have not affected the pharmacokinetics of imatinib in patients with cancer (Eechoute et al. 2011). The SLCO1A2 g.-1105G>A, g.-1032G>A, and g.-361G>A SNPs in the promoter region have been suggested to affect the clearance of imatinib (Yamakawa et al. 2011).

OATP1B1 is localized to the sinusoidal membrane of hepatocytes, where it participates in the hepatic uptake of a broad range of compounds from the portal venous blood (Abe et al. 1999; Hsiang et al. 1999; König et al. 2000a; König et al. 2000b; Niemi et al. 2011). At the mRNA level, OATP1B1 has also been found in small intestinal enterocytes (Glaeser et al. 2007). OATP1B1 transports bile acids, unconjugated and conjugated bilirubin, eicosanoids, steroid conjugates, thyroid hormones, and many drugs, including 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) (Niemi et al. 2011). A common variant of the SLCO1B1 gene, the c.521T>C (p.Val174Ala) SNP, has been associated with a reduced transport activity of OATP1B1 in vitro and markedly increased plasma concentrations of OATP1B1 substrate drugs in vivo (Niemi et al. 2011). In individuals homozygous for the SLCO1B1 c.521T>C SNP, the hepatic influx of active simvastatin acid has been shown to be severely impaired, resulting in a more than 3-fold increased plasma simvastatin acid exposure (Pasanen et al. 2006b), and consequently, a markedly increased risk of simvastatin-induced myopathy, as compared with individuals with the c.521TT genotype (Link et al. 2008). The same variant has also been found to affect the plasma concentrations of other statins, such as atorvastatin (2.4-fold increase in AUC), pravastatin (1.9-fold increase in AUC), and rosuvastatin (1.7-fold increase in AUC), but not fluvastatin (Niemi et al. 2006b; Pasanen et al. 2007). In the Finnish population, the frequency of the SLCO1B1 c.521CC genotype is approximately 4.3% (Pasanen et al. 2006a). Many drugs have been identified as inhibitors of OATP1B1 in vitro, potentially causing important pharmacokinetic interactions in vivo (Niemi et al. 2011). For example, inhibition of OATP1B1 has had a major role in pharmacokinetic interactions in which cyclosporine and gemfibrozil have increased the systemic exposure to many statins (Neuvonen et al. 2006).

OATP1B3 shares 80% amino acid identity with OATP1B1 (König et al. 2000a). It is also localized to the sinusoidal membrane of hepatocytes, but unlike OATP1B1, which shows a uniform expression pattern throughout the liver lobe, OATP1B3 is highly expressed in perivenous regions (König et al. 2000a; Abe et al. 2001). The substrate specificity of OATP1B3 is very similar to that of OATP1B1 and includes bile acids,
bilirubin, eicosanoids, steroid conjugates, thyroid hormones, and numerous drugs (Hagenbuch and Gui 2008; Kalliokoski and Niemi 2009). In addition, OATP1B3 has drug substrates, such as docetaxel and paclitaxel, that are not transported by OATP1B1 (Smith et al. 2005). Many inhibitors of OATP1B1 also inhibit OATP1B3 (Kalliokoski and Niemi 2009; Niemi et al. 2011).

**OATP2B1** is expressed widely in human tissues, including the intestine, liver, brain, heart, pancreas, platelets, skeletal muscle, skin, testis, mammary gland, and placenta (Tamai et al. 2000; Kullak-Ublick et al. 2001; St-Pierre et al. 2002; Kobayashi et al. 2003; Pizzagalli et al. 2003; Schiffer et al. 2003; Steckelbroeck et al. 2004; Englund et al. 2006; Grube et al. 2006; Grube et al. 2007; Niessen et al. 2009; Knauer et al. 2010). At the cellular level, OATP2B1 is localized to the luminal membrane of small intestinal enterocytes, to the sinusoidal membrane of hepatocytes, to the apical membrane of brain endothelial cells, to the vascular endothelium of the heart, to the basal (foetal-facing) membrane of syncytiotrophoblasts, and to the basolateral membrane of the non-pigmented epithelium in the ciliary body (Kullak-Ublick et al. 2001; St-Pierre et al. 2002; Kobayashi et al. 2003; Bronger et al. 2005; Gao et al. 2005; Grube et al. 2006). In the transcription analysis of the **SLCO2B1** gene, five transcriptional variants differing in length and transcription start sites have been found, suggesting that the gene is differently expressed in different tissues (Pomari et al. 2009).

OATP2B1 transports bile acids, steroid conjugates, and such drugs as some statins and montelukast (Kalliokoski and Niemi 2009; Mougey et al. 2009). Grapefruit juice and orange juice have been shown to inhibit OATP2B1 in vitro, and it has been hypothesized that they may reduce the absorption of OATP2B1 substrate drugs (Satoh et al. 2005). However, grapefruit juice has not significantly affected the pharmacokinetics of glibenclamide, an OATP2B1 substrate, in humans (Lilja et al. 2007). Cyclosporine and gemfibrozil have also been found to inhibit OATP2B1 in vitro (Ho et al. 2006b).

Various sequence variants have been described in the **SLCO2B1** gene encoding the OATP2B1 influx transporter, but their clinical relevance has not been clarified (Nozawa et al. 2002; Ho et al. 2006a; Aoki et al. 2009; Mougey et al. 2009). A non-synonymous SNP in the **SLCO2B1** gene, c.935G>A (p.Arg312Gln), has been associated with reduced plasma concentrations of and impaired response to montelukast in patients with asthma (Mougey et al. 2009). By contrast, no relationship has been found between the c.935G>A SNP and the pharmacokinetics of celiprolol (Ieiri et al. 2012). In the Finnish
population, the c.935A variant allele has been reported to have a frequency of 13.6%, and the c.935GA and c.935AA genotypes frequencies of 23.6%, and 1.8%, respectively (Laitinen and Niemi 2011).

Of other sequence variants of \( SLCO2B1 \), the c.43C>T (p.Pro15Ser), c.601G>A (p.Val201Met), and c.1175C>T (p.Thr392Ile) SNPs as well as the p.Glu26_Thr28del deletion variant have been associated with reduced transport activity, and the \( SLCO2B1 \) c.1457C>T (p.Ser486Phe) SNP with both reduced and increased transport activity of the protein in vitro (Nozawa et al. 2002; Ho et al. 2006a). In addition, the \( SLCO2B1 \) g.-282G>A SNP in the promoter region has been associated with increased protein expression level in the human liver (Aoki et al. 2009). The \( SLCO2B1 \) c.1457C>T SNP has been associated with reduced plasma concentrations of celiprolol and fexofenadine in healthy volunteers (Imanaga et al. 2011; Ieiri et al. 2012). Furthermore, the extent of the interaction between apple juice and fexofenadine has been smaller in individuals carrying the c.1457C>T SNP than in individuals with the c.1457CC genotype (Imanaga et al. 2011).

\textbf{OATP4C1} is considered to be a kidney-specific transporter. It is localized to the basolateral membrane of renal proximal tubular cells, where it possibly mediates the uptake of its substrates from the blood into the kidney (Mikkaichi et al. 2004). Among the substrates of OATP4C1 are thyroid hormones, digoxin, methotrexate, and sitagliptin (Mikkaichi et al. 2004; Chu et al. 2007).

3.1.2 Other influx transporters

\textbf{Organic anion transporters (OATs)} 1 and 3 are anion-exchangers expressed mainly in the basolateral membrane of the renal proximal tubular cells, where they may mediate the renal uptake of their substrates from blood (Hosoyamada et al. 1999; Cha et al. 2001; Klaassen and Aleksunes 2010). These substrates include endogenous compounds such as urate, as well as many drugs, including antimicrobial agents, non-steroidal anti-inflammatory drugs (NSAIDs), and diuretics (Rizwan and Burckhardt 2007; Klaassen and Aleksunes 2010). A classic example of a pharmacokinetic drug interaction is the reduction in renal excretion of benzylpenicillin, an OAT3 substrate, caused by probenecid, the inhibitor of OAT1 and OAT3 (Gibaldi and Schwartz 1968). Probenecid and many NSAIDs may also reduce the renal excretion of methotrexate by inhibiting OATs (Takeda et al. 2002). It has been suggested that OAT1 is involved in
nephrotoxicity caused by antiviral drugs adefovir and cidofovir (Ho et al. 2000a; Uwai et al. 2007), and that probenecid could be used to overcome this effect (Zolk and Fromm 2011).

**Organic cation transporters (OCTs) 1 and 2** are uniporters that mediate the facilitated diffusion of a variety of low molecular mass organic cations in either direction, driven by the electrochemical potential (Gorboulev et al. 1997; Koepsell et al. 2007). OCT1 is primarily expressed in the sinusoidal membrane of hepatocytes, where it mediates the exit from portal venous blood (Gorboulev et al. 1997), but is also found in other tissues, such as the apical membrane of renal proximal and distal tubular cells (Koepsell et al. 2007; Tzvetkov et al. 2009). OCT2 is principally expressed in the basolateral membrane of renal proximal tubular cells (Gorboulev et al. 1997), but is also found in neurons, and its endogenous substrates include many neurotransmitters (Koepsell et al. 2007).

A prototypic drug substrate of both of these OCT transporters is metformin (Dresser et al. 2002b; Kimura et al. 2005). Metformin is primarily excreted into urine by a mechanism involving a functional interplay between OCT2-mediated metformin uptake and efflux by multidrug and toxin extrusion protein 1 (MATE1) in the renal proximal tubular cell (Meyer zu Schwabedissen et al. 2010; König et al. 2011). In the liver, i.e. the site of metformin action, the drug transport into and out of the hepatocyte is mediated by OCT1 and MATE1, respectively (König et al. 2011). Certain low-activity genotypes of the **SLC22A1** gene encoding OCT1 have been associated with reduced metformin efficacy, probably as a result of decreased hepatic uptake and increased renal excretion of metformin (Shu et al. 2007; Shu et al. 2008; Tzvetkov et al. 2009). Genetic variation in the **SLC22A2** gene encoding OCT2 may also have a role in the differences in the excretion of metformin (Leabman et al. 2002; Song et al. 2008; Wang et al. 2008; Chen et al. 2009). Moreover, OCT1 transports imatinib into mononuclear cells in chronic myeloid leukaemia, and the functional activity of OCT1 has been shown to be positively associated with the degree to which patients respond to imatinib (White et al. 2006; White et al. 2007; White et al. 2010).

OCTs may contribute to clinical pharmacokinetic interactions (Fahrmayr et al. 2010; Giacomini et al. 2010). Inhibitors of OCT1 and OCT2 include cimetidine and quinidine (Urakami et al. 1998; Zolk et al. 2009), in addition to which, for example, some antiviral drugs and oral antidiabetic drugs have been shown to inhibit OCT1 (Bachmakov et al. 2008; Jung et al. 2008), and some beta blockers OCT2 (Bachmakov et al. 2009; Zolk et al. 2009). Cimetidine has also inhibited the OCT2-mediated renal
excretion of metformin in vivo (AUC of metformin increased by 50%) (Somogyi et al. 1987; Wang et al. 2008). Moreover, OCT2 probably plays an important role in the development of nephro- and ototoxicity caused by cisplatin, and OCT2 inhibitors have been suggested as an option to reduce these adverse effects (Ciarimboli et al. 2005; Ciarimboli et al. 2010; Zolk and Fromm 2011).

The peptide transporters (PEPTs) 1 and 2 are proton-coupled symporters responsible for the cellular uptake of physiologically occurring peptides and structurally related drugs, such as β-lactam antibacterials (Ganapathy and Leibach 1983; Fei et al. 1994; Liu et al. 1995; Brandsch 2009). PEPT1 is primarily expressed in the luminal membrane of intestinal enterocytes, where it mediates the absorption of di- and tripeptides originating from protein digestion (Groneberg et al. 2001; Ziegler et al. 2002). In the renal proximal tubular cells, PEPT2 is responsible for the re-uptake of peptides from the glomerular filtrate, probably accompanied by PEPT1 (Liu et al. 1995; Brandsch 2009).

3.2 Efflux transporters

3.2.1 P-glycoprotein

The multidrug resistance transporter 1 P-glycoprotein (MDR1, P-gp) belongs to the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily and is encoded by the ABCB1 gene (Fairchild et al. 1987). P-gp was first characterized in tumour cells, where it produced resistance to multiple anticancer agents (Biedler and Riehm 1970; Juliano and Ling 1976; Kartner et al. 1983). Subsequently, it was shown to have important physiological functions. P-gp is an efflux pump located in the luminal membrane of enterocytes in the small intestine, in the apical membrane of excretory cells such as hepatocytes and renal proximal tubular cells, and in the apical membrane of endothelial cells that comprise many blood-tissue barriers such as the blood-brain, blood-testes, and blood-placenta barriers (Thiebaut et al. 1987; Cordon-Cardo et al. 1990; Fromm 2004). Energy provided by ATP hydrolysis enables P-gp to work against considerable concentration gradients (Senior and Gadsby 1997). In the small intestine, P-gp prevents the entry of foreign compounds into the systemic circulation by transporting molecules from enterocytes back into the intestinal lumen (Watkins 1997). This protective function is shared with CYP3A4, and many compounds have been identified as substrates of both P-gp and CYP3A4 (Schuetz et al. 1996; Watkins 1997; Kivistö et al. 2004). P-gp also enhances the elimination of its substrates and their
metabolites by facilitating their removal by excretion into bile, urine, and the intestine (Wetterich et al. 1996; Fromm 2004). In addition, P-gp has an important role in drug distribution by limiting entry of various drugs into sensitive tissues such as the central nervous system, foetal circulation, testis, and lymphocytes (Fromm 2004).

P-gp comprises approximately 1280 amino acids and has a molecular mass of 170 kDa. An x-ray crystallographic structure of the mouse P-gp, which has 87% sequence identity to human P-gp, was described in 2009 (Aller et al. 2009). It revealed that in the initial state of the transport cycle, P-gp has an inward-facing conformation formed from two halves, each containing six transmembrane helices and an intracellular nucleotide-binding domain. These two halves form a large internal cavity open both to the inner leaflet of the cell membrane and to the cytoplasm and contain distinct binding sites for drugs. During the catalytic cycle the binding of a substrate stimulates further the binding of ATP, which likely causes dimerization of the nucleotide-binding domains. This produces large structural changes, resulting in an outward-facing conformation. Substrates may be released either as a result of ATP hydrolysis or as a consequence of decreased binding affinity. ATP hydrolysis likely resets the system back to inward facing.

P-gp transports a broad variety of structurally diverse molecules, ranging in size from less than 200 Da to almost 1900 Da (Schinkel and Jonker 2003). Most P-gp substrates are quite hydrophobic and can therefore insert into the inner leaflet of the cell membrane to be transported by P-gp (Aller et al. 2009). The substrates of P-gp include antibacterial, anticancer, and antiviral agents, beta blockers, calcium-channel blockers, glucocorticoids, statins, immunosuppressants, and aliskiren (Fromm 2004; Vaidyanathan et al. 2008b). Digoxin is recommended by regulatory authorities as a probe drug in vivo in pharmacokinetic interaction studies with new molecules that are inhibitors of P-gp (EMA 2010b; Giacomini et al. 2010; FDA 2012). The advantage of digoxin is its negligible metabolism (Iisalo 1977). However, digoxin has a narrow therapeutic index and a high oral bioavailability (60-80%), and it is not entirely safe or very sensitive as a probe drug.

The level of expression and functionality of P-gp can be modulated by induction and inhibition, and P-gp is thus involved in many clinically significant pharmacokinetic interactions (Müller and Fromm 2011). The transcription of ABCB1 is regulated by nuclear receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) (Geick et al. 2001; Burk et al. 2005). Rifampicin induces P-gp via PXR and is
recommended as an inducer in drug interaction studies involving P-gp substrates (Schuetz et al. 1996; Greiner et al. 1999; Geick et al. 2001; FDA 2012). St. John’s wort is another known inducer of P-gp (Johne et al. 1999). In addition, thyroid hormones have been found to induce the expression of ABCB1 mRNA in the intestine and kidney cell lines (Burk et al. 2010). Among the inhibitors of P-gp are cyclosporine, itraconazole, ketoconazole, and verapamil (Naito and Tsuruo 1989; Rao and Scarborough 1994; Wang et al. 2002; Marzolini et al. 2004).

Because of the localization at important blood-tissue barriers, inhibition of P-gp may have a larger effect on tissue drug exposure than on plasma exposure (Eyal et al. 2009). In a study with knockout mice, digoxin concentration in the brain after intravenous administration was about 27-fold higher in mice lacking mdr1-type P-gp transporters, compared with wild-type mice, whereas no significant difference was found in plasma digoxin concentrations (Schinkel et al. 1997). However, prediction of human transporter-mediated drug interactions based on preclinical animal data is challenging (Giacomini et al. 2010).

Many sequence variations have been described in the ABCB1 gene (Hoffmeyer et al. 2000; Fromm 2002; Chinn and Kroetz 2007). However, findings regarding the effect of ABCB1 polymorphisms on P-gp substrates have not been consistently reproduced, and therefore, further studies with larger numbers of samples may be needed to clarify the clinical impact of ABCB1 polymorphisms (Chinn and Kroetz 2007; Giacomini et al. 2010). The most studied sequence variant is a common synonymous ABCB1 c.3435C>T SNP, which has been variably associated with reduced expression and function of P-gp in vitro and increased plasma concentrations of P-gp substrate drugs in vivo (Hoffmeyer et al. 2000; Kimchi-Sarfaty et al. 2007; Keskitalo et al. 2008). This SNP has been suggested to affect ABCB1 mRNA stability (Wang et al. 2005). On the other hand, the associations may also be explained by linkage disequilibrium between c.3435C>T and a functional sequence variation. The ABCB1 c.3435C>T SNP has been found to be in strong linkage disequilibrium with the c.1236C>T (synonymous) and c.2677G>T/A (p.Ala893Ser/Thr) SNPs in the same gene (Kim et al. 2001; Kroetz et al. 2003; Keskitalo et al. 2008). Alternatively, c.3435C>T and sequence variations in linkage disequilibrium with it may form a haplotype conferring altered P-gp activity (Kim et al. 2001; Kimchi-Sarfaty et al. 2007). The variant alleles c.1236T, c.2677T, and c.3435T are common in Europeans and European Americans, with reported frequency ranges of 39-46%, 39-47%, and 54-60%, respectively (Cascorbi et al. 2001; Kim et al. 2001; Kroetz et al. 2003; Leschziner et al. 2007; Keskitalo et al. 2008). In the Finnish
population, the two most common haplotypes are c.1236T-c.2677T-c.3435T (TTT) and c.1236C-c.2377G-c.3435C (CGC), with frequencies of 42.7% and 34.4%, respectively (Keskitalo et al. 2008). The ABCB1 TTT haplotype has been associated with, for example, increased plasma concentrations of and enhanced lipid-lowering response to simvastatin (acid) (AUC increased by 60% in individuals with the ABCB1 TTT/TTT genotype, compared with individuals with the CGC/CGC genotype) and atorvastatin (AUC increased by 55%) (Kajinami et al. 2004; Fiegenbaum et al. 2005; Keskitalo et al. 2008). Studies investigating the effects of the ABCB1 c.3435C>T SNP on the pharmacokinetics of digoxin have revealed conflicting results, with a meta-analysis suggesting that the ABCB1 c.3435C>T SNP does not affect digoxin pharmacokinetics (Greiner et al. 1999; Hoffmeyer et al. 2000; Sakaeda et al. 2001; Johne et al. 2002; Verstuyft et al. 2003; Chowbay et al. 2005). It has been suggested that the ABCB1 c.3435C>T SNP, or its combination with the c.1236C>T or c.2677G>T/A SNP, may alter the substrate specificity of P-gp, and therefore, the effect of the ABCB1 genotype on the pharmacokinetics of different P-gp substrates may vary (Kimchi-Sarfaty et al. 2007).

3.2.2 Other efflux transporters

Breast cancer resistance protein (BCRP), also known as ATP-binding cassette G2 protein encoded by the ABCG2 gene, was first cloned based on its overexpression in a multidrug-resistant Michigan Cancer Foundation (MCF-7) breast cancer cell line (Doyle et al. 1998). BCRP mediates the efflux of its substrates in the physiological barriers and excreting organs, such as the luminal membrane of enterocytes, canalicular membrane of hepatocytes, apical membrane of brain capillary endothelial cells, and maternal side of placental syncytiotrophoblasts (Maliepaard et al. 2001; Schinkel and Jonker 2003; Leslie et al. 2005). The physiological functions of BCRP include the transport of oestradiol conjugates, the extrusion of porphyrins from hematopoietic cells and hepatocytes, as well as the secretion of vitamin B2 and possibly other vitamins, such as biotin and vitamin K, into breast milk (Choudhuri and Klaassen 2006; Giacomini et al. 2010). Among the drug substrates of BCRP are anticancer agents, such as topotecan and methotrexate, antibacterial drugs, and statins (Choudhuri and Klaassen 2006). Cyclosporine is an inhibitor of BCRP (Xia et al. 2007). The sequence variant of BCRP, ABCG2 c.421C>A (p.Gln141Lys) SNP, has been associated with reduced BCRP transport activity in vitro (Imai et al. 2002; Kondo et al. 2004; Mizuurai et al. 2004) and increased plasma concentrations of drugs, such as topotecan, sulphasalazine,
Bile salt export pump (BSEP), encoded by \( ABCB11 \), is expressed in the canalicular membrane of hepatocytes, where it is involved in the secretion of bile salts (Childs et al. 1995; Gerloff et al. 1998). A reduced function of BSEP, caused by inherited mutations or acquired factors, may lead to progressive intrahepatic cholestasis and severe liver disease (Lang et al. 2007; Stieger 2010). BSEP-inhibiting factors include drugs, such as cyclosporine and rifampicin, as well as other xenobiotics, aberrant bile salt metabolites, and pregnancy. Pravastatin is a known drug substrate of BSEP.

Multidrug and toxin extrusion proteins (MATE) are antiporters first identified in bacteria (Morita et al. 1998; Brown et al. 1999). MATE1 transports cationic compounds across the canalicular membrane of hepatocytes into the bile and across the apical membrane of renal proximal tubular cells into urine, but it is also expressed in other tissues such as skeletal muscle (Otsuka et al. 2005; Klaassen and Aleksunes 2010). MATE2-K appears to be specific to the kidney, where it mediates the efflux of organic cations across the apical membrane of proximal tubular cells (Masuda et al. 2006). Metformin is a prototypic substrate of MATE1 and MATE2-K (Tanihara et al. 2007), and cimetidine has been shown to inhibit MATE1 (Matsushima et al. 2009). MATE1 has been demonstrated to act in concert with the OCT1 and OCT2 influx transporters in the liver and kidneys, respectively (Terada and Inui 2008; Meyer zu Schwabedissen et al. 2010; König et al. 2011). A non-coding SNP (rs2289669 G>A) in the \( SLC47A1 \) gene encoding MATE1 has been associated with an increased glucose-lowering efficacy of metformin in individuals homozygous for the non-coding SNP (rs622342 A>C) in the \( SLC22A1 \) gene encoding OCT1 (Becker et al. 2009; Becker et al. 2010).

Multidrug resistance-associated proteins (MRP) 2, 3, and 4 are ATP-binding cassette efflux transporters encoded by the \( ABCC2 \), \( ABCC3 \), and \( ABCC4 \) genes, respectively (Klaassen and Aleksunes 2010). MRP2 is expressed in the canalicular membrane of hepatocytes, in the luminal membrane of enterocytes, in the apical membrane of renal proximal tubular cells, and in the maternal side of placental syncytiotrophoblasts (Mayer et al. 1995; Paulusma et al. 1996; Paulusma et al. 1997; Schaub et al. 1999; Fromm et al. 2000; St-Pierre et al. 2000). It transports glutathione, glucuronide, and sulphate conjugates, but also some unconjugated drugs, e.g. many anticancer drugs (Leslie et al. 2005; Choudhuri and Klaassen 2006). Some antiretroviral drugs have been
shown to inhibit MRP2 (Weiss et al. 2007). Several mutations in the ABCC2 gene, resulting in a lack of functional MRP2 transporter, have been identified to cause the Dubin-Johnson syndrome, which is characterized by conjugated hyperbilirubinaemia and dark pigment deposition in the liver (Dubin and Johnson 1954; Mayer et al. 1995; Paulusma et al. 1996; Paulusma et al. 1997; Tsujii et al. 1999). In addition, loss-of-function mutations in the ABCC2 gene have been suggested to be associated with impaired renal elimination of methotrexate and consequently increased risk of toxicity (Hulot et al. 2005; Zolk and Fromm 2011). The synonymous ABCC2 c.1446C>G SNP has been associated with reduced systemic exposure to pravastatin as a consequence of increased MRP2 expression (Kivistö et al. 2005; Niemi et al. 2006a).

MRP3 mediates the basolateral efflux of its substrates in the liver and intestine (Kool et al. 1997; Uchiumi et al. 1998; Scheffer et al. 2000). These substrates include glucuronide conjugates, such as estradiol-17β-glucuronide, unconjugated and conjugated bile acids, and anticancer drugs (Kool et al. 1999; Choudhuri and Klaassen 2006). MRP3 has been shown to be inhibited by some antiretroviral drugs (Weiss et al. 2007).

MRP4 is expressed in many tissues, including the apical membrane of the renal proximal tubular cells, the luminal membrane of the capillary endothelial cells in the brain, and in the basolateral membrane of hepatocytes (Kool et al. 1997; van Aubel et al. 2002; Rius et al. 2003; Nies et al. 2004; Klaassen and Aleksunes 2010). The transporter contributes to resistance against nucleoside analogue antiviral drugs such as adefovir (Schuetz et al. 1999). Among the substrates of MRP4 are also many anticancer drugs as well as endogenous compounds, including bile acids, urate, prostaglandins, leukotrienes, steroid conjugates, folate, glutathione, and cyclic nucleotides (van Aubel et al. 2002; Reid et al. 2003; Rius et al. 2003; Choudhuri and Klaassen 2006; Klaassen and Aleksunes 2010). Many NSAIDs have been shown to inhibit MRP4 (Reid et al. 2003).

**Multidrug resistance protein 3 (MDR3)**, encoded by the ABCB4 gene, is a phospholipid translocator expressed in the canalicular membrane of hepatocytes (Van der Bliek et al. 1987; Schinkel et al. 1991; van Helvoort et al. 1996). Translocation of phospholipids from the inner to the outer leaflet of the hepatocyte cell membrane is required for the actual secretion process driven by bile acids (Klaassen and Aleksunes 2010). Phospholipids protect the biliary tree from injury by forming micelles with bile acids. Variants of ABCB4 gene have been associated with the progressive familial
intrahepatic cholestasis, the severe form of cholestasis in pregnancy, rare cases of juvenile cholesterol gallstones, and drug-induced hepatocellular and cholestatic injury (Deleuze et al. 1996; de Vree et al. 1998; Jacquemin et al. 1999; Lang et al. 2007; Nakken et al. 2009; Klaassen and Aleksunes 2010). MDR3 also participates in the efflux of drugs into bile and can be inhibited by cyclosporine and verapamil (Smith et al. 2000).
Table 1. Selected influx transporters, their tissue distribution, and examples of their drug substrates and inhibitors.

<table>
<thead>
<tr>
<th>Transporter (Gene)</th>
<th>Tissue distribution</th>
<th>Examples of substrates</th>
<th>Examples of inhibitors</th>
<th>Important roles</th>
</tr>
</thead>
<tbody>
<tr>
<td>OATP1A2 (SLCO1A2)</td>
<td>Intestine, brain, liver, kidney</td>
<td>Acebutolol, aliskiren, atenolol, celiprolol, ciprofloxacin, erythromycin, fexofenadine, imatinib, levofloxacin, methotrexate, pitavastatin, rocuronium, saquinavir, talinolol, thyroxine</td>
<td>Apple juice, grapefruit juice, orange juice, rifampicin, verapamil</td>
<td>Intestinal absorption, central nervous system distribution, pharmacokinetic interactions</td>
</tr>
<tr>
<td>OATP1B1 (SLCO1B1)</td>
<td>Liver</td>
<td>Atorvastatin, atrasentan, benzyl-penicillin, bosentan, caspofungin, cerivastatin, darunavir, enalapril, fexofenadine, fluvastatin, lopinavir, methotrexate, olmesartan, pitavastatin, pravastatin, repaglinide, rifampicin, rosuvastatin, saquinavir, simvastatin, SN-38, temocapril, valsartan</td>
<td>Atazanavir, cyclosporine, gemfibrozil, lopinavir, rifampicin, ritonavir, saquinavir</td>
<td>Hepatic uptake, clinically important polymorphisms, pharmacokinetic interactions</td>
</tr>
<tr>
<td>OATP1B3 (SLCO1B3)</td>
<td>Liver</td>
<td>Atrasentan, bosentan, digoxin, docetaxel, enalapril, erythromycin, fexofenadine, fluvastatin, imatinib, methotrexate, olmesartan, paclitaxel, pitavastatin, pravastatin, repaglinide, rifampicin, rosuvastatin, SN-38, telmisartan, thyrone, valsartan</td>
<td>Cyclosporine, rifampicin</td>
<td>Hepatic uptake</td>
</tr>
<tr>
<td>OATP2B1 (SLCO2B1)</td>
<td>Intestine, liver, brain, heart, pancreas, platelets, skeletal muscle, skin, testis, mammary gland, placenta</td>
<td>Aliskiren, atorvastatin, benzyl-penicillin, bosentan, celiprolol, fexofenadine, fluvastatin, glibenclamide, montelukast, pravastatin, rosuvastatin, talinolol, unoprostone</td>
<td>Cyclosporine, gemfibrozil, grapefruit juice</td>
<td>Intestinal absorption, hepatic uptake, tissue distribution</td>
</tr>
<tr>
<td>OATP4C1 (SLCO4C1)</td>
<td>Kidney</td>
<td>Digoxin, methotrexate, sitagliptin</td>
<td>Renal uptake from blood</td>
<td></td>
</tr>
<tr>
<td>Transporter (Gene)</td>
<td>Tissue distribution</td>
<td>Examples of substrates</td>
<td>Examples of inhibitors</td>
<td>Important roles</td>
</tr>
<tr>
<td>--------------------</td>
<td>---------------------</td>
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<td>------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>OAT1 (SLC22A6)</td>
<td>Kidney</td>
<td>Acyclovir, adeovir, cidovir, cimetidine, furosemide, ganciclovir, ibuprofen, indomethacin, ketoprofen</td>
<td>ketoprofen, lamivudine, methotrexate, ranitidine, tenofovir, tetracycline, zalcitabine, zidovudine</td>
<td>Probenecid Renal uptake from blood, pharmacokinetic interactions</td>
</tr>
<tr>
<td>OAT3 (SLC22A8)</td>
<td>Kidney</td>
<td>Benzyl-penicillin, cimetidine, famotidine, furosemide, ibuprofen</td>
<td>indomethacin, ketoprofen, methotrexate, ranitidine, tetracycline, zidovudine</td>
<td>Probenecid Renal uptake from blood, pharmacokinetic interactions</td>
</tr>
<tr>
<td>OCT1 (SLC22A1)</td>
<td>Liver, intestine, kidney</td>
<td>Acyclovir, cimetidine, cisplatin, famotidine, ganciclovir, imatinib, lamivudine, metformin, oxaliplatin, ranitidine</td>
<td>zalcitabine</td>
<td>Cimetidine, indinavir, nelfinavir, pantamidine, quinidine, repaglinide, ritonavir, rosiglitazone, saquinavir, trimethoprim Hepatic uptake, pharmacokinetic interactions, clinically important polymorphisms</td>
</tr>
<tr>
<td>OCT2 (SLC22A2)</td>
<td>Kidney, neurons</td>
<td>Amantadine, cimetidine, cisplatin, famotidine, metformin, oxaliplatin, ranitidine</td>
<td>Bisoprolol, carvedilol, cimetidine, clonidine, flecainide, imipramine, metoprolol, mexiletine, propranolol, quinidine, verapamil</td>
<td>Renal uptake from blood, pharmacokinetic interactions, clinically important polymorphisms</td>
</tr>
<tr>
<td>PEPT1 (SLC15A1)</td>
<td>Intestine</td>
<td>Cephaclor, cephadroxil, valacyclovir</td>
<td></td>
<td>Intestinal absorption</td>
</tr>
<tr>
<td>PEPT2 (SLC15A2)</td>
<td>Kidney</td>
<td>Cephaclor, cephadroxil, valacyclovir</td>
<td></td>
<td>Renal uptake from urine</td>
</tr>
</tbody>
</table>

The list of substrates and inhibitors is based primarily on in vitro data, and the clinical significance of the transporter on the pharmacokinetics of respective drug is not necessarily stated.

SN-38, active metabolite of irinotecan.
Table 2. Selected efflux transporters, their tissue distribution, and examples of drug substrates and inhibitors.

<table>
<thead>
<tr>
<th>Transporter (Gene)</th>
<th>Tissue distribution</th>
<th>Examples of substrates</th>
<th>Examples of inhibitors</th>
<th>Important roles</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-gp/MDR1 (ABCB1)</td>
<td>Intestine, liver, kidney, brain, testis, placenta</td>
<td>Aliskiren, atorvastatin, ciliprolol, cyclosporine, dexamethasone, digoxin, diltiazem, docetaxel, erythromycin, etoposide, fenoxadine, imatinib, indinavir, itraconazole, levofloxacin, loperamide, lovastatin, methylprednisolone, morphine, nelfinavir, paclitaxel, rifampicin, saquinavir, sirolimus, tacrolimus, talinolol, verapamil, vinblastine, vincristine</td>
<td>Atorvastatin, clarithromycin, cyclosporine, erythromycin, itraconazole, ketoconazole, quinidine, ritonavir, verapamil</td>
<td>Limiting intestinal absorption, limiting tissue distribution, excretion into urine, bile and intestine, pharmacokinetic interactions</td>
</tr>
<tr>
<td>BCRP (ABCG2)</td>
<td>Intestine, liver, brain, mammary gland, placenta, haematopoietic stem cells</td>
<td>Atorvastatin, ciprofloxacin, fluvastatin, imatinib, irinotecan, methotrexate, mitoxantrone, norfloxacin, ofloxacin, pravastatin, rosuvastatin, topotecan</td>
<td>Cyclosporine</td>
<td>Limiting absorption and tissue distribution, excretion into bile, clinically important polymorphisms</td>
</tr>
<tr>
<td>BSEP (ABCB11)</td>
<td>Liver</td>
<td>Bile acids, pravastatin</td>
<td>Bosentan, cyclosporine, glibenclamide, rifampicin</td>
<td>Excretion into bile</td>
</tr>
<tr>
<td>MATE1 (SLC47A1)</td>
<td>Kidney, liver, skeletal muscle, heart, adrenal gland, testis</td>
<td>Metformin</td>
<td>Cimetidine</td>
<td>Excretion into bile and urine</td>
</tr>
<tr>
<td>MATE2-K (SLC47A2)</td>
<td>Kidney</td>
<td>Metformin</td>
<td></td>
<td>Excretion into urine</td>
</tr>
<tr>
<td>Transporter (Gene)</td>
<td>Tissue distribution</td>
<td>Examples of substrates</td>
<td>Examples of inhibitors</td>
<td>Important roles</td>
</tr>
<tr>
<td>-------------------</td>
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<td>-----------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>MRP2 (ABCC2)</td>
<td>Liver, intestine, kidney</td>
<td>Glutathione, glucuronide, and sulphate conjugates, ampicillin, cisplatin, doxorubicin, etoposide, irinotecan and SN-38,</td>
<td>Methotrexate, mitoxantrone, pravastatin, valsartan, vinblastine, vincristine</td>
<td>Excretion into bile and urine, limiting absorption, Dubin-Johnson syndrome</td>
</tr>
<tr>
<td>MRP3 (ABCC3)</td>
<td>Liver, intestine</td>
<td>Glucuronide conjugates, bile acids, etoposide,</td>
<td>Methotrexate, teniposide</td>
<td>Delavirdine, efavirenz, emtricitabine</td>
</tr>
<tr>
<td>MRP4 (ABCC4)</td>
<td>Kidney, brain, liver</td>
<td>Adefovir, furosemide, hydrochlorothiazide, irinotecan and SN-38, methotrexate, tenofovir,</td>
<td>Topotecan, zidovudine,</td>
<td>Ibuprofen, indomethacin, ketoprofen</td>
</tr>
<tr>
<td>MDR3 (ABCB4)</td>
<td>Liver</td>
<td>Digoxin, paclitaxel, vinblastine paclitaxel,</td>
<td>Cyclosporine, verapamil</td>
<td>Transport of phospholipids</td>
</tr>
</tbody>
</table>

The list of substrates and inhibitors is based primarily on in vitro data, and the clinical significance of the transporter on the pharmacokinetics of respective drug is not necessarily stated. SN-38, active metabolite of irinotecan.
4. Drug metabolism

Most drugs are relatively hydrophobic and need to be metabolized in order to terminate their therapeutic effect and facilitate their excretion from the body (Brodie et al. 1958). Drug metabolism occurs mainly in the liver by enzymes of the endoplasmic reticulum, but some drug metabolism also goes on in the gastrointestinal wall, kidneys, skin, and lungs (Remmer 1970; Krishna and Klotz 1994). Drugs are metabolized by two major types of reactions (Meyer 1996). Phase I functionalization reactions, involving oxidation, reduction, and hydrolysis, convert drugs into more polar and usually less active compounds by introducing or exposing a functional group in the drug molecule. However, phase I reactions may also result in the formation of metabolites with enhanced activity (prodrugs) or even toxic effects. During phase II conjugation reactions the functional group of a drug or a metabolite is conjugated with some other substance, such as glucuronic acid, sulphate, or glutathione, to produce a more water-soluble compound. Some drugs undergo only phase I or II reactions, while others can simultaneously undergo metabolism by several pathways or even stay unmetabolized (Krishna and Klotz 1994).

4.1 CYP enzymes

The superfamily of cytochrome P450 (CYP) is responsible for the majority of the phase I oxidation reactions (Wrighton and Stevens 1992). Cytochrome P450, a cellular chromophore, was first named after the characteristic peak absorption wavelength (450 nm) of the pigment (P) when reduced and bound to carbon monoxide (Omura and Sato 1962). The human CYP superfamily comprises 57 genes encoding haem-containing mono-oxygenases, which are divided into 18 families sharing greater than 40% amino acid sequence homology (e.g. CYP3) and further into 44 subfamilies sharing greater than 55% amino acid homology (e.g. CYP3A) (Nelson et al. 1996). The catalytic mechanism appears to be common to all CYPs and involves a two-electron reduction of molecular oxygen to form a reactive oxygen species and water (Lin and Lu 1998). In addition to a major role in the metabolism of drugs and other xenobiotics (Wrighton and Stevens 1992), CYPs have important endogenous functions, including biosynthesis of bile acids, metabolism of cholesterol, and biosynthesis and metabolism of steroids and vitamin D3 (Nebert and Russell 2002). The CYP enzymes belonging to the CYP1, CYP2, and CYP3 families are principally responsible for the drug metabolism in humans (Table 3) (Zanger et al. 2008).
Non-genetic factors affecting CYP activity. In newborns, CYP activity is still relatively undeveloped (Tanaka 1998). In neonates, a specific foetal form, CYP3A7, has been found, but its activity disappears soon after birth (Lacroix et al. 1997). The metabolic activity of CYPs matures during the first year (Tanaka 1998), and in infants and young children the elimination half-lives of drugs are generally shorter than in adults due to the relatively large-sized liver and higher clearance of drugs per kilogram of body weight (Rowland and Tozer 2011). The hepatic clearance of many drugs diminishes with age, but this seems to reflect more a decrease in liver volume and hepatic blood flow than a decrease in liver CYP activity (Parkinson et al. 2004).

CYP enzymes can be induced or inhibited by numerous drugs and other xenobiotics (Table 3) (Pelkonen et al. 2008). Drug metabolism may also be affected by disease states and physiological factors. For instance, cytokines released during inflammation can inhibit CYP activity and raise the concentrations of drugs (Aitken et al. 2006) such as CYP1A2 substrate clozapine (Raaska et al. 2002). Liver diseases may reduce both the amount of microsomal protein and the activity of CYP enzymes (Parkinson et al. 2004; Frye et al. 2006). In addition, hormones from the pituitary, sex, adrenal, and thyroid glands as well as from the pancreas are involved in the regulation of CYPs, and changes and differences in hormonal status may have some role in intra-individual and interindividual variability in drug metabolism (Gibson and Skett 2001).

The induction of drug metabolism usually results in reduced plasma concentrations of the parent compound, which may lead to loss of efficacy (Pelkonen et al. 2008). The induction occurs to a major extent by a mechanism where ligand activation of transcription factors leads to increased gene transcription and further increased protein synthesis (Handschin and Meyer 2003). These intracellular receptors include aryl hydrocarbon receptor (AhR), PXR, and CAR (Waxman 1999). In addition, oestrogen receptor α and glucocorticoid receptor (GR) may be involved in some induction phenomena (Gerbal-Chaloin et al. 2002; Hukkanen et al. 2003; Higashi et al. 2007).

The inhibition of drug metabolism can result in increased exposure to the parent compound, potentially leading to enhanced drug efficacy and even to severe adverse drug reactions (Pelkonen et al. 2008). The inhibition of CYP enzymes can occur in either reversible or irreversible (mechanism-based) way (Hollenberg 2002). Reversible inhibitors bind non-covalently to the enzyme and are able to dissociate from it. The inhibition disappears soon after the removal of inhibitor, and the enzyme is not permanently destroyed. Reversible inhibition can be further divided into competitive,
non-competitive, and uncompetitive inhibition, depending on whether the inhibitor binds to the same position of the enzyme as the substrate, to a different position of the enzyme as the substrate, or to the enzyme-substrate complex, respectively. Mixed-type inhibition displays elements of both competitive and non-competitive inhibition. Irreversible inhibitors need to undergo metabolic activation to form intermediates that further inactivate the enzyme, usually by forming covalent bonds. Resynthesis of the enzyme is required before the activity is restored.

Pharmacogenetics of CYP enzymes. The genetic variability of CYP enzymes is a significant cause of interindividual differences in drug response. The clinically most important polymorphisms are those in genes encoding CYP2C9, CYP2C19, and CYP2D6 (Zanger et al. 2008). CYP2C9 is involved in the metabolism of approximately 10-20% of drugs on the market, with several NSAIDs and S-warfarin being examples of CYP2C9 substrates (Kirchheiner and Brockmöller 2005; Zanger et al. 2008). The two most investigated variant alleles of the CYP2C9 gene, CYP2C9*2 (g.3608C>T, p.Arg144Cys) and CYP2C9*3 (g.42614A>C, p.Ile359Leu), are common in Caucasians, with frequencies of approximately 13-17% and 6-7%, respectively, but are rarer in African and Asian populations, with frequencies of 0-4%. Both of these variants yield enzymes with reduced activity and are of clinical relevance. The carriers of CYP2C9*2 and *3 alleles have been reported to experience higher incidences of adverse drug reactions, such as gastrointestinal bleeding from NSAIDs (Martínez et al. 2004), hypoglycaemia from sulphonylurea antidiabetic drugs (Holstein et al. 2005), and serious bleeding from warfarin treatment (Aithal et al. 1999), in which anticoagulant response also depends on variants in genes encoding vitamin K epoxide reductase complex subunit 1 and CYP4F2 (Takeuchi et al. 2009).

CYP2C19 contributes to the metabolism of about 10% of commonly used drugs such as many proton-pump inhibitors and antidepressants (Zanger et al. 2008). The genetic variation in the CYP2C19 gene leads to distinct phenotypes, termed poor (PM) and extensive (EM) metabolizers. The PM phenotype results from two null alleles, leading to the absence of a functional CYP2C19 protein, whereas EMs carry at least one functional allele. About 3–5% of Caucasians and Africans, and up to 20% of Asians exhibit the PM phenotype. Moreover, the CYP2C19*17 allele (g.-806C>T SNP in the promoter region) has been found to cause increased CYP2C19 activity due to an increase in CYP2C19 transcription (Sim et al. 2006). The frequency of this allele is about 15-25% in Caucasians and Africans, but only about 4% in Asians (Sim et al. 2006; Zanger et al. 2008). In vivo, the CYP2C19 polymorphism has been shown to
affect, for example, the responsiveness to Helicobacter pylori eradication therapy that includes proton-pump inhibitors (Furuta et al. 2001).

CYP2D6 participates in the metabolism of about 15-25% of clinically used drugs, among which are beta blockers, antipsychotics, antidepressants, and opioids (Dahl and Bertilsson 1993; Ingelman-Sundberg 2005; Zanger et al. 2008). CYP2D6 is the only non-inducible enzyme among the drug-metabolizing CYPs, and therefore, genetic variation is of major importance for the interindividual variability in its activity. The genetic variants of the CYP2D6 gene have been divided into alleles causing four phenotypes: PM (no activity, results from two null alleles), intermediate metabolizer (IM; reduced activity, results from one partially defective allele in combination with a null allele or a partially defective allele), EM (normal activity), and ultrarapid metabolizer (UM; high activity, results from enzyme overexpression due to duplication or multiplication of the active gene) (Dahl et al. 1992; Johansson et al. 1993; Dahl et al. 1995; Ingelman-Sundberg 2005; Zanger et al. 2008). The frequencies of PM, IM, EM, and UM phenotypes in Caucasians are approximately 5-10%, 10-15%, 70-85%, and 1-5%, respectively (Zanger et al. 2008). In Asians and Africans, the UM phenotype is common, with a frequency of about 30%, whereas the PM phenotype exhibits a frequency of only 0-1%. The polymorphism of CYP2D6 has clinically significant consequences (Ingelman-Sundberg 2005). PMs may experience adverse drug reactions due to increased exposure to CYP2D6 substrates, whereas UMs may not achieve sufficient clinical response. However, in the case of prodrugs, such as codeine, which is metabolized into morphine, the UM phenotype may be associated with adverse effects as a result of high concentrations of the active metabolite (Gasche et al. 2004). The CYP2D6 polymorphism has also been shown to have a major impact on, for instance, the clinical outcomes of the adjuvant treatment of breast cancer with the prodrug tamoxifen (Schroth et al. 2009).

4.1.1 CYP3A4

CYP3A4 is the most abundant CYP enzyme in the liver and small intestine (Bork et al. 1989; Guengerich 1989; Kolars et al. 1992), and it has been estimated to be involved in the metabolism of approximately 50% of the drugs in clinical use (Wrighton et al. 2000). CYP3A4 was one of the first P450 enzymes purified from the human liver in the 1980s (Wang et al. 1983; Watkins et al. 1985; Guengerich et al. 1986). Subsequently, the crystal structures of bound and unbound CYP3A4 have been constructed, and they
have revealed that the active site of CYP3A4 is large and flexible, explaining the broad substrate specificity (Williams et al. 2004; Yano et al. 2004; Ekroos and Sjögren 2006). The substrates of CYP3A4 vary widely in size and structure and include endogenous compounds, such as steroid hormones, as well as several clinically important drugs, e.g. midazolam (a probe substrate) and triazolam, cyclosporine, calcium-channel blockers, statins, and antiretroviral drugs (Dresser et al. 2000; Wrighton et al. 2000).

CYP3A4 is regulated by PXR, CAR, and GR and is induced by many compounds such as rifampicin, phenobarbital, phenytoin, carbamazepine, dexamethasone, and St. John's wort (Combalbert et al. 1989; Kolars et al. 1992; Backman et al. 1996b; Lehmann et al. 1998; Goodwin et al. 1999; Sueyoshi et al. 1999; Moore et al. 2000). CYP3A4 is also prone to both reversible and mechanism-based inhibition (Dresser et al. 2000; Wrighton et al. 2000). The most well-established inhibitors of CYP3A4 include azole antifungal agents (itraconazole, ketoconazole, voriconazole) (Back and Tjia 1991; Jurima-Romet et al. 1994; Olkkola et al. 1994; Saari et al. 2006), macrolide antibacterials (clarithromycin, erythromycin, telithromycin) (Olkkola et al. 1993; Jurima-Romet et al. 1994; Gorski et al. 1998; Kajosaari et al. 2006), antiretroviral drugs (indinavir, nelfinavir, ritonavir, saquinavir) (Ernest et al. 2005), calcium-channel blockers (diltiazem and verapamil) (Backman et al. 1994), and grapefruit juice (Lown et al. 1997; Schmiedlin-Ren et al. 1997). Several CYP3A4 variant alleles have been found, but they appear to have limited clinical significance (Lamba et al. 2002; Zanger et al. 2008).

In addition to CYP3A4, the CYP3A subfamily comprises the CYP3A5 and CYP3A7 (foetal form) enzymes (Wrighton et al. 2000) as well as CYP3A43, which appears to be of minor clinical relevance (Westlind et al. 2001). CYP3A5 has overlapping substrate specificity with CYP3A4 (Daly 2006). Its expression is highly variable due to CYP3A5 polymorphism, and only individuals with the CYP3A5*1 allele express significant amounts of the CYP3A5 enzyme (Hustert et al. 2001; Kuehl et al. 2001). The frequency of the CYP3A5*1 allele is about 5-20% in Caucasians, 25-30% in Asians, and 40-55% in Africans (Lamba et al. 2002; Daly 2006). In addition to the liver and intestine, CYP3A5 is expressed in such tissues as the kidney and lung (Haehner et al. 1996; Kivistö et al. 1996a; Kivistö et al. 1996b; Daly 2006).

<table>
<thead>
<tr>
<th>SUBSTRATES</th>
<th>CYP1A2</th>
<th>CYP2C8</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>CYP2D6</th>
<th>CYP3A4</th>
</tr>
</thead>
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<tr>
<td>Caffeine,</td>
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<td>Diclofenac,</td>
<td>Citalopram,</td>
<td>Amitriptyline,</td>
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<td>glibenclamide,</td>
<td>clomipramine,</td>
<td>codeine,</td>
<td>budesonide,</td>
<td>methyl-</td>
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<tr>
<td>duloxetine,</td>
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<td>diazepam,</td>
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<td>buspirone,</td>
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<td>midazolam,</td>
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<td>ibuprofen,</td>
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<td>S-warfarin</td>
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<td></td>
<td>indinavir,</td>
<td>simvastatin,</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>lercanidipine,</td>
<td>triazolam</td>
</tr>
</tbody>
</table>

| INHIBITORS | Ciprofloxacin, | Gemfibrozil, | Amiodarone, | Fluconazole, | Buprobion, | Amiodarone, | ketoconazole, |
|           | fluvoxamine, | trimethoprim | fluconazole, | omeprazole | fluoxetine, | clarithromycin, | nelfinavir, |
|           | oral contraceptives | | metronidazole, | | paroxetine, | diltiazem, | ritonavir, |
|           |            | | miconazole, | | quinidine, | erythromycin, | saquinavir, |
|           |            | | voriconazole | | terbinafine | grapefruit juice, | telithromycin, |
|           |            | | | | | indinavir, | verapamil, |
|           |            | | | | | itraconazole, | voriconazole |

| INDUCERS | Carbamazepine, | Rifampicin | Carbamazepine, | Phenobarbital, | Non-inducible | Carbamazepine, | St. John's wort |
|          | charcoal-grilled | | phenobarbital, | phenytoin, | | dexamethasone, | |
|          | food, | | phenytoin, | rifampicin, | rifampicin, | phenobarbital, | |
|          | cigarette smoke, | | St. John's wort | St. John's wort | | phenytoin, | |
|          | rifampicin | | | | | rifampicin, | |

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5. Interacting drugs investigated

5.1 Rifampicin

Rifampicin (C_{43}H_{58}N_{4}O_{12}, 822.95 g/mol) is a broad-spectrum antibacterial drug. It was discovered in the 1960s as the first orally active derivative of rifamycin B, a product of the bacterium Amycolatopsis mediterranei, and was introduced into clinical use in 1968 (Sensi 1983). Rifampicin is a bactericidal drug that acts by inhibiting bacterial DNA-dependent RNA polymerase activity, thus suppressing the initiation of RNA synthesis (Campbell et al. 2001; Kohanski et al. 2010). Rifampicin is used to treat tuberculosis (Gyselen et al. 1968; Koul et al. 2011). It is also an alternative treatment for staphylococcal infections (Turnidge and Grayson 1993). The usual dosage of rifampicin in adults is 450-600 mg once daily, and it is principally used in combination with other antibacterial drugs to prevent the development of rifampicin resistance (Douglas and McLeod 1999). Rifampicin is generally well tolerated, and the most common adverse effects are rash, fever, and gastrointestinal disturbances. In addition, rifampicin may produce a benign reddish coloration of body fluids such as urine and tears. In rare cases, rifampicin has been shown to cause hepatotoxicity, particularly in patients with chronic liver disease, alcoholism, or poor nutritional status, or in patients taking other hepatotoxic agents (Grosset and Leventis 1983). Intermittent rifampicin therapy has been associated with an immune-mediated reaction showing flu-like symptoms, potentially leading to acute renal failure.

Rifampicin is well absorbed from the gastrointestinal tract (Acocella 1978), and the C_{max} is achieved in 2-4 hours (Douglas and McLeod 1999). Food delays the absorption and has been found to reduce the C_{max} by 36% (Peloquin et al. 1999). The oral bioavailability of rifampicin is approximately 68% during continuous treatment (Loos et al. 1987). About 80% of the drug is bound to plasma protein (Acocella 1978), and the V_d at steady state is about 0.8 l/kg (Holdiness 1984). Rifampicin is metabolized by deacetylation to an antibacterially active metabolite (Acocella 1978). Both unchanged and metabolized rifampicin is excreted into bile and urine, and the unchanged form undergoes enterohepatic circulation. The t_{1/2} varies between 2 and 5 hours (Burman et al. 2001); this shortens during continuous treatment because of autoinduction (Loos et al. 1987).

Rifampicin is a potent inducer of several drug-metabolizing enzymes and some drug transporters (Niemi et al. 2003). The mechanism of induction involves binding of
rifampicin to the nuclear receptor PXR, which leads to increased gene transcription and subsequently to increased protein synthesis (Lehmann et al. 1998). The PXR-target genes induced by rifampicin include CYP3A4 (Combalbert et al. 1989; Morel et al. 1990; Kolars et al. 1992; Schuetz et al. 1996; Lehmann et al. 1998; Goodwin et al. 1999; Glaeser et al. 2005), ABCB1 encoding P-gp (Schuetz et al. 1996; Geick et al. 2001), CYP2A6 (Meunier et al. 2000; Rae et al. 2001; Itoh et al. 2006), CYP2B6 (Chang et al. 1997; Goodwin et al. 2001; Rae et al. 2001), CYP2C8, CYP2C9, and CYP2C19 (Morel et al. 1990; Chang et al. 1997; Gerbal-Chaloin et al. 2001; Raucy et al. 2002; Chen et al. 2003; Madan et al. 2003; Chen et al. 2004; Ferguson et al. 2005; Glaeser et al. 2005), CYP3A5 (Burk et al. 2004), ABCC2 encoding MRP2 (Fromm et al. 2000; Kast et al. 2002), and some genes encoding phase II drug-metabolizing enzymes such as members of the UDP-glucuronosyltransferase and sulphotransferase families (Rae et al. 2001; Sonoda et al. 2002). Because the induction requires de novo protein synthesis, full induction is reached in about one week after beginning rifampicin treatment, and baseline activity after discontinuing rifampicin is attained in roughly two weeks (Fromm et al. 1996; Niemi et al. 2003).

Rifampicin is involved in many clinically important pharmacokinetic interactions, having its greatest effects on the pharmacokinetics of orally administered drugs that are substrates of CYP3A4 (Barbarash et al. 1988; Fromm et al. 1996; Niemi et al. 2003). For instance, rifampicin reduces the AUC of oral verapamil by 93% (Barbarash et al. 1988), cyclosporine by 70% (Hebert et al. 1992), midazolam by 96% (Backman et al. 1996a), saquinavir by 80% (Vella and Floridia 1998), and simvastatin (acid) by 93% (Kyrklund et al. 2000). Rifampicin also reduces the plasma concentrations of CYP2C substrates, such as the CYP2C9 substrate S-warfarin (74% reduction in AUC) (Heimark et al. 1987), as well as those of repaglinide, rosiglitazone, and pioglitazone, all of which are metabolized mainly by CYP2C8 (over 50% reductions in AUC) (Niemi et al. 2000; Niemi et al. 2004; Jaakkola et al. 2006). As an example of the effect of rifampicin on P-gp in vivo, rifampicin reduces the AUC of oral digoxin by 30% by inducing P-gp in the small intestine, but has no effect on renal clearance (Greiner et al. 1999). Interestingly, PXR mRNA is found in marked quantities in the liver and small intestine, but to a much smaller extent in the kidneys (Kliewer et al. 1998), possibly explaining the lack of effect on the renal clearance of P-gp substrates. Regulatory authorities recommend rifampicin (600 mg orally once daily over several days) as an inducer for drug interaction studies involving substrates of P-gp, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4/5 (FDA 2012).
5.2 Itraconazole

Itraconazole (C₃₅H₃₈Cl₂N₈O₄, 705.64 g/mol) is a broad-spectrum triazole antifungal agent synthesized at the beginning of the 1980s (Van Cutsem et al. 1984). It inhibits the growth of fungi by inhibiting CYP enzyme lanosterol 14α-demethylase, which impairs fungal cell membrane ergosterol biosynthesis (Grant and Clissold 1989). The usual dosage for the treatment of fungal infections in adults is 100-400 mg daily. The most common adverse effects are gastrointestinal disturbances, rash, and a mild increase in liver enzymes (Grant and Clissold 1989).

Itraconazole is available both as an oral capsule, better absorbed on a full stomach, and as a solution, better absorbed in the fasting state (Van Peer et al. 1989). The oral bioavailability is about 55% (Heykants et al. 1989), and the Cmax is achieved in 2-5 hours (Van Peer et al. 1989). Itraconazole is highly (99.8%) bound to plasma protein (Heykants et al. 1989). The drug is widely distributed to the tissues, especially those rich in keratin, such as skin and nails, with an apparent Vd of 10.7 l/kg. Itraconazole is both a substrate and an inhibitor of CYP3A4 (Tucker et al. 1992; Olkkola et al. 1994; Ishigam et al. 2001; Isoherranen et al. 2004), and it undergoes extensive biotransformation in the liver into metabolites excreted into urine and bile (Heykants et al. 1989). The major metabolite hydroxyitraconazole is antifungally active. Itraconazole exhibits non-linear pharmacokinetics, probably as a result of saturation of the first-pass metabolism (Hardin et al. 1988; Van Peer et al. 1989). The half-life of itraconazole is 15-25 hours after single dosing; this is prolonged to 34-42 hours with repeated dosing (Hardin et al. 1988).

Itraconazole was initially believed not to interfere with mammalian drug-metabolizing enzymes (Heykants et al. 1989). Subsequently, itraconazole has been shown to be a potent competitive inhibitor of CYP3A4, with an inhibition constant (Ki) of 0.016-11 μmol/l (Kwan et al. 1987; Back and Tjia 1991; Jurima-Romet et al. 1994; Olkkola et al. 1994; von Moltke et al. 1996a; von Moltke et al. 1996b; Venkatakrishnan et al. 2000; Ishigam et al. 2001; Isoherranen et al. 2004). Moreover, itraconazole metabolites, hydroxyitraconazole (Ki 0.038 μmol/l), keto-itraconazole (IC₅₀ for unbound form 7.0 nmol/l), and N-desalkyl-itraconazole (IC₅₀ for unbound form 0.4 nmol/l), have been found to inhibit CYP3A4 (Isoherranen et al. 2004), and they thus contribute to the effect of itraconazole on CYP3A4 in vivo (Templeton et al. 2008). The inhibitory effect of itraconazole on CYP3A4 begins rapidly and is dependent on its plasma concentrations, as evidenced by interaction studies involving itraconazole with triazolam or midazolam.
A single dose of itraconazole, taken concomitantly with or 3, 12, or 24 hours before triazolam, increased the AUC of triazolam 3.1-, 4.8-, 4.6-, and 3.8-fold, respectively (Neuvonen et al. 1996). When a single dose of itraconazole was ingested 2 hours before midazolam, the increase in the AUC of midazolam was 3.4-fold compared with placebo, but after a 6-day repeated administration of itraconazole, the increase was 6.6-fold (Olkkola et al. 1996). Itraconazole also inhibits the P-gp transporter, with an IC₅₀ of 2 μmol/l (Wang et al. 2002), and regulatory authorities recommend itraconazole as an inhibitor in drug interaction studies involving substrates of CYP3A4 or P-gp (FDA 2012).

Itraconazole markedly raises the AUC and enhances the effects of several CYP3A4 and/or P-gp substrates (Table 4). In 1993, itraconazole was found to greatly increase the plasma concentrations of parent drug terfenadine, a non-sedating antihistamine, resulting in prolongation of the QT-interval in ECG and leading to torsades de pointes ventricular tachycardia (Honig et al. 1993; Pohjola-Sintonen et al. 1993). This hazardous drug interaction with CYP3A4 inhibitors led to removal of terfenadine from the market. Itraconazole can also increase the exposure to other QT-interval-prolonging agents such as cisapride and quinidine (Kaukonen et al. 1997; Wysowski et al. 2001).

Table 4. Examples of pharmacokinetic interactions of itraconazole.

<table>
<thead>
<tr>
<th>Interacting drug</th>
<th>Effect of itraconazole</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triazolam</td>
<td>27.1-fold increase in AUC</td>
<td>Varhe et al. 1994</td>
</tr>
<tr>
<td>Lovastatin (acid)</td>
<td>22.1-fold increase in AUC</td>
<td>Neuvonen and Jalava 1996</td>
</tr>
<tr>
<td>Simvastatin (acid)</td>
<td>18.6-fold increase in AUC</td>
<td>Neuvonen et al. 1998</td>
</tr>
<tr>
<td>Midazolam</td>
<td>10.8-fold increase in AUC</td>
<td>Olkkola et al. 1994</td>
</tr>
<tr>
<td>Felodipine</td>
<td>6.3-fold increase in AUC</td>
<td>Jalava et al. 1997a</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>3.9-fold increase in AUC</td>
<td>Varis et al. 1998</td>
</tr>
<tr>
<td>Atorvastatin (acid)</td>
<td>3.3-fold increase in AUC</td>
<td>Kantola et al. 1998a</td>
</tr>
<tr>
<td>Fexofenadine</td>
<td>2.7-fold increase in AUC</td>
<td>Shimizu et al. 2006</td>
</tr>
<tr>
<td>Celiprolol</td>
<td>1.8-fold increase in AUC</td>
<td>Lilja et al. 2003</td>
</tr>
<tr>
<td>Digoxin</td>
<td>1.7-fold increase in AUC</td>
<td>Partanen et al. 1996</td>
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<td>Jalava et al. 1997b</td>
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</table>
6. Fruit juices investigated

6.1 Grapefruit juice

Effect on CYP3A4. The ability of grapefruit juice to produce clinically important pharmacokinetic interactions was discovered by chance in 1991, when grapefruit juice was used to mask the taste of ethanol in a study involving the calcium-channel blocker felodipine (Bailey et al. 1989; Bailey et al. 1991). Unexpectedly, grapefruit juice raised the AUC of felodipine 2.8-fold (Bailey et al. 1991). The interaction was found to result from a mechanism-based inhibition of CYP3A4, principally in the small intestine (Lown et al. 1997; Schmiedlin-Ren et al. 1997). Furanocoumarins bergamottin and its derivative, 6′,7′-dihydroxybergamottin, were identified as the primary components responsible for inhibition, with IC50 values (for midazolam 1′-hydroxylation) of 10-30 μmol/l and 0.3-1 μmol/l, respectively (Edwards et al. 1996; Fukuda et al. 1997; Schmiedlin-Ren et al. 1997; He et al. 1998; Guo et al. 2000; Greenblatt et al. 2003; Kakar et al. 2004). The concentrations of bergamottin and 6′,7′-dihydroxybergamottin vary considerably among different grapefruit juice brands and have been reported to be 4-30 μmol/l and 1-44 μmol/l, respectively (Edwards et al. 1996; Schmiedlin-Ren et al. 1997; He et al. 1998; Bailey et al. 2000; Guo et al. 2000; Kakar et al. 2004).

The inhibitory effect of grapefruit juice on intestinal CYP3A4 begins rapidly. In vitro, the maximal inhibition of CYP3A4 by 6′,7′-dihydroxybergamottin has been found to occur within 30 minutes, whereas bergamottin has had a slower onset of action (maximum within 3 hours) (Paine et al. 2005). In vivo, the concentration of CYP3A4 protein in the duodenum has been found to be reduced by an average of 30% even 1-2 hours after ingestion of a single glass of grapefruit juice, compared with ingestion of water (Glaeser et al. 2007). After a 6-day ingestion of grapefruit juice three times a day, the reduction in the concentration of CYP3A4 in the small intestine has been 62% (Lown et al. 1997). The interaction between grapefruit juice and felodipine has been shown to reach its maximum already after the first glass of grapefruit juice taken concomitantly with the drug (Lundahl et al. 1998). By contrast, in a study with triazolam, multiple doses of double-strength grapefruit juice (three times a day for two days) had a greater effect on the AUC of triazolam (2.4-fold increase) than single doses of normal-strength or double-strength grapefruit juice (1.5-fold increases) (Lilja et al. 2000a). Multiple doses also prolonged the t½ of triazolam, suggesting inhibition of both intestinal and hepatic CYP3A4. Repeated consumption of grapefruit juice has also prolonged the t½ of other CYP3A4 substrates, including atorvastatin, buspirone,
cisapride, midazolam, and oxycodone, and in one study, a decrease in the amount of exhaled $^{14}$CO$_2$ in the erythromycin breath test was also seen (Lilja et al. 1998b; Kivistö et al. 1999; Lilja et al. 1999; Veronese et al. 2003; Nieminen et al. 2010). However, grapefruit juice has not inhibited hepatic CYP3A4, reflected as an effect on the pharmacokinetics of intravenously administered CYP3A4 substrates midazolam, cyclosporine, felodipine, or saquinavir (Ducharme et al. 1995; Kupferschmidt et al. 1995; Lundahl et al. 1997; Kupferschmidt et al. 1998).

In studies concerning the duration of the effect, the inhibitory effect of grapefruit juice on CYP3A4 has lasted at least 24 hours, and in some cases, some effect was seen up to three days after grapefruit juice ingestion (Lundahl et al. 1995; Lilja et al. 2000b; Takanaga et al. 2000; Greenblatt et al. 2003; Culm-Merdek et al. 2006). For instance, grapefruit juice taken concomitantly with, 24 hours before, or 3 days before the drug increased the AUC of simvastatin 3.5-, 2.1-, and 1.4-fold, respectively, compared with water, whereas no effect was observed when grapefruit juice was taken 7 days before the drug (Lilja et al. 2000b).

Nowadays, grapefruit juice is known to enhance the oral bioavailabilities of several CYP3A4 substrate drugs (1.3 to 16-fold increases in AUC), including hypnotics (midazolam, triazolam) (Kupferschmidt et al. 1995; Hukkinen et al. 1995), calcium-channel blockers (felodipine, nicardipine, nifedipine, nimodipine, nisoldipine, nitrendipine, verapamil) (Bailey et al. 1991; Soons et al. 1991; Bailey et al. 1993; Fuhr et al. 1998; Ho et al. 2000b; Uno et al. 2000; Bailey and Dresser 2004), statins (atorvastatin, lovastatin, simvastatin) (Kantola et al. 1998b; Lilja et al. 1998a; Lilja et al. 1999), amiodarone (Libersa et al. 2000), buspirone (Lilja et al. 1998b), carbamazepine (Garg et al. 1998), cisapride (Kivistö et al. 1999), cyclosporine (Yee et al. 1995), diazepam (Ozdemir et al. 1998), erythromycin (Kanazawa et al. 2001), methylprednisolone (Varis et al. 2000), nilotinib (Yin et al. 2010), oxycodone (Nieminen et al. 2010), saquinavir (Kupferschmidt et al. 1998), tacrolimus (2-fold increase in trough concentrations in liver transplant patients) (Liu et al. 2009), and tolvaptan (Shoaf et al. 2012).

**Effect on P-glycoprotein.** The effect of grapefruit juice on P-gp activity has been widely studied, but the results have been somewhat conflicting. Based on some early in vitro studies, constituents of grapefruit juice seemed to have no significant effect on P-gp activity (Eagling et al. 1999; Edwards et al. 1999). However, these studies have been criticized for technical issues and for using inadequate constituent concentrations.
Grapefruit juice has subsequently been shown to inhibit P-gp in many in vitro studies using colchicine, talinolol, saquinavir, vinblastine, and vincristine as probes (Takanaga et al. 1998; Ikegawa et al. 2000; Honda et al. 2004; de Castro et al. 2007; Dahan and Amidon 2009). 6’,7’-dihydroxybergamottin and naringin have inhibited P-gp, with IC$_{50}$ values of 33 μmol/l and 3000 μmol/l, respectively (Dresser et al. 2002a). Bergamottin, by contrast, has not altered P-gp activity at concentrations of up to 50 μmol/l. However, 6’,7’-dihydroxybergamottin and naringin occur in grapefruit juice at relatively low concentrations in relation to IC$_{50}$ values, 1-44 μmol/l and 130-1230 μmol/l, respectively (Ameer et al. 1996; Edwards et al. 1996; Schmiedlin-Ren et al. 1997; Bailey et al. 2000; Guo et al. 2000; Tomás-Barberán and Clifford 2000; Kakar et al. 2004; Vanamala et al. 2006; Bailey et al. 2007), suggesting that they are not likely to produce relevant inhibition of P-gp activity in vivo.

In vivo, grapefruit juice has had no significant effect on the total exposure of the P-gp probe digoxin (Becquemont et al. 2001; Parker et al. 2003). Moreover, no effect on the P-gp-protein expression level in the small intestine has been seen (Lown et al. 1997; Glaeser et al. 2007). In case of a substrate of both P-gp and OATP transporter, the OATP-inhibiting effect of grapefruit juice appears to predominate, as evidenced by interaction studies with fexofenadine, celiprolol, and talinolol (Karlsson et al. 1993; Wetterich et al. 1996; Spahn-Langguth et al. 1998; Cvetkovic et al. 1999; Dresser et al. 2002a; Lilja et al. 2003; Nozawa et al. 2004; Schwarz et al. 2005; Glaeser et al. 2007; Kato et al. 2009; Shirasaka et al. 2010).

Effect on OATPs. A new type of pharmacokinetic food-drug interaction emerged when grapefruit, orange, and apple juices were discovered to reduce the systemic exposure to fexofenadine by about 60-70% (Dresser et al. 2002a). The metabolism of fexofenadine is negligible, and it is eliminated unchanged into bile and urine (Russell et al. 1998). Fexofenadine is a substrate of P-gp (Cvetkovic et al. 1999), OATP1A2 (Cvetkovic et al. 1999; Dresser et al. 2002a; Glaeser et al. 2007), OATP2B1 (Nozawa et al. 2004), OATP1B1 (Niemi et al. 2005), and OATP1B3 (Shimizu et al. 2005). Interestingly, grapefruit and orange juices were found to inhibit OATP1A2-mediated fexofenadine uptake in vitro in human cervical carcinoma (HeLa) cells (Dresser et al. 2002a). Thus, the suggested mechanism of these interactions was the inhibition of OATP1A2-mediated uptake of fexofenadine in the small intestine by these juices.

Subsequently, grapefruit juice has been found to reduce the oral bioavailabilities of other OATP substrate drugs. Celiprolol and talinolol are substrates of both OATP1A2
and OATP2B1 (Kato et al. 2009; Shirasaka et al. 2010; Ieiri et al. 2012), and grapefruit juice has reduced their AUC values by 87% and 36%, respectively (Lilja et al. 2003; Schwarz et al. 2005). In addition, grapefruit juice has had a minor, probably not clinically relevant, effect on the oral bioavailabilities of the OATP1A2 substrates acebutolol (7% reduction in AUC) and levothyroxine (9% reduction in AUC) (Fujiwara et al. 2001; Lilja et al. 2005a; Lilja et al. 2005b; Kato et al. 2009).

Several constituents of grapefruit juice have been screened for inhibitory effects on intestinal OATP transporters. The foremost flavonoid in grapefruit juice, naringin, is a potent inhibitor of OATP1A2, with an IC\textsubscript{50} value of 3.6 µmol/l (Bailey et al. 2007), which also is significant relative to its corresponding concentration in grapefruit juice (130-1230 µmol/l) (Ameer et al. 1996; Bailey et al. 2000; Tomás-Barberán and Clifford 2000; Vanamala et al. 2006; Bailey et al. 2007). A drug is generally considered to be an inhibitor of OATP transporter in vivo if it shows an IC\textsubscript{50} value of less than 10 times its unbound peak concentration (Giacomini et al. 2010). Thus, naringin is believed to be mainly responsible for the inhibition of OATP1A2 in vivo (Bailey et al. 2007). However, pure naringin solution has decreased the bioavailability of fexofenadine by only half the amount seen with whole grapefruit juice containing the same naringin concentration, suggesting that grapefruit juice also contains other OATP1A2 inhibitors. Of other constituents, quercetin, reported to occur in grapefruit juice at concentrations of 17-26 µmol/l (Vanamala et al. 2006), has been noted to inhibit OATP1A2, with an IC\textsubscript{50} of 12.6 µmol/l (Mandery et al. 2010).

Naringin, at a concentration of 10 µmol/l, has inhibited the OATP2B1-mediated uptake of estrone-3-sulphate by 39% in vitro in HEK293 cells, and it probably contributes to the inhibition of OATP2B1 by grapefruit juice in vivo (Satoh et al. 2005). Furthermore, naringenin (an aglycone form of naringin), quercetin, bergamottin, and 6’,7’-dihydroxybergamottin, all at concentrations of 10 µmol/l, have been found to inhibit the OATP2B1-mediated transport by 28%, 21%, 60%, and 43%, respectively (Satoh et al. 2005). Moreover, naringenin has inhibited the OATP1B1- and OATP1B3-mediated bromosulphophthalein transport in HEK293 cells, with IC\textsubscript{50} values of 81.6 µmol/l and 101.1 µmol/l, respectively (Mandery et al. 2012).

The duration of the effect of grapefruit juice on OATP transporters appears to be much shorter than on CYP3A4, indicating a different mechanism of action. Grapefruit juice taken concomitantly with or 2 hours before fexofenadine has decreased the AUC of fexofenadine by 52% and 38%, respectively, compared with water, but it has had no
effect when taken 4 hours before the drug (Glaeser et al. 2007). Furthermore, no change in the duodenal OATP1A2-protein expression level has been seen 1-2 hours after grapefruit juice ingestion. Moreover, repeated consumption of grapefruit juice seems to have a similar effect on OATP1A2 as a single dose. Ingestion of a single 300-ml dose of grapefruit juice concomitantly with talinolol decreased the AUC of talinolol to a similar extent as the ingestion of 300 ml of grapefruit juice three times daily for 6 days, prior to talinolol administration (Schwarz et al. 2005). The effect of grapefruit juice volume on the reduction of fexofenadine bioavailability has also been studied. Ingestion of 300 or 1200 ml of grapefruit juice concomitantly with fexofenadine led to 42% and 64% decreases in the AUC values of fexofenadine, respectively, compared with water (Dresser et al. 2005).

6.2 Orange juice

Orange juice appears to be a potent inhibitor of intestinal OATP1A2 in vivo, as observed in an interaction study, in which it had a similar effect on fexofenadine exposure as grapefruit juice (60-70% reduction in AUC) (Dresser et al. 2002a). Orange juice has also reduced the AUC values of OATP1A2 and OATP2B1 substrate celiprolol as well as those of OATP1A2 substrates atenolol and ciprofloxacin by 83%, 40%, and 22%, respectively (Neuhofel et al. 2002; Lilja et al. 2004; Lilja et al. 2005c; Maeda et al. 2007; Kato et al. 2009; Ieiri et al. 2012). However, in contrast to grapefruit juice, orange juice has had no effect on CYP3A4 (Bailey et al. 1991; Yee et al. 1995; Edwards et al. 1996; Kakar et al. 2004).

The major flavonoid in orange juice, hesperidin, is most probably mainly responsible for the inhibition of OATP1A2 (Bailey et al. 2007). Hesperidin has been found to inhibit OATP1A2 in vitro, with an IC50 of 2.7 μmol/l (Bailey et al. 2007), which is much lower than the concentration normally occurring in orange juices (106-1965 μmol/l) (Ameer et al. 1996; Tomás-Barberán and Clifford 2000; Vanamala et al. 2006; Bailey et al. 2007). No published studies exist on the possible effects of hesperidin on OATP2B1. However, tangeritin and nobiletin, occurring in orange juice at concentrations of 0.35-1.6 μmol/l and 2.5-11 μmol/l, respectively (Rouseff and Ting 1979; Sendra et al. 1988), have been found to inhibit the OATP2B1-mediated uptake of estrone-3-sulphate in HEK293 cells by 42% and 60%, respectively, at 10 μmol/l concentrations (Satoh et al. 2005).
6.3 Apple juice

Studies regarding the pharmacokinetic interactions of apple juice are sparse. Similarly to grapefruit and orange juices, apple juice has reduced the AUC fexofenadine by 73% (Dresser et al. 2002a). In addition, apple juice has decreased the exposure to OATP1A2 substrate atenolol by 82% (Jeon et al. 2012). Thus, these studies suggest the inhibition of intestinal OATPs by apple juice. At 5% concentration, apple juice has only slightly inhibited OATP1A2 in vitro (Dresser et al. 2002a), and it has had no effect on OATP2B1 (Satoh et al. 2005). However, at 20% concentration, apple juice has inhibited OATP2B1-mediated transport of fexofenadine in cRNA-injected oocytes by approximately 70% (Imanaga et al. 2011). Moreover, apple juice has reduced the C\text{max} of midazolam by 20%, with no effect on the other pharmacokinetic variables, suggesting a negligible effect on CYP3A4 (Imanaga et al. 2011).

Among the flavonoids occurring in apple juice are phlorizin (6-450 µmol/l), epicatechin (41 µmol/l), quercetin (4-23 µmol/l), and kaempferol (0.5 µmol/l) (Young et al. 1999; Tomás-Barberán and Clifford 2000; Schieber et al. 2001). Of these quercetin and kaempferol have inhibited the OATP1A2-mediated uptake of bromosulphophthalein in HEK293 cells, with K\text{i} values of 22.0 µmol/l and 25.2 µmol/l, respectively, and OATP2B1-mediated uptake, with K\text{i} values of 8.7 µmol/l and 15.1 µmol/l, respectively (Mandery et al. 2010). Furthermore, extracts of green tea, containing catechin and epicatechin, and Ginkgo biloba, containing quercetin and kaempferol, have inhibited OATP2B1 in vitro (Fuchikami et al. 2006). Quercetin has also been reported to inhibit hepatic OATP transporters OATP1B1 and OATP1B3, with K\text{i} values of 8.8 µmol/l and 7.8 µmol/l, respectively, and kaempferol, with K\text{i} values of 32.4 µmol/l and 24.4 µmol/l, respectively (Mandery et al. 2012).
AIMS OF THE STUDY

Considerable interindividual variability exists in the pharmacokinetics of the direct renin inhibitor aliskiren. Aliskiren is a substrate of the P-gp efflux and OATP influx transporters, and its pharmacokinetic properties, such as low oral bioavailability and minor metabolism, render it susceptible to transporter-mediated pharmacokinetic interactions. Moreover, several sequence variations have been discovered in the ABCC1 and SLCO2B1 genes encoding P-gp and OATP2B1, respectively, some of which have been suggested to be associated with altered transporter activity. These studies were conducted to investigate the effects of induction and inhibition of P-gp on the pharmacokinetics and pharmacodynamics of aliskiren. Furthermore, we investigated whether certain OATP-inhibiting fruit juices affect aliskiren exposure and pharmacodynamic factors. In addition, the effects of ABCC1 and SLCO2B1 polymorphisms on the pharmacokinetics and pharmacodynamics of aliskiren were studied.

Specific aims of the study were as follows:

Study I To investigate the effects of rifampicin, an inducer of P-gp and CYP3A4, on the pharmacokinetics and pharmacodynamics of aliskiren.

Study II To investigate the effects of itraconazole, an inhibitor of P-gp and CYP3A4, on the pharmacokinetics and pharmacodynamics of aliskiren.

Study III To investigate the effects of grapefruit juice, an inhibitor of intestinal CYP3A4, OATP1A2, and OATP2B1 in vitro, on the pharmacokinetics and pharmacodynamics of aliskiren.

Study IV To investigate the effects of orange juice and apple juice on the pharmacokinetics and pharmacodynamics of aliskiren.

Study V To investigate whether the common ABCC1 haplotypes affect the pharmacokinetics and pharmacodynamics of aliskiren.

Study VI To investigate whether the SLCO2B1 c.935G>A SNP affects the pharmacokinetics and pharmacodynamics of aliskiren.
MATERIALS AND METHODS

1. Subjects

A total of 93 (39 female and 54 male) healthy Finnish volunteers participated in the studies (Table 5). Some of the volunteers participated in two studies. The number of participants for each study was estimated to be sufficient to detect at least a 50% difference in the AUC of aliskiren between phases or groups, with a power of 80% (α-level 5%), except in Study VI, in which the number of participants in each genotype group was sufficient to detect a 50% higher or 33% lower difference in the AUC of aliskiren between the $SLCO2B1$ c.935GA and c.935GG groups and a 100% higher or 50% lower difference between the c.935AA and c.935GG groups. Originally, 12 volunteers were recruited for each pharmacokinetic interaction study (Studies I–IV), but in Study II, one subject dropped out after the first phase for personal reasons, and in Study III, one subject was subsequently excluded because of non-compliance with the diet.

The participants for the pharmacogenetic studies (Studies V and VI) were recruited from a pool of over 800 genotyped individuals. For Study V, the participants were selected and divided into two groups based on the genotypes composed of the $ABCB1$ c.1236C>T (rs1128503), c.2677G>T/A (p.Ala893Ser/Thr; rs2032582), and c.3435C>T (rs1045642) SNPs; both the $ABCB1$ c.1236T-c.2677T-c.3435T/c.1236T-c.2677T-c.3435T and c.1236C-c.2677G-c.3435C/c.1236C-c.2677G-c.3435C groups comprised 11 participants. To minimize variability in aliskiren pharmacokinetics due to other genetic variants, only non-carriers of the $ABCB1$ c.1199G>A (p.Ser400Asn; rs2229109) and $CYP3A5^*1$ (g.6986A; CYP3A5 expressor) alleles were recruited (Fanta et al. 2008). For Study VI, the participants were selected based on the $SLCO2B1$ c.935G>A (p.Arg312Gln; rs12422149) SNP, and they were allocated into one of three groups according to genotype; the $SLCO2B1$ c.935AA group comprised five participants, the c.935GA group 12 participants, and the c.935GG (control) group 16 participants.

The participants were ascertained to be healthy by medical history, clinical examination, and laboratory tests. Female subjects gave a negative pregnancy test before entering the studies. Subjects with a systolic blood pressure of less than 110 mmHg were not included in Studies I, II, and V, and those with less than 100 mmHg were not included in Studies III, IV, and VI. None of the participants was on any continuous medication,
including hormonal contraceptives, and none was a tobacco smoker. Use of other drugs was prohibited for one week before and one week after the day of administration of aliskiren (Studies I and II), or for one week before and three days after the day of administration of aliskiren (Studies III-VI). Use of grapefruit products was prohibited for 2-5 days before and three days after the day of administration of aliskiren in Studies I, II, V, and VI. In Study III, use of grapefruit products was prohibited during the entire study, starting from two weeks before the first day of administration of aliskiren, as was the use of orange juice and apple juice for two days before and three days after the day of administration of aliskiren. In Study IV, use of fruit juices and grapefruit, orange, and apple products was prohibited during the entire study, starting from one week before the first day of administration of aliskiren. Use of alcohol was prohibited for 1-5 days prior to the day of administration of aliskiren and during the days of blood sampling. Participation in any other trial or blood donation within three months before and after each study was also prohibited.

Table 5. Characteristics of participants.

<table>
<thead>
<tr>
<th>Study no. (n)</th>
<th>Genotype (n)</th>
<th>Sex (f/m)</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
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<tbody>
<tr>
<td>I (12)</td>
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<td>7/5</td>
<td>21 (20-25)</td>
<td>174 ±  10</td>
<td>66 ± 11</td>
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<td>II (11)</td>
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<td>25 (21-34)</td>
<td>174 ± 11</td>
<td>68 ± 13</td>
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<td>5/6</td>
<td>22 (20-28)</td>
<td>178 ±  8</td>
<td>71 ± 10</td>
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<tr>
<td>IV (12)</td>
<td></td>
<td>5/7</td>
<td>22 (20-28)</td>
<td>173 ±  7</td>
<td>69 ± 10</td>
</tr>
<tr>
<td>V (22)</td>
<td>ABCB1 TTT/TTT (11)</td>
<td>5/6</td>
<td>24 (21-27)</td>
<td>175 ± 10</td>
<td>69 ± 12</td>
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<tr>
<td></td>
<td>ABCB1 CGC/CGC (11)</td>
<td>4/7</td>
<td>26 (22-37)</td>
<td>178 ±  9</td>
<td>74 ± 11</td>
</tr>
<tr>
<td>VI (33)</td>
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<td>69 ±  7</td>
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<td></td>
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<td>3/9</td>
<td>25 (21-31)</td>
<td>177 ±  8</td>
<td>73 ± 13</td>
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<td></td>
<td>SLCO2B1 c.935GG (16)</td>
<td>8/8</td>
<td>23 (20-30)</td>
<td>174 ± 10</td>
<td>67 ± 10</td>
</tr>
</tbody>
</table>

Age data are means (range), height and weight data means ± SD. F, females; m, males.
1.1 DNA preparation and genotyping

For Studies V and VI, 10-ml ethylenediaminetetraacetic acid (EDTA) blood samples were obtained from healthy volunteers and stored at -20ºC until DNA extraction. Genomic DNA was extracted with standard methods (QIAamp DNA Mini Kit; Qiagen, Hilden, Germany). The subjects were genotyped for the alleles described above (see Subjects, Section 1), and the genotyping was carried out by allelic discrimination with Taqman® Genotyping Assays (Table 6). Polymerase chain reactions (PCRs) with a reaction volume of 10 μl were performed in an Applied Biosystems 7300 Real-Time PCR System or 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Pre- and post-PCR fluorescence measurements and genotype calls were made with the 7300 Real-Time PCR System. PCR cycling conditions were as follows: 1 cycle at 95ºC for 10 minutes, followed by 40 cycles of melting at 95ºC for 15 seconds, and annealing and extending at 60ºC for 1 minute.

Table 6. Single-nucleotide polymorphisms investigated and sequences of primers and probes or Applied Biosystems Assay identification numbers (AB Assay ID) for the genotyping assays used.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Primers and probes or AB Assay ID</th>
</tr>
</thead>
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<tr>
<td>ABCB1</td>
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<tr>
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<td>ABCB1</td>
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<td></td>
<td>5'GTAGGGAGTAACAAAATAAACA-3'</td>
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<td></td>
<td>5'-NED-CTTCCACGC-3'</td>
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<td>ABCB1</td>
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<td>c.601G&gt;A</td>
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<td></td>
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In Study IV, genomic DNA was extracted from the 10-ml EDTA blood samples, and the participants were genotyped for the \textit{SLCO2B1} g.-282G>A (rs2712807), c.601G>A (p.Val201Met; rs35199625), c.935G>A, and c.1457C>T (p.Ser486Phe; rs2306168) alleles for exploratory purposes.

2. Study designs

Studies were carried out at the Department of Clinical Pharmacology, University of Helsinki, and Helsinki University Central Hospital, in 2008-2010. Studies I-IV were randomized, placebo-controlled cross-over studies with 2-3 phases and washout periods of 2-4 weeks (Table 7). Studies V and VI were prospective genotype panel studies. Study VI was a part of a 2-phase study investigating the effects of the \textit{SLCO2B1} polymorphism on the pharmacokinetics of montelukast and aliskiren, with a washout period of at least one week.

Aliskiren (Rasilez 150-mg tablet; Novartis, Horsham, UK) was supplied by the Pharmacy of Helsinki University Central Hospital. The pretreatment medications (rifampicin: Rimpen 600-mg tablet; Orion Pharma, Espoo, Finland; itraconazole: Sporanox 100-mg capsule; Janssen-Cilag, Borgo San Michele, Italy) and matched placebos were also supplied, packed, and labelled according to a randomization list for each participant by the Pharmacy of Helsinki University Central Hospital. Grapefruit juice (Valio Greippitäysmehu; Valio, Helsinki, Finland), orange juice (Valio Appelsiinitäysmehu; Valio, Helsinki, Finland), and apple juice (Valio Omenatäysmehu; Valio, Helsinki, Finland) were purchased from local grocery stores and picked from the same production batch whenever possible (Table 8). 200-ml doses of juice, taken unsupervised at home, were bottled beforehand by the researchers. The randomization for Studies III and IV was done by the researchers.

On the study days, following at least an 8-hour overnight fast, the participants ingested a single 150-mg oral dose of aliskiren with 150 ml of water (Studies I, II, V, and VI) or with 200 ml of water or study juice (Studies III and IV) at 08:00 (Studies I and III-VI) or at 09:00 (Study II). A standardized warm meal was served 4 hours after the administration of aliskiren, and a standardized light meal after 7 hours and 10 hours. The participants were under direct medical supervision for 12 hours after the administration of aliskiren. Fluids for intravenous infusion were available for immediate use in the event of hypotension, but were not needed.
### Table 7. Structure of the pharmacokinetic interaction studies.

<table>
<thead>
<tr>
<th>Study no.</th>
<th>Pretreatment medication / juice</th>
<th>Administration of a single 150-mg dose of aliskiren</th>
<th>Washout period (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>600 mg of rifampicin or placebo once daily at 20:00 for 5 days</td>
<td>On day 6 at 08:00</td>
<td>4</td>
</tr>
<tr>
<td>II</td>
<td>100 mg of itraconazole (first dose 200 mg) or placebo twice daily at 09:00 (on day 3 at 08:00) and 21:00 for 5 days</td>
<td>On day 3 at 09:00</td>
<td>4</td>
</tr>
<tr>
<td>III</td>
<td>200 ml of grapefruit juice or water three times a day at 08:00, 12:00, and 20:00 for 5 days</td>
<td>On day 3 at 08:00</td>
<td>2</td>
</tr>
<tr>
<td>IV</td>
<td>200 ml of orange juice, apple juice, or water three times a day at 08:00, 12:00, and 20:00 for 5 days</td>
<td>On day 3 at 08:00</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 8. Characteristics of the fruit juices investigated.

<table>
<thead>
<tr>
<th>Nutritional value /100 g</th>
<th>Grapefruit juice</th>
<th>Orange juice</th>
<th>Apple juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.3</td>
<td>3.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>8 g</td>
<td>9 g</td>
<td>10 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>~ 1.8 g*</td>
<td>~ 3.6 g*</td>
<td>~ 1.4 g*</td>
</tr>
<tr>
<td>Fructose</td>
<td>~ 3.4 g*</td>
<td>~ 2.7 g*</td>
<td>~ 5.7 g*</td>
</tr>
<tr>
<td>Glucose</td>
<td>~ 2.8 g*</td>
<td>~ 2.7 g*</td>
<td>~ 2.9 g*</td>
</tr>
<tr>
<td>Organic acids</td>
<td>0.3 g*</td>
<td>0.6 g*</td>
<td>0.4 g*</td>
</tr>
<tr>
<td>Proteins</td>
<td>&lt; 1 g</td>
<td>&lt; 1 g</td>
<td>&lt; 1 g</td>
</tr>
<tr>
<td>Fibre</td>
<td>&lt; 1 g</td>
<td>&lt; 1 g</td>
<td>&lt; 1 g</td>
</tr>
</tbody>
</table>

Data are from the producer, except for values indicated with an asterisk, which are from Fineli 2010.

### 3. Blood and urine sampling and blood pressure measurements

On the days of administration of aliskiren, a forearm vein of each participant was cannulated for blood sampling. Timed blood samples for drug concentration
measurements (5 or 10 ml each) were drawn prior to and 0.5, 1, 2, 3, 4, 5, 7, 9, 12, 24, 34, 48, and 72 hours after aliskiren administration into tubes containing EDTA. Blood samples for the determination of plasma renin activity (5 ml each) were drawn before the administration of aliskiren and 4 hours and 24 hours thereafter into chilled EDTA tubes, which were placed on ice immediately after sampling, except in Study III, in which the blood samples were kept at room temperature according to the instructions of Medix Laboratories (Espoo, Finland). Plasma was separated by centrifugation within 30 minutes. Urine was collected up to 12 hours after the administration of aliskiren. Urine aliquots and plasma were stored at -70°C until analysis. Sitting systolic and diastolic blood pressures, as well as heart rates, were measured twice (mean value used in the calculations) from the forearm with an automatic oscillometric blood pressure monitor (Omron M5-I; Omron Healthcare Europe BV, Hoofddorp, The Netherlands) prior to and 2, 4, 7, 9, 12, and 24 hours after the administration of aliskiren.

4. Determination of drug concentrations and renin activity

4.1 Aliskiren

Plasma concentrations of aliskiren were quantified by use of solid-phase extraction and liquid chromatography-tandem mass spectrometry (LC/MS/MS) (Waldmeier et al. 2007). An aliquot of 700 µl of each plasma sample was mixed with 100 µl of 10% phosphoric acid and 50 µl of internal standard solution (1000 ng/ml acebutolol in 1:1 methanol:water). The mixture was then loaded to the extraction cartridge (Oasis MCX 1cc/30 mg; Waters, Milford, MA, USA), which had been preconditioned with 1 ml of methanol and 1 ml of 1% acetic acid. The cartridge was subsequently washed with 1 ml of methanol and 1 ml of 1% acetic acid. The analytes were eluted out twice with 0.6 ml of 2% ammonium hydroxide in methanol. The eluent was dried at 50°C under a nitrogen stream. The residue was dissolved in 100 µl of the mobile phase, and 15 µl of the sample was injected into the LC/MS/MS system. The chromatographic separation was carried out on an AtlantisT3 column (2.1×100 mm, 3 µm) protected by an AtlantisT3 (2.1×10 mm, 3 µm) guard cartridge (Waters). The mobile phase consisted of (A) 2 mmol/l ammonium acetate (pH 4.90) and (B) methanol. The total running time was 15 minutes with the mobile phase gradient of 0.5 minute at 70% A, from 70 to 5% A in 0.5 minute, 3 minutes at 5% A, from 5 to 70% A in 0.1 minute, and 10.9 minutes at 70% A. The flow rate of the mobile phase was 250 µl/min. The column temperature was 40°C. Mass spectra were obtained using an Applied Biosystems SCIEX API 2000 Q
Trap mass spectrometer operated in positive ion mode (Sciex Division of MDS, Toronto, ON, Canada). The ion transitions were mass-to-charge ratio (m/z) 552.4 → m/z 436.3 for aliskiren and m/z 337.3 to m/z 116.3 for acebutolol. The lower limit of quantification of plasma aliskiren was 0.24 ng/ml (Study IV) or 0.25 ng/ml (Studies I-III, V, and VI), and the between-day CV was 6.1% at 1.8 ng/ml, 4.6% at 18 ng/ml, and 7.0% at 180 ng/ml (n = 10). The calibration curve for plasma aliskiren was linear over the range 0.24-600 ng/ml (r > 0.999, weighting 1/x), and the extraction recoveries were over 80%.

For the determination of aliskiren concentrations in urine, an aliquot of 300 µl of each urine sample was mixed with 300 µl of fresh frozen plasma, 100 µl of 10% phosphoric acid, and 50 µl internal standard solution (1000 ng/ml acebutolol in 1:1 methanol:water). The extraction, chromatographic separation, and mass spectrometric detection were performed as described for plasma aliskiren. The lower limit of quantification of urine aliskiren was 9 ng/ml (Studies I-III and V) or 21 ng/ml (Studies IV and VI), and the intra-day CV was below 5% at relevant concentrations. The calibration curve for urine aliskiren was linear over the range 9-1800 ng/ml (r > 0.999, weighting 1/x), and the extraction recoveries were over 80%.

4.2 Itraconazole and hydroxyitraconazole

Plasma itraconazole and hydroxyitraconazole concentrations were determined by high-performance liquid chromatography with fluorescence detection (Dionex Ultimate 3000 HPLC system and Dionex RF 2000 Fluorescence Detector; Dionex Softron GmbH, Germering, Germany), as described previously (Remmel et al. 1988; Allenmark et al. 1990). The lower limit of quantification was 20 ng/ml for both compounds, and the between-day CV was 8.6% at 192 ng/ml and 3.3% at 1200 ng/ml for itraconazole, and 10.2% at 192 ng/ml and 4.3% at 1200 ng/ml for hydroxyitraconazole (n = 4).

4.3 Renin activity

Plasma renin activity was determined by radioimmunoassay of generated angiotensin I with a commercially available method (RENCTK; DiaSorin, Saluggia, Italy) at the HUSLAB laboratory (Helsinki, Finland) (Studies I, II, and V) or at Medix Laboratories (Studies III, IV, and VI). The lower limit of quantification was 0.1 µg/l/h (Studies I, II,
and V) or 0.2 μg/l/h (Studies III, IV, and VI). In Studies I, II, and V, the renin activity data were multiplied by 1.449 according to earlier HUSLAB practice.

5. Pharmacokinetics

In all studies, the pharmacokinetics of aliskiren were characterized by $C_{\text{max}}$, time to peak concentration ($t_{\text{max}}$), $t_{1/2}$, AUC values from 0 to 72 hours ($\text{AUC}_{0-72}$) and from time 0 to infinity ($\text{AUC}_{0-\infty}$), the amount of unchanged aliskiren excreted into urine from 0 to 12 hours ($A_e$), and renal clearance ($\text{Cl}_{\text{renal}}$), and also by oral clearance ($\text{Cl}/F$) in Study III. The pharmacokinetics of itraconazole and hydroxyitraconazole (Study II) were characterized by $C_{\text{max}}$ and $\text{AUC}_{0-13}$ on day three and the morning trough concentration on days 3-6. Pharmacokinetic parameters were calculated by conventional non-compartmental methods using MK-Model, version 5.0 (Biosoft, Cambridge, UK). The terminal log-linear part of each concentration-time curve was identified visually. The elimination rate constant ($k_e$) was determined by linear regression analysis of the log-linear part of the plasma drug concentration-time curve, using a minimum of three data points and nominal sampling times. The $t_{1/2}$ was calculated by the equation $t_{1/2} = \ln2/k_e$. The AUC values were calculated by a combination of the linear (for increasing concentrations) and log-linear (for decreasing concentrations) trapezoidal rules with extrapolation to infinity, when appropriate, by division of the last measured concentration by $k_e$. The $\text{Cl}_{\text{renal}}$ of aliskiren was calculated by the equation $\text{Cl}_{\text{renal}} = A_e/\text{AUC}_{0-12}$, and the $\text{Cl}/F$ by the equation $\text{Cl}/F = \text{dose}/\text{AUC}_{0-\infty}$.

6. Pharmacodynamics

The pharmacodynamics of aliskiren were characterized by estimating plasma renin activity at 4 hours and 24 hours after the administration of aliskiren, as well as by average sitting systolic and diastolic blood pressures, and heart rate. Average values of systolic and diastolic blood pressures and heart rate were calculated by dividing the area under the effect-time curve from 0 to 24 hours by 24 hours.
7. Statistical analysis

The data were analysed using the statistical program SPSS for Windows, version 16.0, 17.0, or 19.0 (SPSS, Chicago, IL, USA). Differences were considered significant when \( P < 0.05 \). In Study I, results are expressed as geometric means with 95% confidence intervals (CIs) \((C_{\text{max}}; \text{AUC; } \text{Ae; and } \text{Cl}_{\text{renal}})\) or means ± SDs \((t_{\frac{1}{2}; \text{and } \text{pharmacodynamic variables}})\). In Study II, results (except \(t_{\text{max}}\)) are expressed as means ± SDs. In Study III, results are expressed as geometric means with 90% CIs \((C_{\text{max}}; \text{AUC; } \text{Cl}/\text{F; Ae; and } \text{Cl}_{\text{renal}})\) or means ± SDs \((t_{\frac{1}{2}; \text{and } \text{pharmacodynamic variables}})\). In Study IV, results (except \(t_{\text{max}}\)) are expressed as geometric means with 95% CIs. In Study V, results are expressed as estimated marginal means with 95% CIs adjusting for body weight \((C_{\text{max}}; \text{AUC; and Ae})\) or estimated marginal means with 95% CIs \((t_{\frac{1}{2}; } \text{Cl}_{\text{renal}; } \text{and plasma renin activity})\) in text and tables, and as weight-adjusted means ± standard error of the means (SEM) or means ± SEMs in figures. In Study VI, results (except \(t_{\text{max}}\)) are expressed as geometric means with CVs (adjusting \(C_{\text{max}}; \text{AUC; and Ae for body weight}\) in text and tables, and as weight-adjusted geometric means with 90% CIs in figures. In all studies, \(t_{\text{max}}\) data are expressed as medians with ranges. Geometric mean ratios with 90% or 95% CIs or mean differences with 95% CIs were calculated for the pharmacokinetic and pharmacodynamic variables, except for \(t_{\text{max}}\). Furthermore, to enable comparison, the results are also expressed as percentage changes in geometric means in Table 9 and as percentage changes in geometric means with 90% CIs in Figures 5-8 (CPT 2010).

In case an individual plasma renin activity value was below the lower limit of quantification, it was replaced with zero (Studies I and V) or half of the lower limit of quantification (Studies II-IV, VI, and calculations for summarizing report). Logarithmic transformation was performed before statistical analysis for \(C_{\text{max}}; \text{AUC; Ae; } \text{Cl}_{\text{renal}; } \text{and } \text{Cl}/\text{F data in Studies I-III and V, and for all data, except } t_{\text{max}}, \text{ in Studies IV and VI.}"

In pharmacokinetic interaction studies, statistical comparisons of the pharmacokinetic (except \(t_{\text{max}}\)) and pharmacodynamic variables between phases were carried out using repeated-measures analysis of variance (ANOVA) with treatment phase as a within-subjects factor and treatment sequence as a between-subjects factor (Studies I-III) or using repeated-measures ANOVA with a priori pairwise comparisons with the Fisher’s Least Significant Difference method (Study IV). In pharmacogenetic studies, statistical comparisons of the pharmacokinetic (except \(t_{\text{max}}\)) and pharmacodynamic variables between participants with the different genotypes were made using ANOVA with a priori pairwise comparisons with the Fisher’s Least Significant Difference method,
when appropriate (Studies V and VI). Body weight was set as a covariate for $C_{\text{max}}$, AUC, and $Ae$ (Jarugula et al. 2010). The $t_{\text{max}}$ data were compared using the Wilcoxon signed-rank test (Studies I-III), Friedman’s ANOVA with a priori pairwise comparisons with the Wilcoxon signed-rank test (Study IV), the Mann-Whitney test (Study V), or the Kruskall-Wallis test with a priori pairwise comparisons with the Mann-Whitney U-test (Study VI). In Study I, the frequencies of individuals with a double peak in the plasma aliskiren concentration-time curve were compared between the phases using McNemar’s test. Possible correlations between subject characteristics and pharmacokinetic variables and between pharmacokinetic and pharmacodynamic variables and their changes were investigated with the Pearson correlation coefficient (Studies I, II, and IV).

8. Ethical considerations

The study protocols were approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District, and the National Agency for Medicines in Finland. All participants received both oral and written information and gave written informed consent before they were enrolled in the studies.
RESULTS

1. Effects of rifampicin on the pharmacokinetics and pharmacodynamics of aliskiren (Study I)

Rifampicin markedly reduced the plasma concentrations of aliskiren (Figure 5, Table 9). Aliskiren $C_{\text{max}}$ was reduced by 39% (effect on $C_{\text{max}}$ ranged from a 78% decrease to a 9% increase, $P = 0.017$), AUC$_{0-\infty}$ by 56% (range, 20-76%, $P < 0.001$), and Ae by 48% (effect on Ae ranged from a 73% decrease to a 4% increase, $P = 0.001$) by rifampicin. Rifampicin had no significant effect on the $t_{\text{max}}$, $t_{1/2}$, or $\text{Cl}_{\text{renal}}$ of aliskiren. The plasma aliskiren concentration-time curve showed a double peak in 11 out of 12 participants during the placebo phase and in 6 out of 12 participants during the rifampicin phase ($P = 0.063$). None of the pharmacokinetic variables of aliskiren correlated with subject's body weight during either the placebo or the rifampicin phase ($r < 0.38$, $P > 0.23$).

The reduction in plasma aliskiren concentrations resulted also in attenuation of the renin-inhibiting effect of aliskiren (Figure 5, Table 9). Plasma renin activity 24 hours after aliskiren ingestion was 61% higher during the rifampicin phase than during the placebo phase ($P = 0.008$). No significant differences existed in blood pressure or heart rate between the rifampicin and placebo phases.

![Figure 5](image)

**Figure 5.** Effects of rifampicin on the $C_{\text{max}}$, AUC$_{0-\infty}$, $t_{1/2}$, and renin-inhibiting effect of aliskiren. Bars represent percentage changes in geometric means with 90% confidence intervals.

* $P < 0.05$ compared with placebo phase.

** $P < 0.001$ compared with placebo phase.
2. Effects of itraconazole on the pharmacokinetics and pharmacodynamics of aliskiren (Study II)

Itraconazole markedly raised the plasma concentrations of aliskiren (Figure 6, Table 9). Aliskiren $C_{\text{max}}$ was raised 5.8-fold (range, 1.1-24.3-fold, $P < 0.001$) and $\text{AUC}_{0-\infty}$ 6.5-fold (range, 2.6-20.5-fold, $P < 0.001$) by itraconazole. The $C_{\text{max}}$ of aliskiren occurred later during the itraconazole phase (median, 4 hours) than during the placebo phase (median, 1 hour) ($P = 0.006$). Itraconazole had no significant effect on the $t_{1/2}$ of aliskiren. The $C_{\text{max}}$ of aliskiren was increased 8.0-fold (range, 3.6-19.9-fold, $P < 0.001$) and its $C_{\text{renal}}$ 1.2-fold (effect on $C_{\text{renal}}$ ranged from a 21% decrease to a 74% increase, $P = 0.042$).

In agreement with the effects of itraconazole on the pharmacokinetics of aliskiren, the renin-inhibiting effect of aliskiren was also enhanced by itraconazole (Figure 6, Table 9). Plasma renin activity 24 hours after aliskiren intake was 68% lower during the itraconazole phase than during the placebo phase ($P = 0.011$). No significant difference existed in blood pressure or heart rate between the itraconazole and placebo.

The $C_{\text{max}}$ and $\text{AUC}_{0-13}$ of itraconazole on day three were 275 ± 85 ng/ml and 2355 ± 631 ng•h/ml, respectively, and those of hydroxyitraconazole were 619 ± 122 ng/ml and 6964 ± 1385 ng•h/ml, respectively. The $C_{\text{max}}$ and $\text{AUC}_{0-13}$ of itraconazole and hydroxyitraconazole showed no significant correlation with the relative increase in the $C_{\text{max}}$ or $\text{AUC}_{0-\infty}$ of aliskiren ($r < 0.52, P > 0.10$).

![Figure 6](image6.png)

**Figure 6.** Effects of itraconazole on the $C_{\text{max}}$, $\text{AUC}_{0-\infty}$, $t_{1/2}$, and renin-inhibiting effect of aliskiren. Bars represent percentage changes in geometric means with 90% confidence intervals. **$P < 0.001$ compared with placebo phase.**
3. Effects of grapefruit juice, orange juice, and apple juice on the pharmacokinetics and pharmacodynamics of aliskiren (Studies III and IV)

Three glasses a day of normal-strength grapefruit juice, orange juice, or apple juice for five days greatly reduced the plasma concentrations of aliskiren (Figure 7, Table 9). Grapefruit juice reduced aliskiren $C_{\text{max}}$ by 81% (range, 42-91%, $P < 0.001$), AUC$_{0-\infty}$ by 61% (range, 15-72%, $P < 0.001$), and $A_{\text{e}}$ by 66% (range, 6-81%, $P < 0.001$). Orange juice reduced aliskiren $C_{\text{max}}$ by 80% (effect on $C_{\text{max}}$ ranged from a 95% decrease to a 24% increase, $P < 0.001$), AUC$_{0-\infty}$ by 62% (range, 9-87%, $P < 0.001$), and $A_{\text{e}}$ by 67% (range, 32-87%, $P < 0.001$). Apple juice reduced aliskiren $C_{\text{max}}$ by 84% (range, 2-96%, $P < 0.001$), AUC$_{0-\infty}$ by 63% (range, 13-87%, $P < 0.001$), and $A_{\text{e}}$ by 69% (range, 14-87%, $P < 0.001$). The $C_{\text{max}}$ of aliskiren occurred later during the grapefruit juice phase (median, 1 hour) than during the water phase (median, 0.5 hour) ($P = 0.016$), and the $t_{1/2}$ of aliskiren was shortened from 26.1 to 23.6 hours by grapefruit juice ($P = 0.020$). Grapefruit juice had no significant effect on the $C_{\text{renal}}$ of aliskiren. Neither orange juice nor apple juice had an effect on the $t_{\text{max}}$, $t_{1/2}$, or $C_{\text{renal}}$ of aliskiren.

In Study III, aliskiren AUC$_{0-\infty}$ values varied 6.1-fold and 5.8-fold between individual participants during the water and grapefruit juice phases, respectively. In Study IV, aliskiren AUC$_{0-\infty}$ values varied 7.9-fold, 4.0-fold, and 4.2-fold between individual participants during the water, orange juice, and apple juice phases, respectively, and the changes in aliskiren AUC$_{0-\infty}$ by orange juice and apple juice correlated with each other, and with the AUC$_{0-\infty}$ of aliskiren during the water phase ($r = 0.98$, $P < 0.001$ for all pairs).

No significant differences existed in plasma renin activity between the grapefruit juice and water phases (Figure 8, Table 9). Orange juice and apple juice significantly reduced the renin-inhibiting effect of aliskiren. Plasma renin activity 4 hours and 24 hours after aliskiren administration was 63% ($P = 0.023$) and 87% ($P = 0.037$) higher, respectively, during the orange juice phase than during the water phase. During the apple juice phase plasma renin activity was 67% higher 24 hours after aliskiren administration than during the water phase ($P = 0.036$). Moreover, in Study IV, during the water phase the plasma aliskiren concentration at 24 hours correlated significantly with the plasma renin activity at 24 hours ($r = -0.615$, $P = 0.033$), and the relative change in plasma aliskiren concentration at 24 hours by orange juice or apple juice correlated with the corresponding relative change in plasma renin activity (orange juice: $r = -0.580$, $P =$
0.048; apple juice: \( r = -0.716, P = 0.009 \). No significant differences in blood pressure or heart rate were observed between the phases in either of these studies.

In Study IV, no obvious differences were seen in the pharmacokinetics of aliskiren between participants with different \( SLCO2B1 \) genotypes, but the number of individuals with different genotypes was too small to draw any conclusions.

**Figure 7.** Effects of grapefruit juice (GFJ), orange juice (OJ), and apple juice (AJ) on the \( C_{\text{max}} \), \( \text{AUC}_{0-\infty} \), and \( t_{1/2} \) of aliskiren. Bars represent percentage changes in geometric means with 90% confidence intervals.

* \( P < 0.05 \) compared with water phase.

** \( P < 0.001 \) compared with water phase.

**Figure 8.** Effects of grapefruit juice (GFJ), orange juice (OJ), and apple juice (AJ) on the renin-inhibiting effect of aliskiren. Bars represent percentage changes in geometric means with 90% confidence intervals.

* \( P < 0.05 \) compared with water phase.
4. Effects of the *ABCB1* haplotypes on the pharmacokinetics and pharmacodynamics of aliskiren (Study V)

The *ABCB1* haplotypes were not significantly associated with the pharmacokinetics or pharmacodynamics of aliskiren. The geometric mean ratios (95% CI) of aliskiren C\(_{\text{max}}\) and AUC\(_{0-\infty}\) in participants homozygous for the *ABCB1* c.1236T-c.2677T-c.3435T haplotype to those in participants homozygous for the c.1236C-c.2677G-c.3435C haplotype were 1.14 (0.66, 1.96; \(P = 0.631\)) and 1.01 (0.58, 1.76; \(P = 0.960\)), respectively. As a post-hoc analysis, fractional AUC values (AUC\(_{0-1}\), AUC\(_{0-2}\), AUC\(_{0-3}\), AUC\(_{0-4}\), and AUC\(_{0-5}\)) were also calculated for aliskiren, but no tendency for a difference between the genotype groups was observed in these values (\(P > 0.3\), data not shown). Aliskiren C\(_{\text{max}}\), AUC\(_{0-\infty}\), Ae, and Cl\(_{\text{renal}}\) varied 14.1-, 16.2-, 10.4-, and 3.1-fold, respectively, between individual participants.

5. Effects of the *SLCO2B1* c.935G>A SNP on the pharmacokinetics and pharmacodynamics of aliskiren (Study VI)

The *SLCO2B1* c.935G>A SNP had no significant effect on the pharmacokinetics or pharmacodynamics of aliskiren. The geometric mean ratios (90% CI) of aliskiren C\(_{\text{max}}\) in participants with the *SLCO2B1* c.935GA or c.935AA genotype to those with the c.935GG genotype were 1.11 (0.78, 1.60) and 1.47 (0.91, 2.39), respectively (ANOVA \(P = 0.408\)), and the corresponding geometric mean ratios (90% CI) of aliskiren AUC\(_{0-\infty}\) were 0.98 (0.74, 1.30) and 1.24 (0.85, 1.80) (ANOVA \(P = 0.576\)). Aliskiren C\(_{\text{max}}\), AUC\(_{0-\infty}\), Ae, and Cl\(_{\text{renal}}\) varied 8.8-, 5.0-, 6.0-, and 4.2-fold, respectively, between individual participants.
6. Summary

Rifampicin, grapefruit juice, orange juice, and apple juice markedly reduced the plasma concentrations of aliskiren. Grapefruit juice also slightly shortened the $t_{1/2}$ of aliskiren. The reduction in aliskiren exposure by rifampicin, orange juice, and apple juice resulted in attenuation of the renin-inhibiting effect of aliskiren. Itraconazole greatly raised the plasma concentrations of aliskiren, with no effect on $t_{1/2}$, and, consistent with increased exposure, enhanced the renin-inhibiting effect of aliskiren. The results of the pharmacokinetic interaction studies with aliskiren are summarized in Table 9.

The $ABCB1$ c.1236C-c.2677G-c.3435C and c.1236T-c.2677T-c.3435T haplotypes as well as the $SLCO2B1$ c.935G>A SNP were not significantly associated with the pharmacokinetics or pharmacodynamics of aliskiren.

Table 9. Results of the pharmacokinetic interaction studies with aliskiren.

<table>
<thead>
<tr>
<th>Pretreatment medication / juice</th>
<th>Effect on aliskiren pharmacokinetics</th>
<th>Effect on aliskiren pharmacodynamics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin</td>
<td>$C_{\text{max}}$ 39% ↓ *</td>
<td>Renin activity</td>
</tr>
<tr>
<td></td>
<td>$AUC$ 56% ↓ **</td>
<td>at 4 hours ← ←</td>
</tr>
<tr>
<td></td>
<td>$t_{1/2}$ ↔</td>
<td>at 24 hours 117% ↑ *</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>$C_{\text{max}}$ 481% ↑ **</td>
<td>Renin activity</td>
</tr>
<tr>
<td></td>
<td>$AUC$ 554% ↑ **</td>
<td>at 4 hours ← ←</td>
</tr>
<tr>
<td></td>
<td>$t_{1/2}$ ↔</td>
<td>at 24 hours 72% ↓ **</td>
</tr>
<tr>
<td>Grapefruit juice</td>
<td>$C_{\text{max}}$ 81% ↓ **</td>
<td>Renin activity</td>
</tr>
<tr>
<td></td>
<td>$AUC$ 61% ↓ **</td>
<td>at 4 hours ← ←</td>
</tr>
<tr>
<td></td>
<td>$t_{1/2}$ 10% ↓ *</td>
<td>at 24 hours ← ←</td>
</tr>
<tr>
<td>Orange juice</td>
<td>$C_{\text{max}}$ 80% ↓ **</td>
<td>Renin activity</td>
</tr>
<tr>
<td></td>
<td>$AUC$ 62% ↓ **</td>
<td>at 4 hours ← ←</td>
</tr>
<tr>
<td></td>
<td>$t_{1/2}$ ← ←</td>
<td>at 24 hours 63% ↑ *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>at 24 hours 87% ↑ *</td>
</tr>
<tr>
<td>Apple juice</td>
<td>$C_{\text{max}}$ 84% ↓ **</td>
<td>Renin activity</td>
</tr>
<tr>
<td></td>
<td>$AUC$ 63% ↓ **</td>
<td>at 4 hours ← ←</td>
</tr>
<tr>
<td></td>
<td>$t_{1/2}$ ← ←</td>
<td>at 24 hours 67% ↑ *</td>
</tr>
</tbody>
</table>

Data are given as percentage change in geometric means. Therefore, the results may slightly differ from those presented in the text.

* $P < 0.05$ compared with placebo/water phase.
** $P < 0.001$ compared with placebo/water phase.
← No statistically significant difference.
DISCUSSION

1. Methodological considerations

These studies investigated the pharmacokinetic interactions and pharmacogenetic factors affecting the pharmacokinetics and pharmacodynamics of aliskiren. All six studies were carried out with healthy, relatively young (20-37 years) volunteers to minimize possible confounding factors such as chronic diseases or long-term medication. Based on the variation in the AUC of aliskiren in previous studies, the number of participants for each study was estimated to be sufficient to detect possible clinically meaningful differences in the AUC of aliskiren between phases or groups, while avoiding unnecessary exposure of healthy volunteers to drugs. The pharmacokinetics of aliskiren has been shown to be similar in normotensive and hypertensive individuals (Vaidyanathan et al. 2008c; EMA 2012), making it possible to extrapolate the pharmacokinetic data from these studies to patient populations. However, hypertensive patients may exhibit characteristics that increase the extent of the interactions.

The pharmacokinetic interaction studies (I-IV) were placebo- or water-controlled, randomized cross-over studies with washout periods of 2-4 weeks. The cross-over design was used to minimize variation and to reduce sample size, as participants served as their own controls. Balanced randomization and adequate washout periods minimized the risk of a possible carry-over effect. The pharmacogenetic studies (V and VI) were prospective genotype panel studies. In these studies, no significant differences in age, height, or weight were observed between groups. Furthermore, the pharmacokinetic variables were adjusted for body weight, when appropriate (Jarugula et al. 2010). To reduce further variation in all studies, restrictions were imposed concerning the use of drugs, alcohol, and certain fruit juices and fruits before and during the studies. To minimize variability due to the food effect, aliskiren was administered after an overnight fast and food intake during the study days was standardized and controlled.

In all studies, aliskiren was administered at the lowest recommended dose (150 mg). In previous studies with healthy volunteers, once-daily doses of up to 640 mg have been well tolerated (Nussberger et al. 2002). In addition, in an interaction study with the P-gp and CYP3A4 inhibitor ketoconazole, aliskiren was well tolerated at a dose of 300 mg once daily for several days (Vaidyanathan et al. 2008b).
In clinical practice, aliskiren is administered on a regular basis. In these studies, a single dose, instead of multiple dosing, was chosen for safety and practical reasons. The single-dose study design enabled the participants to be kept under medical supervision and their blood pressure to be monitored during the greatest exposure to aliskiren (the first 12 hours after the administration). A single dose of aliskiren was considered safe because it is not expected to have a marked effect on blood pressure in healthy subjects, although the response in renin activity is detectable already 30 minutes after aliskiren ingestion (Nussberger et al. 2002). According to pharmacokinetic theory, the $AUC_{0-\infty}$ after a single dose is equal to the dose-interval $AUC$ at steady state (Rowland and Tozer 2011). However, the pharmacokinetics of aliskiren is slightly non-linear (Vaidyanathan et al. 2008c), potentially interfering with the direct extrapolation of the results from the single-dose studies to the steady state.

The sampling schedule was selected to cover the plasma concentration-time curve sufficiently long to provide a reliable estimate of the extent of exposure. According to the guidelines for bioequivalence studies, this is achieved if $AUC_{0-t}$ covers at least 80% of $AUC_{0-\infty}$ (EMA 2010a). In all of these studies, $AUC_{0-72}$ covered at least 82% of $AUC_{0-\infty}$. Urine was collected only during the time spent at the Clinical Research Unit to ensure compliance.

Rifampicin is widely used and recommended as a model inducer of drug-metabolizing enzymes and transporters in pharmacokinetic drug interaction studies in humans (FDA 2012). Because the induction by rifampicin requires the synthesis of new enzyme or transporter protein, it takes about one week to achieve maximal induction (Fromm et al. 1996; Niemi et al. 2003). Baseline activity returns two weeks after discontinuing rifampicin. Thus, in Study I, with the administration of rifampicin for five days, nearly maximal induction of intestinal CYP3A4 and P-gp was attained. To avoid the possible competitive inhibitory effect of rifampicin (Vavricka et al. 2002; Kajosaari et al. 2005a), the last dose of rifampicin was administered 12 hours before aliskiren ingestion.

Itraconazole is recommended as an inhibitor in drug interaction studies involving substrates of CYP3A4 and P-gp (FDA 2012). Inhibition by itraconazole is dependent on its plasma concentrations (Back and Tjia 1991; Jurima-Romet et al. 1994; Neuvonen et al. 1996; Olkkola et al. 1996; von Moltke et al. 1996a). In Study II, after a loading dose on day one, the administration of itraconazole for five days was sufficient to achieve nearly maximal inhibition of CYP3A4 and P-gp, thus revealing a potentially clinically important pharmacokinetic interaction. This dosing regimen was also used to ensure the
tissue distribution of itraconazole to achieve a systemic effect in addition to the effect at the intestinal level. The timing of administration of itraconazole (one hour before aliskiren) was chosen to ensure its adequate absorption before aliskiren ingestion.

In Study III, a 5-day regimen of grapefruit juice three times daily was used to ensure nearly maximal inhibition of CYP3A4. Ingestion of aliskiren on day three corresponded to the clinical situation in which subjects consume grapefruit juice daily. Because aliskiren has a long $t_{1/2}$ and is known to undergo enterohepatic circulation, the administration of grapefruit juice was continued after aliskiren ingestion to investigate the effect of grapefruit juice on the elimination of aliskiren. For comparison, the same protocol was used in Study IV with orange and apple juices.

Compliance with pretreatment drugs and juices in pharmacokinetic interaction studies was assessed by records of participants in their pretreatment diaries. Furthermore, in Study II, the concentrations of itraconazole and hydroxyitraconazole were measured on study days.

The common synonymous $ABCB1$ c.3435C>T SNP, forming a part of the haplotype investigated in Study V, has been variably associated with reduced expression and function of P-gp in vitro and increased plasma concentrations of P-gp substrate drugs in vivo (Hoffmeyer et al. 2000; Kimchi-Sarfaty et al. 2007; Keskitalo et al. 2008). However, the mechanism underlying these associations is not completely understood. Because the $ABCB1$ c.3435C>T SNP is in strong linkage disequilibrium with the c.1236C>T (synonymous) and c.2677G>T/A (p.Ala893Ser/Thr) SNPs within the same gene (Kim et al. 2001; Kroetz et al. 2003; Keskitalo et al. 2008), we chose to study the two most common haplotypes in the Finnish population based on these SNPs (Keskitalo et al. 2008), instead of a single variant allele. In addition, these haplotypes have affected the pharmacokinetics of simvastatin (acid) and atorvastatin in the Finnish population (Keskitalo et al. 2008). Only non-carriers of the $ABCB1$ c.1199A and $CYP3A5$ g.6986A (CYP3A5 expressor) alleles were included in the study to exclude other polymorphisms possibly affecting the pharmacokinetics of aliskiren.

The $SLCO2B1$ c.935G>A SNP, investigated in Study VI, was selected based on a previous study in which it had been associated with reduced plasma concentrations of and impaired response to montelukast in patients with asthma (Mougey et al. 2009). In the Finnish population, the frequency of this variant allele is 13.6% (Laitinen and Niemi 2011).
2. Effects of rifampicin on the pharmacokinetics and pharmacodynamics of aliskiren (Study I)

In Study I, rifampicin reduced aliskiren $C_{\text{max}}$, $AUC_{0-\infty}$, and $Ae$ by 39%, 56%, and 48%, respectively. In line with the reduced exposure to aliskiren, plasma renin activity 24 hours after aliskiren administration was 61% higher during the rifampicin phase than during the placebo phase. Decreased $C_{\text{max}}$, $AUC_{0-\infty}$, and $Ae$ of aliskiren, without a change in $t_{1/2}$ and $Cl_{\text{renal}}$, are consistent with increased first-pass elimination of aliskiren with no or minimal effect on its systemic clearance.

Aliskiren is a substrate of P-gp and is slightly metabolized by CYP3A4 (Waldmeier et al. 2007; Vaidyanathan et al. 2008b), both of which are induced by rifampicin (Combalbert et al. 1989; Kolars et al. 1992; Backman et al. 1996a; Schuetz et al. 1996; Greiner et al. 1999). Because aliskiren has a low hepatic extraction ratio (0.10) (Azizi et al. 2006) and its $t_{1/2}$ was not shortened by rifampicin, it is unlikely that induction of its hepatic elimination would explain this interaction. Furthermore, the incidence of double peaks in the plasma aliskiren concentration-time curve, a characteristic of enterohepatic circulation, appeared to diminish during the rifampicin phase compared with the placebo phase. This finding could be explained by reduced intestinal reabsorption of aliskiren due to induction of intestinal P-gp and CYP3A4. Thus, the interaction between rifampicin and aliskiren most likely occurred as a result of induction of P-gp-mediated efflux and CYP3A4-catalysed metabolism of aliskiren in the small intestine. It is noteworthy that intestinal P-gp and CYP3A4 act synergistically to reduce the oral bioavailability of their joint substrates (Kivistö et al. 2004).

Similarly to rifampicin, a high-fat meal can reduce the AUC of aliskiren by about 70% (Vaidyanathan et al. 2008c). To minimize variability due to the food effect, aliskiren is recommended to be taken with a light meal or on a regular schedule with regard to meals (EMA 2012; Novartis 2012). No published studies exist on the interactions of aliskiren with other dual inducers of P-gp and CYP3A4 such as phenytoin or St. John's wort (Rameis 1985; Backman et al. 1996b; Johne et al. 1999; Wang et al. 2001). Based on the results of Study I, these other inducers might also reduce the concentrations and effects of aliskiren.
3. Effects of itraconazole on the pharmacokinetics and pharmacodynamics of aliskiren (Study II)

In Study II, itraconazole raised the $C_{\text{max}}$, $\text{AUC}_{0-\infty}$, and $\text{Ae}$ of aliskiren 5.8-, 6.5-, and 8.0-fold, respectively, and also considerably enhanced the renin-inhibiting effect of aliskiren. The unaffected $t_{1/2}$ of aliskiren indicates that the interaction occurred mainly during the first-pass phase. This is also supported by the finding that the extent of the interaction did not correlate with the systemic (plasma) concentrations of itraconazole and hydroxyitraconazole.

Itraconazole is a potent inhibitor of P-gp and CYP3A4 (Jurima-Romet et al. 1994; Olkkola et al. 1994; Jalava et al. 1997b; Wang et al. 2002). Because aliskiren has a low hepatic extraction ratio and is eliminated mainly unchanged (Azizi et al. 2006; Waldmeier et al. 2007), the mechanism of this interaction is probably inhibition of P-gp in the small intestine. Inhibition of the CYP3A4-catalysed metabolism of aliskiren by itraconazole may, however, also contribute to the interaction. In a previous study, itraconazole reduced the $\text{Cl}_{\text{renal}}$ of digoxin by 20% by inhibiting P-gp in renal tubule cells (Jalava et al. 1997b). The minor increase in the $\text{Cl}_{\text{renal}}$ of aliskiren by itraconazole, seen in this study, probably results from other mechanisms such as saturation of reuptake systems in the kidneys due to a high aliskiren concentration.

The interaction between itraconazole and aliskiren was much stronger (6.5-fold increase in aliskiren AUC) than previously described between the otherazole antifungal agent ketoconazole and aliskiren (76% increase in aliskiren AUC) (Vaidyanathan et al. 2008b). Itraconazole and ketoconazole are similarly potent inhibitors of P-gp ($\text{IC}_{50}$ 2 μmol/l and 6 μmol/l, respectively) and CYP3A4 ($K_i$ 0.27-11 μmol/l and 0.0037-10 μmol/l, respectively) in vitro (Back and Tjia 1991; Jurima-Romet et al. 1994; von Moltke et al. 1996a; von Moltke et al. 1996b; Venkatakrishnan et al. 2000; Wang et al. 2002), and their effects on P-gp and CYP3A4 activity have also been quite similar in vivo (Olkkola et al. 1994; Shon et al. 2005; Sanofi-Aventis 2007). One explanation for the different effects of itraconazole and ketoconazole on the pharmacokinetics of aliskiren could be the non-optimal dosage of ketoconazole in the previous study (200 mg twice daily). Because the interaction between ketoconazole and aliskiren probably occurred at the intestinal level (Vaidyanathan et al. 2008b), the 400 mg of ketoconazole once daily could have produced a larger interaction (Zhao et al. 2009). On the other hand, several active metabolites and the long $t_{1/2}$ of itraconazole relative to ketoconazole,
as well as their different tissue distributions, could explain the differences in extent of the interaction.

Another dual inhibitor of P-gp and CYP3A4, verapamil, has raised the AUC of aliskiren 2-fold (Rebello et al. 2011b). Furthermore, the concomitant administration of cyclosporine with a small (75-mg) dose of aliskiren has raised the AUC of aliskiren 5-fold (Rebello et al. 2011a). In contrast to itraconazole, cyclosporine has also prolonged the t½ of aliskiren.

4. Effects of grapefruit juice, orange juice, and apple juice on the pharmacokinetics and pharmacodynamics of aliskiren (Studies III and IV)

Studies III and IV demonstrated that the intake of grapefruit juice, orange juice, or apple juice three times a day can markedly reduce the plasma concentrations of aliskiren. The reduction in plasma aliskiren concentrations by orange and apple juices also resulted in attenuation of the renin-inhibiting effect of aliskiren, whereas grapefruit juice had no significant effect on the renin activity response to aliskiren.

Grapefruit juice, orange juice, and apple juice decreased the Cmax, AUC, and Ae of aliskiren, without affecting its Clrenal, consistent with a reduction in oral bioavailability. In previous studies, these juices have decreased the oral bioavailabilities of some substrates of intestinal OATPs such as fexofenadine (Dresser et al. 2002a; Bailey 2010). In addition, certain constituents of these juices have been found to inhibit OATP2B1 and/or OATP1A2 in vitro (Dresser et al. 2002a; Satoh et al. 2005; Fuchikami et al. 2006; Bailey et al. 2007; Mandery et al. 2010). Aliskiren has been shown to be a substrate of OATP2B1, with a Km of 72 μmol/l (Vaidyanathan et al. 2008b), and more recently, a substrate of OATP1A2 (Km could not be estimated at concentrations of up to 50 μmol/l) (Rebello et al. 2011c). Therefore, inhibition of OATP2B1- and/or OATP1A2-mediated absorption of aliskiren from gut lumen by grapefruit, orange, and apple juices probably mainly explains the interactions seen in Studies III and IV. This is supported by the finding that orange juice and apple juice had no effect on the t½ of aliskiren. Grapefruit juice, by contrast, slightly shortened aliskiren t½. In previous studies, repeated consumption of grapefruit juice has had the opposite (slightly prolonging) effect on the t½ of some CYP3A4 substrate drugs (Lilja et al. 1998b; Kivistö et al. 1999; Lilja et al. 1999; Lilja et al. 2000a; Veronese et al. 2003; Nieminen
et al. 2010). The shortened $t_{1/2}$ seen in Study III may have resulted from inhibition of the reabsorption of aliskiren during enterohepatic circulation. Moreover, the $t_{\text{max}}$ of aliskiren was extended during the grapefruit juice phase, consistent with delayed absorption.

To our knowledge, Studies III and IV were the first to suggest that grapefruit juice, orange juice, and apple juice may be clinically important inhibitors of OATP2B1, although inhibition of OATP1A2 may also have contributed to these interactions. The results of Study III contradicted the findings of previous studies in which grapefruit juice increased the AUC of the OATP2B1 substrates atorvastatin (2.5-fold) and amiodarone (1.5-fold) (Lilja et al. 1999; Libersa et al. 2000; Grube et al. 2006; Seki et al. 2009). Both atorvastatin and amiodarone are, however, significantly metabolized by CYP3A4 (Libersa et al. 2000; Neuvonen et al. 2006), and the inhibition of intestinal CYP3A4 by grapefruit juice probably mainly explains these interactions. Moreover, grapefruit juice has had no significant effect on the pharmacokinetics of the OATP2B1 substrates glibenclamide and pravastatin (Lilja et al. 1999; Nozawa et al. 2004; Satoh et al. 2005; Lilja et al. 2007), suggesting that OATP2B1 is not important for the intestinal absorption of these drugs.

Several constituents of fruit juices have been screened for inhibitory effects on intestinal OATP transporters. However, the prediction of in vivo pharmacokinetic interactions from in vitro data is challenging because flavonoids in these juices are usually found as glycoside conjugates that require hydrolysis to active aglycones in vivo (Erlund et al. 2001; Walle 2004; Walle et al. 2005). Naringin is considered to be the major constituent of grapefruit juice responsible for inhibition of OATP1A2 in vivo (Bailey et al. 2007), and it could also play an important role in the inhibition of OATP2B1 in vivo. In addition, naringin and its aglycone form naringenin, as well as quercetin, bergamottin, and 6’,7’-dihydroxybergamottin, all occurring in grapefruit juice, have inhibited OATP2B1 in vitro (Satoh et al. 2005). The major flavonoid in orange juice, hesperidin, has inhibited OATP1A2 in vitro (Bailey et al. 2007), but it is not known whether it also inhibits OATP2B1. However, tangeritin and nobiletin, also found in orange juice, have been shown to have an inhibitory effect on OATP2B1 (Satoh et al. 2005). Possible OATP2B1- and/or OATP1A2-inhibiting flavonoids in apple juice include epicatechin, quercetin, and kaempferol (Fuchikami et al. 2006; Mandery et al. 2010).

In addition to the direct inhibition of OATP2B1 and/or OATP1A2 by fruit juice constituents, other theoretical mechanisms of interaction may be suggested. These include formation of insoluble complexes between fruit juice constituents and aliskiren,
increased ionization of aliskiren because of an alteration in intestinal pH by organic acids, an increase in intestinal fluid volume by an osmotic effect of carbohydrates that could decrease the intestinal aliskiren concentration, or delayed gastric emptying caused by carbohydrates. Further studies are required to clarify the exact mechanisms of the pharmacokinetic interactions between fruit juices and aliskiren.

As evidenced by a previous study with fexofenadine, the duration of the inhibitory effect of grapefruit juice on OATP1A2 is more than 2 hours but less than 4 hours (Glaeser et al. 2007). Furthermore, a concomitantly ingested single dose of grapefruit juice has decreased talinolol exposure to a similar extent as grapefruit juice ingested three times daily for six days (Schwarz et al. 2005). Thus, it appears possible that ingestion of only a single 200-ml dose of grapefruit, orange, or apple juice concomitantly with or a few hours before has a similar effect on the exposure to aliskiren as ingestion of 200 ml three times daily.

5. Lack of effect of the ABCB1 haplotypes and the SLCO2B1 c.935G>A SNP on the pharmacokinetics and pharmacodynamics of aliskiren (Studies V and VI)

In Studies V and VI, no pharmacogenetic explanation for the high interindividual variability seen in aliskiren pharmacokinetics was found, as neither the ABCB1 c.1236C-c.2677G-c.3435C and c.1236T-c.2677T-c.3435T haplotypes nor the SLCO2B1 c.935G>A SNP were associated with altered pharmacokinetics or pharmacodynamics of aliskiren. Study V had a sufficient power to detect a 50% difference in the \( \text{AUC}_{0-\infty} \) of aliskiren between \( \text{ABCB1} \) genotype groups, and in Study VI, the power was sufficient to detect a 50% higher or 33% lower difference in the \( \text{AUC}_{0-\infty} \) of aliskiren between the \( \text{SLCO2B1} \) c.935GA and c.935GG groups and a 100% higher or 50% lower difference between the c.935AA and c.935GG groups. Thus, a small difference cannot be ruled out. However, the mean \( \text{AUC}_{0-\infty} \) values of aliskiren were almost identical in the genotype groups in both studies.

The lack of effect of these genetic variants does not indicate that P-gp or OATP2B1 is not otherwise important for aliskiren pharmacokinetics. Based on in vitro studies, aliskiren is a substrate of P-gp \((K_m = 2.1 \ \mu\text{mol/l})\) and OATP2B1 \((K_m = 72 \ \mu\text{mol/l})\) (Vaidyanathan et al. 2008b). The importance of P-gp has also been demonstrated in vivo, e.g. in Studies I and II, in which both the induction and inhibition of P-gp
markedly affected the pharmacokinetics of aliskiren. In Studies III and IV, the OATP2B1- and OATP1A2-inhibiting fruit juices greatly reduced the exposure to aliskiren, supporting important roles of these transporters in aliskiren pharmacokinetics.

In previous studies, ABCB1 genotypes have explained only a fraction of the variability in P-gp expression (Hoffmeyer et al. 2000). In addition, the findings from many studies on the effect of ABCB1 polymorphisms on drug pharmacokinetics have not been consistently reproduced (Chinn and Kroetz 2007; Giacomini et al. 2010). For instance, the results from studies investigating the effects of the ABCB1 c.3435C>T SNP on the pharmacokinetics of digoxin have been conflicting, with a meta-analysis suggesting no effect at all (Greiner et al. 1999; Hoffmeyer et al. 2000; Sakaeda et al. 2001; Johne et al. 2002; Verslyuyt et al. 2003; Chowbay et al. 2005). Moreover, the effect of ABCB1 haplotypes has been modest. In a previous study, the differences in the AUC values of simvastatin (acid) and atorvastatin were 60% and 55%, respectively, between the ABCB1 TTT/TTT and CGC/CGC genotype groups (Keskitalo et al. 2008). It has been suggested that the ABCB1 c.3435C>T SNP, or its combination with the c.1236C>T or c.2677G>T/A SNP, may alter the substrate specificity of P-gp (Kimchi-Sarfaty et al. 2007). Therefore, the effect of the ABCB1 genotype on the pharmacokinetics of different P-gp substrates may vary, which might also explain the lack of effect on aliskiren pharmacokinetics.

At the time that Study VI was performed, the c.935G>A SNP was the only sequence variant in the SLCO2B1 gene reported to have affected the pharmacokinetics and efficacy of an OATP2B1 substrate drug in vivo in studies involving montelukast (Mougey et al. 2009; Mougey et al. 2011). Subsequently, no relationship has been found between c.935G>A and the pharmacokinetics of celiprolol (Ieiri et al. 2012). There are no in vitro studies investigating the effect of this SNP on the expression or function of OATP2B1. The findings from Study VI do not support functional significance of the SLCO2B1 c.935G>A SNP on OATP2B1 activity in vivo. In addition to the c.935G>A SNP, various other sequence variants have been described in the SLCO2B1 gene with yet unknown clinical relevance (Nozawa et al. 2002; Ho et al. 2006a; Aoki et al. 2009; Mougey et al. 2009). Overall, further pharmacogenetic studies are needed to identify the factors explaining the large interindividual variability in the pharmacokinetics of aliskiren.
6. Clinical implications

Aliskiren proved to be susceptible to transporter-mediated pharmacokinetic interactions of clinical significance. In Studies I and II, both induction and inhibition of P-gp efflux transporter had a marked effect on the systemic exposure to aliskiren, although a minor part of the effect may also have resulted from induction and inhibition of CYP3A4. Thus, considering the pharmacokinetic and pharmacodynamic properties of aliskiren, it could be a suitable probe drug for evaluating P-gp-mediated drug interactions in vivo in humans. Studies III and IV suggested that aliskiren may also be prone to inhibition of the OATP2B1 influx transporter. However, the common haplotypes of ABCB1 gene encoding P-gp (Study V) and the polymorphism in SLCO2B1 gene encoding OATP2B1 (Study VI) were not associated with altered pharmacokinetics of aliskiren.

In all pharmacokinetic interaction studies (I-IV), the extent of the interaction showed marked interindividual variability even among young healthy volunteers. The variability is likely even larger in patient populations using aliskiren because of comorbidities, concomitant medications, and increasing age (Vaidyanathan et al. 2007b).

In Studies I, II, and IV, in line with the alterations in pharmacokinetics of aliskiren, changes in the pharmacodynamic response to aliskiren were also seen, as rifampicin, orange juice, and apple juice all attenuated and itraconazole enhanced the renin-inhibiting effect of aliskiren. Grapefruit juice, in turn, had no significant effect on plasma renin activity, although it greatly reduced the plasma aliskiren concentrations. Because of the delayed start of the blood pressure-lowering effect of aliskiren (EMA 2012; Novartis 2012), no differences in haemodynamic effects were observed between different phases or groups in these single-dose studies with healthy volunteers. In any case, the pharmacodynamic response to aliskiren may be different in hypertensive patients using aliskiren daily than in normotensive individuals (Vaidyanathan et al. 2008c; EMA 2012).

Aliskiren shows a dose-response relationship in the range of 150-300 mg, but has no clear effect on blood pressure at lower doses (Oh et al. 2007; EMA 2012). Therefore, the decreased exposure to aliskiren by rifampicin, grapefruit juice, orange juice, or apple juice may lead to a reduced antihypertensive effect of aliskiren. In clinical practice, the interaction between rifampicin and aliskiren may result in a need to adjust the aliskiren dose. In addition, the intake of aliskiren concomitantly with grapefruit,
orange, or apple juice is best avoided. Accordingly, after publishing Study III, EMA has recommended that aliskiren not be taken together with grapefruit juice (EMA 2012).

The antihypertensive efficacy of aliskiren 600 mg once daily has been similar to 300 mg once daily (Gradman et al. 2005; Weir et al. 2007). Thus, the increased systemic exposure to aliskiren by itraconazole does not necessarily result in an enhanced antihypertensive effect, but may increase the risk of adverse reactions. It is noteworthy that inhibition of P-gp by itraconazole may potentially increase the tissue permeability of aliskiren (Schinkel et al. 1997; Eyal et al. 2009). The inhibition of P-gp at the blood-brain barrier might increase brain exposure to aliskiren. The concomitant use of aliskiren with cyclosporine has been contraindicated by the EMA because of a similar pharmacokinetic interaction (EMA 2012). Thus, the concomitant use of itraconazole and aliskiren is best avoided; it has also been contraindicated by EMA after Study II was published (EMA 2012).
CONCLUSIONS

The following conclusions can be drawn on the basis of the results of these six studies:

Study I  Rifampicin, an inducer of P-gp and CYP3A4, considerably reduces the plasma concentrations and the renin-inhibiting effect of aliskiren by decreasing its oral bioavailability, and therefore, rifampicin may reduce the antihypertensive efficacy of aliskiren.

Study II  Itraconazole markedly raises the plasma concentrations of aliskiren and enhances its renin-inhibiting effect, probably mainly by inhibiting the P-gp-mediated efflux of aliskiren in the small intestine, with a minor contribution from inhibition of CYP3A4. Concomitant use of aliskiren and itraconazole is best avoided.

Study III  Grapefruit juice greatly reduces the plasma concentrations of aliskiren, probably by inhibiting its OATP2B1- and/or OATP1A2-mediated influx in the small intestine. The concomitant use of aliskiren and grapefruit juice is best avoided. To our knowledge, this is the first published study suggesting that grapefruit juice may be a clinically important inhibitor of OATP2B1.

Study IV  Orange juice and apple juice greatly reduce the plasma concentrations of aliskiren and its renin-inhibiting effect, probably by reducing its absorption from the gastrointestinal tract. The interactions may be caused by inhibition of the OATP2B1- and/or OATP1A2-mediated intestinal absorption of aliskiren by constituents of these juices, but further studies are required to clarify the mechanisms involved. Concomitant intake of aliskiren with orange or apple juice is best avoided.

Study V  The ABCB1 c.1236C-c.2677G-c.3435C and c.1236T-c.2677T-c.3435T haplotypes have no clinically meaningful effect on the pharmacokinetics or pharmacodynamics of aliskiren.

Study VI  The SLCO2B1 c.935G>A SNP does not significantly affect the pharmacokinetics or pharmacodynamics of aliskiren. Further studies are needed to clarify the possible pharmacogenetic factors explaining the considerable interindividual variability in the pharmacokinetics of aliskiren.
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