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# Effects of grapefruit juice on the pharmacokinetics of selected CYP3A4 substrate drugs

by
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ACADEMIC DISSERTATION
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**ABBREVIATIONS**

| | |
|---|---|
| ANOVA | analysis of variance |
| AUC(0-t) | area under the concentration-time curve from 0 to t hours |
| AUC(0-∞) | total area under the concentration-time curve |
| CFFT | critical flicker fusion test |
| $Cl_{oral}$ | oral clearance |
| $C_{max}$ | peak concentration in serum or plasma |
| CNS | central nervous system |
| CYP | cytochrome P-450 enzyme |
| CV | coefficient of variation |
| DSST | digit symbol substitution test |
| ECG | electrocardiogram |
| ERMBT | erythromycin breath test |
| GABA | gamma-aminobutyric acid |
| HMG-CoA | 3-hydroxy-3-methylglutaryl-coenzyme A |
| HPLC | high performance liquid chromatography |
| 5-HT | serotonin |
| $k_{el}$ | elimination rate constant |
| LC-MS-MS | liquid chromatography-tandem mass spectrometry |
| MDR | multiple drug resistance |
| mRNA | messenger ribonucleic acid |
| OC | oral contraceptive steroid |
| P-gp | P-glycoprotein |
| $QT_c$ | heart rate-corrected QT interval |
| REA | radioenzyme inhibition assay |
| SD | standard deviation |
| SEM | standard error of the mean |
| $t_{½}$ | half-life |
| t.i.d. | three times a day |
| $t_{max}$ | time of peak concentration |
| VAS | visual analogue scale |

## LIST OF ORIGINAL PUBLICATIONS

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

I Lilja JJ, Kivistö KT, Backman JT, Lamberg TS, Neuvonen PJ. Grapefruit juice substantially increases plasma concentrations of buspirone. *Clin Pharmacol Ther* 1998;64:655-60

II Kivistö KT, Lilja JJ, Backman JT, Neuvonen PJ. Repeated consumption of grapefruit juice considerably increases plasma concentrations of cisapride. *Clin Pharmacol Ther* 1999;66:448-53

III Lilja JJ, Kivistö KT, Neuvonen PJ. Grapefruit juice-simvastatin interaction: effect on serum concentrations of simvastatin, simvastatin acid, and HMG-CoA reductase inhibitors. *Clin Pharmacol Ther* 1998;64:477-83

IV Lilja JJ, Kivistö KT, Neuvonen PJ. Grapefruit juice increases serum concentrations of atorvastatin and has no effect on pravastatin. *Clin Pharmacol Ther* 1999;66:118-27

V Lilja JJ, Backman JT, Kivistö KT, Neuvonen PJ. Effect of grapefruit juice dose on grapefruit juice-triazolam interaction: repeated consumption prolongs triazolam half-life. *Eur J Clin Pharmacol* 2000;56:411-15

VI Lilja JJ, Kivistö KT, Neuvonen PJ. Duration of effect of grapefruit juice on the pharmacokinetics of the CYP3A4 substrate simvastatin. *Clin Pharmacol Ther* 2000;68:384-90

**ABSTRACT**

About ten years ago, grapefruit juice was observed to increase the plasma concentrations and effects of felodipine. Since then, grapefruit juice has been found to interact with more than 20 different drugs. However, many factors affecting the susceptibility of drugs to interaction with grapefruit juice are still unknown. In this series of investigations, the potential of grapefruit juice to interact with the CYP3A4 substrates buspirone, cisapride, simvastatin, atorvastatin, and pravastatin was studied in healthy human volunteers in randomized cross-over studies. In addition, the effect of grapefruit juice dose in pharmacokinetic interaction and the duration of grapefruit juice effect were studied, with triazolam and simvastatin, respectively, as the model drugs. In each study, 10 to 12 volunteers participated. Subjects ingested grapefruit juice 200 ml or water (control) t.i.d. for 2 days and then received a single dose of study drug with grapefruit juice or water. The effect of dose of grapefruit juice was studied by giving the volunteers the study drug with 200 ml water, normal or double-strength grapefruit juice, or on the third day of double-strength grapefruit juice, 200 ml t.i.d. Duration of effect of grapefruit juice was studied by administering the study drug after multiple-dose grapefruit juice (200 ml t.i.d. for 3 days) or 1, 3, or 7 days after the last dose of multiple-dose grapefruit juice. Following administration of the study drug, timed blood samples were taken for up to 12 to 72 hours for determination of plasma or serum drug concentrations by means of gas and liquid chromatographic, mass spectrometric, or radioenzyme inhibition assay.

Grapefruit juice substantially increased plasma concentrations of buspirone. The AUC of buspirone was increased by grapefruit juice about 9-fold ($p < 0.01$). The increased buspirone plasma concentrations were associated with increased subjective drug effect. The pharmacokinetics of cisapride were considerably affected by grapefruit juice. The AUC of cisapride was increased by about 140% by grapefruit juice ($p < 0.01$), but it did not enhance the effect of a single dose of cisapride on the $QT_c$ interval. Grapefruit juice increased the AUC of simvastatin and simvastatin acid about 16-fold ($p < 0.05$) and 7-fold ($p < 0.01$), respectively. The AUC of atorvastatin acid was increased by about 150% ($p < 0.01$). The AUC of 2-hydroxyatorvastatin acid, the main active metabolite of atorvastatin, was decreased by grapefruit juice ($p < 0.01$). Grapefruit juice did not affect the pharmacokinetics of pravastatin. All of these interactions were subject to large interindividual variation. A single dose of either normal- or double-strength grapefruit juice and the multiple dose of double-strength grapefruit

juice increased triazolam AUC by about 50% ($p < 0.01$) and 140% ($p < 0.001$), respectively. Multiple-dose ingestion of grapefruit juice affected triazolam pharmacokinetics significantly more than did a single dose of normal- or double-strength grapefruit juice ($p < 0.001$). The elimination $t_{½}$ of triazolam was significantly prolonged by multiple dose ($p < 0.001$), but not by a single dose of grapefruit juice. Triazolam effects were increased by multiple-dose grapefruit juice. The effect of grapefruit juice on the AUC of simvastatin was approximately one-tenth of its maximum 24 hours after termination of repeated intake of grapefruit juice. The effect of grapefruit juice on the pharmacokinetics of simvastatin disappeared within 3 to 7 days.

It is probable that in these studies the pharmacokinetic interactions between grapefruit juice and CYP3A4 substrates mostly occurred during their first pass, because both the $C_{max}$ and AUC values substantially increased. However, it appears that the systemic elimination as well may be inhibited by multiple-dose grapefruit juice, as evidenced by significantly increased elimination $t_{½}$ of some of the drugs. Susceptibility of CYP3A4 substrates to interaction with grapefruit juice reflects the extent of their first-pass metabolism. This long-lasting effect of grapefruit juice is in line with the proposal that the mechanism of action of grapefruit juice is irreversible inactivation of CYP3A4. However, other mechanisms such as modulation of function of P-glycoprotein may have contributed. Large interindividual variation in pharmacokinetics of the CYP3A4 substrates observed in these studies makes prediction of the magnitude of interaction for an individual difficult. Concomitant ingestion of grapefruit juice with CYP3A4 substrates with extensive first-pass metabolism and a narrow therapeutic range can increase the risk for adverse effects of these drugs.

## INTRODUCTION

Drugs and other exogenous lipophilic compounds usually have to be metabolized before they can be excreted from the body. This metabolism occurs by phase I and phase II reactions. In phase I, the majority of drugs undergo biotransformation reactions that are generally mediated by CYP enzymes, oxidative reactions being the most common. In phase II reactions, drug molecules or their metabolites are usually conjugated. There is considerable interindividual variation in activity of drug-metabolizing enzymes caused by both genetic and exogenous factors. Concomitant administration of two or more drugs may alter their pharmacokinetics, especially if they are metabolized by the same isoenzyme. There are many well-characterized pharmacokinetic drug-drug interactions which can have highly significant clinical consequences due to increased therapeutic effects or toxicity (Olkkola et al. 1993; Neuvonen and Jalava 1996). Smoking and dietary factors can also alter significantly drug metabolism (Conney et al. 1977; Grygiel and Birkett 1981; Singh 1999).

A serendipitous finding about 10 years ago in a clinical drug study, when grapefruit juice was used to mask the taste of ethanol, led to the discovery of a grapefruit juice-felodipine interaction. Felodipine is a dihydropyridine calcium-channel antagonist used for the treatment of hypertension. Simultaneous administration of felodipine with a single glass of grapefruit juice increased significantly the plasma concentration of felodipine and also increased its hypotensive effect (Bailey et al. 1991). Since then, grapefruit juice has been shown to increase plasma concentrations of several drugs, e.g., cyclosporin, midazolam, and terfenadine (Yee et al. 1995; Kupferschmidt et al. 1995; Benton et al. 1996). In some instances, coadministration of drugs with grapefruit juice has increased significantly the intensity and duration of effects of these drugs. Most of the drugs reported to be susceptible to interaction with grapefruit juice have variable oral bioavailability due to moderate or extensive first-pass metabolism. Almost exclusively, these drugs are also substrates for CYP3A4, the most important drug-metabolizing member of the CYP enzyme family that participates in the metabolism of about 50% of drugs (Wrighton et al. 1992). However, relatively few published studies adress the question of the dose-relationship of the grapefruit juice effect and duration of the effect of grapefruit juice. In this series of investigations, the purpose was to study to what degree grapefruit juice can interfere with the pharmacokinetics of drugs metabolized to varying degrees by CYP3A4, and also to discover factors governing these interactions.

# REVIEW OF THE LITERATURE

## 1. Drug metabolism and interactions

### 1.1. Cytochrome P450 (CYP) enzymes and drug interactions

Drug molecules are often lipophilic, which allows their absorption from the gastrointestinal tract and also their entry to the site of action, e.g., in the brain. Excretion of drugs and other, perhaps toxic, lipophilic foreign compounds (xenobiotics) from the body in the bile and urine is facilitated by metabolic reactions that transform them into being more hydrophilic, i.e., soluble in water (Morselli 1995). These reactions comprise oxidation, hydroxylation, dealkylation, and reduction. They are called phase I reactions. In phase II reactions, drug molecules or their metabolites are conjugated with endogenous molecules such as glucuronate, sulfate, acetate, or an amino acid. Usually drugs undergo reactions of both phases before their elimination. Drug metabolism occurs mainly in the liver, although organs such as the gastrointestinal tract, kidneys, and lungs also contribute to biotransformation reactions (Krishna and Klotz 1994).

Orally administered drugs must traverse the intestinal wall, the hepatic portal system, and the liver before they reach the systemic circulation. If a drug is subject to extensive biotransformation in the liver, only a fraction of its dose will reach the systemic circulation and, ultimately, its site of action. This phenomenon is referred to as first-pass metabolism. By the term *extraction ratio* is meant the fraction of drug removed from the blood during a single transit through the liver. Correspondingly, drugs that undergo extensive first-pass metabolism have high extraction ratios (i.e. > 0.7). First-pass metabolism can reduce in a significant way the bioavailability of some drugs, despite complete absorption. As well as in the liver, orally administered drugs can be subject to metabolism in the intestinal wall before they reach the systemic circulation. It has been shown that for some drugs (mostly CYP3A4 substrates) presystemic metabolism in the intestine can be a significant determinant of their bioavailability (Paine et al. 1996; Thummel et al. 1996).

Drugs and other xenobiotics are mainly transformed in phase I reactions by human cytochrome P-450 enzymes (CYP enzymes). CYP enzymes constitute an enzyme superfamily of hem-containing mono-oxygenases, which have an absorption maximum at wavelength 450 nm in

the presence of carbon monoxide; hence the name P-450. Besides drug metabolism, CYP enzymes are important in the synthesis and metabolism of different endogenous compounds like steroid hormones, fatty acids, arachidonic acid, and bile acids. The classification of CYP enzymes is based on homology of their deduced amino acid sequences (Nelson et al. 1996). Within a single CYP family, enzymes show amino acid sequence homology greater than 40% and are denoted in the nomenclature by a common Arabic number (e. g., CYP3). Within one subfamily, enzymes share a homology greater than 55% and are denoted by a common letter (e. g., CYP3A). Single isoforms are labeled with a second Arabic number (e. g., CYP3A4).

In humans, 14 CYP gene families are known to date, of which the first three families are important in the metabolism of drugs and xenobiotics. The other CYP gene families are responsible for the biotransformation of endogenous compounds like steroids.

Human CYP1A1 demonstrates almost 70% sequence similarity to CYP1A2. In humans, CYP1A1 has been found almost exclusively in extrahepatic tissues such as lung, small intestine, and placenta (Pasanen et al. 1988; Shimada et al. 1992; McLemore et al. 1994). Without induction, it is often not expressed. It is induced by cigarette smoke and polycyclic aromatic hydrocarbons (PAHs) (Wrighton and Stevens 1992). A prototype substrate for CYP1A1 is benzo(a) pyrene (Shimada et al. 1989). Unlike CYP1A1, in humans CYP1A2 appears to be present only in the liver, where it represents 10 to 15% of the total amount of CYP (Shimada et al. 1994). It catalyzes 3-demethylation of caffeine and is also responsible for L-demethylation of theophylline and O-de-ethylation of phenacetin (Butler et al. 1989; Sesardic et al. 1990; Brosen et al. 1993). Most substrates of CYP1A1 appear also to be substrates of CYP1A2 (Tassaneeyakul et al. 1993), which is induced by cigarette smoke, physical exercise, charcoal broiled meat, or cruciferous vegetables (Wrighton and Stevens 1992). Human CYP1A2 is inhibited by α-naphtoflavone, 7-ethoxycoumarin, furafylline, and fluvoxamine (Tassaneeyakul et al. 1993; Jeppesen et al. 1996).

In humans at least four members of the CYP2C subfamily are expressed, and they participate in drug metabolism, namely, CYP2C8, CYP2C9, CYP2C18, and CYP2C19. After the CYP3A subfamily, the CYP2C enzymes form the second largest CYP subfamily in human liver, comprising 20% of its total amount of CYP protein (Shimada et al. 1994). CYP2C enzymes are also expressed to a minor extent in the small intestine (Zhang et al. 1999). CYP2C8 catalyzes

the biotransformation of retinal, retinoic acid, and taxol (Leo et al. 1989; Rahman et al. 1994). CYP2C9 metabolizes phenytoin, tolbutamide, warfarin, and NSAIDs (Knodell et al. 1987; Veronese et al. 1991; Rettie et al. 1992; Leemann et al. 1993). Both CYP2C8 and CYP2C9 are induced by rifampicin and phenytoin. CYP2C9 is inhibited by sulphaphenazole, fluconazole, amiodarone, and metronidazole (Back 1988). CYP2C18 catalyzes biotransformation of such drugs as diazepam. There is large interindividual variability in drug-metabolizing capacity mediated by CYP2C19 that is caused by genetic polymorphisms. About 3% of Caucasians and 20% of Asians are poor metabolizers of CYP2C19 substrates (Wedlund et al. 1984; Nakamura et al. 1985). CYP2C19 metabolizes a wide variety of drugs, such as mephenytoin, amitriptyline, imipramine, and diazepam (Breyer-Pfaff et al. 1992; Skjelbo et al. 1993; Bertilsson et al. 1989). Fluvoxamine and fluoxetine are inhibitors of CYP2C19 (Jeppesen et al. 1996).

Expression of CYP2D6 has been shown to be determined by genetic polymorphisms (Bertilsson 1995). About 7% of Caucasians and 1% of Asian populations are poor metabolizers of CYP2D6 substrates. Although CYP2D6 constitutes only about 2% of the hepatic CYP protein, it participates in the biotransformation of a range of widely used drugs such as tricyclic antidepressants, neuroleptics, and β-blockers (Cholerton et al. 1992). CYP2D6 is not inducible, but can be inhibited by such drugs as quinidine, paroxetine and fluoxetine (Inaba et al. 1986; Jeppesen et al. 1996).

The subfamily CYP3A consists of at least three functional human genes: CYP3A4, CYP3A5, and CYP3A7. It comprises about 30% of the total hepatic protein and is also the most prevalent CYP enzyme in the small intestine (Shimada et al. 1994; Paine et al. 1997). In humans, CYP3A4 is both qualitatively and quantitatively the most important CYP isoform in drug metabolism. Besides the liver, it is abundantly expressed in the duodenum, jejunum, and ileum where it represents a respective 63%, 49%, and 88% of total CYP protein (Paine et al. 1997). It has been shown that the cDNAs of intestinally and hepatically expressed forms of human CYP3A4 are identical (Lown et al. 1998); however, they are possibly not co-regulated (Lown et al. 1994). An at least 10-fold interindividual variation has been found in the amount of CYP3A4 both in the liver and in the small intestine (Lown et al. 1994). The molecular basis for this wide interindividual variation in expression and function has thus far remained unexplained. In some but not all studies, females have shown greater CYP3A4 activity than

males (Watkins et al. 1989; Kashuba et al. 1998). Some studies suggest that the phases of the menstrual cycle have no effect on the pharmacokinetics of CYP3A4 substrates (Kashuba et al. 1998; Kamimori et al. 2000).

CYP3A5 shows a sequence homology of 84% with CYP3A4, but unlike the latter it is polymorphically expressed. In the majority of humans, CYP3A5 is expressed in the liver and small intestine at low levels, but it is the major CYP3A in the colon (Kolars et al. 1994). CYP3A5 has relatively widely overlapping substrate specificity with CYP3A4 (Wrighton et al. 1990).

CYP3A7 is the major CYP3A isoform in the fetal liver, but CYP3A7 mRNA has been found also in adult livers (Hakkola et al. 1998).

In humans, CYP3A4 catalyzes the biotransformation of a very large number of drugs that are mostly lipohilic but otherwise often very dissimilar structurally. The list of CYP3A4 substrates includes, from different therapeutic areas: midazolam (Gorski et al. 1994), triazolam (Kronbach et al. 1989), cyclosporin (Kronbach et al. 1988), diltiazem (Pichard et al. 1990), dihydropyridine calcium-channel antagonists (Guengerich et al. 1991), lovastatin (Wang et al. 1991), simvastatin (Prueksaritanont et al. 1997), cisapride (Bohets et al. 2000), erythromycin (Hunt et al. 1992), saquinavir (Fitzsimmons and Collins 1997), and itraconazole (Ducharme et al. 1995a). CYP3A4 is inhibited for instance by such drugs as itraconazole (Back and Tjia 1991; Olkkola et al. 1994; Neuvonen and Jalava 1996), erythromycin (Olkkola et al. 1993), diltiazem (Backman et al. 1994), verapamil, and ritonavir (Eagling et al. 1997). Prototype inducers of CYP3A4 include rifampicin, phenytoin, and carbamazepine (Backman et al. 1996; Bertilsson et al. 1997; Capewell et al. 1988).

### 1.2. Mechanisms of CYP inhibition

Drugs and chemicals may cause reversible or irreversible inhibition of CYP enzymes and thus prevent biotransformation of substrates. In reversible inhibition, a preferentially lipophilic compound binds tightly within the active site of the enzyme. This prevents binding of other substrate molecules to active hydrophobic regions on the CYP apoprotein or oxygen activation

by CYP heme, both actions resulting in transient although sometimes potent inhibition of enzyme activity (Murray 1999). Competitive inhibitors are often but not necessarily substrates for the enzyme they inhibit. Typical competitive inhibitors of CYP enzymes include the imidazole antifungals ketoconazole and itraconazole and the protease inhibitor, ritonavir (Back and Tjia 1991; Eagling et al. 1997)

Irreversible inhibition of CYP enzymes, also called mechanism-based inhibition, can be divided into two categories: autocatalytic inactivation and metabolite intermediate (MI)-complexation. Autocatalytic inactivation or suicide inhibition takes place when a reactive drug metabolite, formed in previous catalytic steps by a CYP enzyme, binds to this enzyme and alters its structure, resulting in irreversible loss of function. This can lead to rapid destruction of enzyme protein. Gestodene (Guengerich 1990a), ethinyl estradiol (Guengerich 1988), spironolactone (Decker et al. 1989), furanocoumarin methoxsalen (Tinel et al. 1987), and furafylline (Kunze and Trager 1993) are examples of suicide inhibitors. Alkylamines are probably the most significant class of compounds able to cause irreversible inhibition of CYPs through complexation of a metabolite intermediate with an enzyme. Putative oxidized metabolite intermediates include nitroso analogues of substrate molecules. These analogues bind tightly to the CYP heme, preventing oxygen binding and activation. Elicited inhibition can be protracted although CYP protein is not degraded. The macrolide antibiotics erythromycin and troleandomycin are known inhibitors of CYPs that form MI-complexes (Periti et al. 1992).

### 1.3. P-glycoprotein

P-glycoproteins (P-gp) are plasma membrane glycoproteins that belong to the superfamily of ATP-binding cassette (ABC) transporters (Preiss 1998). They mediate the transport of xenobiotics and endogenous substrates from the inner to the outer surface of cell membranes. P-glycoproteins can be expressed in the luminal surfaces of the small and large intestine, biliary tract, hepatocytes, endothelial cells that contribute to the blood brain barrier, and the proximal tubule of the kidneys (Lum and Gosland 1995). A possible physiological role for P-gp is to secrete endogenous and xenobiotic compounds and thus contribute to protective barrier mechanisms. P-gp has also been found to be a cause for MDR (multiple drug resistance) as it is overexpressed in cancer cells that are resistant to chemotherapeutic agents. In these cells, P-gp

functions as an efflux pump that decreases intracellular drug concentrations and cytotoxicity (Lum and Gosland 1995). P-gp and CYP3A4 have widely overlapping substrate specificity (Wacher et al. 1995). Many of the inhibitors and inducers of CYP3A4 are also capable of altering the activity of P-gp (Relling 1996; Schuetz et al. 1996).

## 2. Grapefruit juice

### 2.1. Effects of grapefruit juice on drug metabolism and its mechanism of action

Approximately 10 years ago, Bailey et al. reported on the first grapefruit juice-drug interaction observed between grapefruit juice and felodipine, a dihydropyridine Ca-channel blocker (Bailey et al. 1991). This was recognized as a chance finding during an ethanol-felodipine interaction study. In a study with six borderline hypertensive patients, 250 ml double-strength grapefruit juice, but not orange juice, increased three-fold the mean plasma felodipine area under the curve (AUC) compared with the AUC achieved with water (Bailey et al. 1991). Grapefruit juice elevated the peak plasma concentration ($C_{max}$) of felodipine, whereas the elimination $t_{½}$ remained unchanged. The AUC of dehydrofelodipine, the primary metabolite of felodipine, was also elevated by grapefruit juice. However, the dehydrofelodipine/felodipine AUC ratio was lower with grapefruit juice than with water.

Felodipine is usually completely absorbed from the gastrointestinal tract (Edgar et al. 1985). However, due to its presystemic metabolism, its absolute bioavailability is about 15%, with considerable interindividual variation. Oxidation of felodipine into dehydrofelodipine and that of dehydrofelodipine into a secondary metabolite are both catalyzed by the CYP3A4 that is abundantly expressed both in the apical enterocytes of the small bowel and in the hepatocytes of the liver. Thus, the results from Bailey et al. indicate that grapefruit juice inhibits both the primary metabolic pathway of felodipine and the subsequent metabolic pathway of dehydrofelodipine. In addition to the plasma concentrations of felodipine, also the pharmacodynamic effects of felodipine: blood pressure reduction, heart rate increase, and frequency of vasodilatation-related adverse effects, were greater during the grapefruit juice phase (Bailey et al. 1991). Because grapefruit juice did not alter intravenous felodipine pharmacokinetics, it was hypothesized that the interaction with grapefruit juice results from

inhibition of presystemic drug metabolism (Lundahl et al. 1997). Thus, it seemed that the main mechanism of action of grapefruit juice is inhibition of the CYP3A4-mediated first-pass metabolism in the intestinal wall.

Recently, results of an investigation by Lown et al. (1997) gave further support to this hypothesis. In this *in vivo* study, consumption of grapefruit juice t.i.d. for 5 days resulted in a mean 62% reduction in small intestinal enterocyte CYP3A4 protein content and a 3-fold and 5-fold increase in felodipine AUC and $C_{max}$, respectively. In contrast, no changes occurred in small-intestine levels of CYP3A4 mRNA, or liver CYP3A4 activity as measured by the erythromycin breath test (ERMBT), in small-intestine levels of P-gp, or in colon levels of CYP3A5. Further, intestinal CYP2D6 and CYP1A1 protein content remained unaltered. It was concluded from the decreased CYP3A4 expression in the gut wall that the grapefruit-juice effect is not only based on competitive inhibition. Because small intestine CYP3A4 mRNA was not altered, grapefruit juice probably reduced CYP3A4 protein content by a post-transcriptional mechanism, possibly by accelerated CYP3A4 degradation through mechanism-based (suicide) enzyme inhibition. Thus, the restoration of CYP3A4 activity would require *de novo* enzyme synthesis (Lown et al. 1997). This is in line with results of a study by Lundahl et al. (1995) which showed the effect of a single dose of grapefruit juice on felodipine pharmacokinetics to be still present, even when the juice was ingested 24 hours before the drug.

Although grapefruit juice did not affect concentrations of P-gp in the small intestine it may modify in some other way the function of this transporter. Grapefruit juice components have been shown to inhibit the P-gp function in Caco-2 cells *in vitro*, and it has also been suggested that inhibition of P-gp may be responsible for the effect of grapefruit juice on cyclosporine pharmacokinetics *in vivo* (Takanaga et al. 1998; Edwards et al. 1999).

### 2.2. Active ingredients in grapefruit juice

Grapefruit juice (*Citrus paradisi*) contains an abundance of structurally differing flavonoids, several of which are found also in other plants and fruits. The most prevalent flavonoid in grapefruit juice is naringin, which is responsible for the bitter taste, and the concentrations of which are reported to range from 100 to 800 mg/l, but usually reach 200 mg/l to 500 mg/l in

commercial grapefruit-juice preparations (Ameer et al. 1996; Hagen et al. 1965; Kupferschmidt et al. 1995). Other flavonoids present mainly as glycosides in grapefruit juice include narirutin, hesperidin, quercetin, kaempferol, and apigenin (Guengerich et al. 1990b).

Naringin and its aglycone naringenin are known competitive inhibitors of CYP3A4-mediated drug metabolism in human liver microsomes (Guengerich et al. 1990b; Miniscalco et al. 1992). This fact, together with the high content of naringin in grapefruit juice, led to the proposal that this flavonoid is the active component. Moreover, naringin is absent from orange juice, which leaves unaffected the pharmacokinetics of felodipine or cyclosporin (Bailey et al. 1991; Yee et al. 1995). It is suggested that the aglycone form of naringin, naringenin, is produced from naringin by enzymatic cleavage of the sugar moiety, possibly in the small intestine, and subsequently is glucuronized (Fuhr and Kummert 1995). *In vitro*, naringenin is more potent as an inhibitor of drug metabolism than is naringin. In *in vivo* studies, naringenin was not found in plasma, and the amount excreted renally was small relative to the administered naringin dose, whereas the amount of naringenin glucuronide was higher in plasma and urine. It was suggested that the inhibitory effect of this grapefruit juice flavonoid localizes in the small intestine. However, naringin administered as an aqueous solution or in an encapsulated preparation in the amount found in grapefruit juice did not affect the pharmacokinetics of dihydropyridines *in vivo* (Bailey et al. 1993a; Bailey et al. 1993b). Quercetin is another flavonoid compound in grapefruit juice that acts as a potent CYP3A4 inhibitor *in vitro*. However, it is not specific for grapefruit juice. Furthermore, *in vivo* it did not affect the pharmacokinetics of nifedipine (Rashid et al. 1993).

Recently, it has been suggested that furanocoumarins (psoralens) are the active ingredients in grapefruit juice. Edwards et al. (1996) demonstrated that 6',7'-dihydroxybergamottin, the major furanocoumarin in grapefruit juice (extractable in methylene chloride) can inhibit CYP3A4-mediated 6β-hydroxytestosterone formation in rat microsomes. A subsequent study showed that 6',7'-dihydroxybergamottin causes a dose-dependent fall in human CYP3A4 catalytic activity and immunoreactive CYP3A4 concentration (Schmiedlin-Ren et al. 1997). These results are in line with those of the study of Lown et al. (1997), in which grapefruit juice caused down-regulation of the small intestinal CYP3A4, probably due to suicide inhibition. Furthermore, Schmiedlin-Ren et al. (1997) found that, although the concentration of 6',7'-dihydroxybergamottin in grapefruit juice varies significantly among different grapefruit juice

preparations, it exceeds the $IC_{50}$ for midazolam 1'-OH formation. Bailey et al. (1998) performed a study in which grapefruit juice was separated into supernatant and particulate fractions, which were then assayed for naringin and 6',7'-dihydroxybergamottin. The amounts of naringin and 6',7'-dihydroxybergamottin were higher in the supernatant than in the particulate fraction. However, after oral coadministration of felodipine with these fractions, the particulate fraction had a significantly greater AUC for felodipine than did the supernatant fraction. The authors concluded that neither naringin nor 6',7'-dihydroxybergamottin is the major active ingredient in grapefruit juice, although each may contribute to the interaction.

He et al. (1998) showed that bergamottin, a major furanocoumarin in grapefruit juice, may act as a mechanism-based inhibitor of CYP3A4. The inhibition of CYP3A4 required metabolism of bergamottin and appeared to involve modification of apoprotein rather than either modification of the heme or heme fragmentation. Recently, it has been demonstrated that grapefruit juice contains furanocoumarin dimers that are potent inhibitors of CYP3A4 (Fukuda et al. 1997; Guo et al. 2000). Both competitive and mechanism-based inhibition seem to be involved. Although the concentrations of these dimers plus an epoxide of bergamottin in grapefruit juice are lower than those of the monomers (bergamottin or 6',7'-dihydroxybergamottin), their contributions to the interaction appear comparable, due to the higher inhibitory potencies of the dimers relative to those of the monomers against microsomal CYP3A activity (Guo et al. 2000). Furthermore, these furanocoumarins reside in the precipitate of grapefruit juice, a fact in line with results observed *in vivo* (Bailey et al. 1998). The grapefruit-juice effect seems to be dependent on the presence of all the furanocoumarin components and is weaker if some of them are absent from the juice (Guo et al. 2000).

### 2.3. Interactions between grapefruit juice and drugs

Almost exclusively, all drugs shown to have a pharmacokinetic interaction with grapefruit juice are CYP3A4 substrates. In the first reported grapefruit juice-drug interaction, the drug under study was felodipine. Since then, more grapefruit juice interaction studies have been conducted with felodipine than with any other drug (Fuhr 1998; Bailey et al. 1998). In studies in which felodipine has been coadministered with a single dose of grapefruit juice, the mean increase in

the AUC of felodipine has ranged from 43 to 234% (Fuhr 1998; Edgar et al. 1992; Lundahl et al. 1995). The mean increase in felodipine $C_{max}$ has ranged from 70 to 225%.

In all these studies, grapefruit juice has not affected the $t_{max}$ in a consistent manner. The elimination $t_{½}$ of felodipine has usually not been changed by grapefruit juice. In those studies where measured, grapefruit juice has increased concentrations of the main metabolite of felodipine, dehydrofelodipine, although to a lesser degree than concentrations of the parent compound (Edgar et al. 1992; Bailey et al. 1993a; Bailey et al. 1995).

In a study by Lown et al. (1997), repeated consumption of grapefruit juice increased the AUC and $C_{max}$ of felodipine by 211% and 335%, respectively. Increased felodipine concentrations due to ingestion of grapefruit juice have been associated with increased effects of felodipine. Thus, a pronounced blood pressure-lowering or heart rate-increasing effect of felodipine, or both have been observed when hemodynamic monitoring has been performed (Bailey et al. 1991; Edgar et al. 1992). Coadministration of felodipine with grapefruit juice has also resulted in increased incidence of adverse effects such as headache and flushing (Edgar et al. 1992; Bailey et al. 1991).

In addition to felodipine, some other dihydropyridine calcium-channel antagonists have been found to interact significantly with grapefruit juice. The $C_{max}$ and AUC of nisoldipine were increased by 306% and 98%, respectively, by double-strength grapefruit juice 250 ml (Bailey et al. 1993b). High plasma concentrations of nisoldipine with grapefruit juice did not result in significant effects on blood pressure and produced only a slightly higher heart rate than did its administration with water at 4 hours. The AUC of nifedipine after coadministration with grapefruit juice was 134% of that with water (Bailey et al. 1991). Pharmacodynamic effects of nifedipine were not changed significantly by grapefruit juice. A single dose of grapefruit juice increased the AUC of nitrendipine by 106% (Soons et al. 1991) but did not alter its hemodynamic effects. The $C_{max}$ and AUC of nimodipine were increased by 24% and 51% by a single dose of grapefruit juice (Fuhr et al. 1998). Grapefruit juice 250 ml induced minor changes in the pharmacokinetics of amlodipine but did not significantly affect its hemodynamic effects (Josefsson et al. 1996). A single dose of grapefruit juice was not found to change significantly the pharmacokinetics of verapamil (Zaidenstein et al. 1998). In a study by Sigusch

et al. (1994), repeated doses of grapefruit juice (200 ml at 0, 2, 4, 8, and 12 h) did not significantly change the AUC of diltiazem.

Cyclosporine is an immunosuppressive agent with a narrow therapeutic range and variable oral bioavailability. It is a substrate for CYP3A4 (Kronbach et al. 1988). The majority of interaction studies between cyclosporine and grapefruit juice have been performed with patients who take cyclosporine on a regular basis. In young, healthy adults, a single dose of grapefruit juice increased cyclosporine AUC and $C_{max}$ by 43% and 18%, respectively, after oral administration (Yee et al. 1995). In a study by Ducharme et al. (1995b), a single dose of grapefruit juice given twice increased cyclosporine AUC significantly after oral administration but not after intravenous administration. Grapefruit juice given at 3-hour intervals for a period of 30 hours increased the $C_{max}$ of cyclosporine by 22% in kidney transplant patients (Hollander et al. 1995). In a study by Proppe et al. (1995), a mean increase of 77% and of 62% in the trough concentrations of cyclosporine and its metabolites was observed after administration of grapefruit juice 175 ml at an interval of 12 hours. Grapefruit juice increased significantly the AUC of both cyclosporine and its metabolites in patients with autoimmune diseases (Ioannides-Demos et al. 1997).

The non-sedating antihistamine terfenadine is a prodrug that has extensive CYP3A4-mediated first-pass metabolism. The parent terfenadine can prolong the QT interval, which may result in a life-threatening ventricular arrhythmia, torsades de pointes. In a study by Benton et al. (1996), 12 healthy volunteers received terfenadine 60 mg twice daily for 7 days. They were then randomized to ingest grapefruit juice 250 ml with terfenadine twice daily or 2 hours after drug for an additional 7 days. The AUC of the terfenadine acid metabolite increased 55% in the simultaneous and 22% in the delayed group. The mean QT interval increased significantly in the simultaneous group only. In another study, poor metabolizers of terfenadine received first terfenadine 60 mg twice daily for 7 days and then terfenadine twice daily with grapefruit juice (Honig et al. 1996). Coadministration of terfenadine with grapefruit juice resulted in accumulation of parent terfenadine, and QT interval was significantly prolongated after coingestion as compared with the baseline without terfenadine. In a randomized study in healthy volunteers, a single dose of grapefruit juice raised the AUC and $C_{max}$ of terfenadine carboxylate (Rau et al. 1997); the mean QT interval was not changed.

Cisapride is a widely used gastrointestinal prokinetic agent that can cause prolongation of QT interval. Coadministration of a single dose of cisapride with grapefruit juice increased cisapride AUC by about 50% (Gross et al. 1999).

Midazolam and triazolam are short-acting benzodiazepine derivatives that have oral bioavailability of 40 to 50%. Grapefruit juice 200 ml ingested 60 minutes and 15 minutes before orally administered midazolam increased the AUC of midazolam by about 50% and enhanced the pharmacodynamic effects of midazolam (Kupferschmidt et al. 1995). A single dose of grapefruit juice increased triazolam AUC by approximately 50% and slightly increased the effects of triazolam (Hukkinen et al. 1995). Vanakoski et al. (1996) concluded that grapefruit juice may not have an important interaction with midazolam or triazolam.

Coadministration of estradiol derivatives with grapefruit juice has increased significantly concentrations of the parent drug (Weber et al. 1996) or those of estrogen metabolites (Schubert et al. 1995). Grapefruit juice has not affected the AUC of prednisone or prednisolone (Hollander et al. 1995). However, methylprednisolone AUC was increased by 75% by grapefruit juice (Varis et al. 2000).

The pharmacokinetics of lovastatin, an HMG-CoA reductase inhibitor, were considerably changed after coadministration with grapefruit juice. The mean $C_{max}$ and AUC of lovastatin were increased approximately 12-fold and 15-fold by grapefruit juice given t.i.d. for 3 days (Kantola et al. 1998a). Saquinavir is a potent HIV protease inhibitor whose effectiveness is limited by its low and variable oral bioavailability. Its AUC was increased by 50% by grapefruit juice 200 ml given twice before administration of this drug (Kupferschmidt et al. 1998).

Grapefruit juice 300 ml increased the AUC(0-8) of carbamazepine by about 41% in epileptic patients (Garg et al. 1998). Grapefruit juice has been found to reduce oral clearance of the CYP1A2 substrate caffeine (Fuhr et al. 1993). However, pharmacokinetics of another CYP1A2 substrate, theophylline, remained unaffected (Fuhr et al. 1995).

In the grapefruit juice-drug studies performed, type (fresh squeezed vs. reconstituted frozen concentrate), concentration (normal vs. double strength), volume and scheme of administration

(single dose vs. repeated doses) of grapefruit juice have varied. Also, with respect to administration of study drug, the protocols have not been uniform. Most studies utilize a single drug dose. However, in some studies drugs have been given to reach a steady state. This lack of standardization of experimental conditions makes comparison of study results difficult.

## 3. CYP3A4 substrates studied

### 3.1. Buspirone

Buspirone is an anxiolytic agent structurally different from the benzodiazepines; it is an azaspirodecanedione derivative. Buspirone has been shown to be equipotent as an anxiolytic with benzodiazepines, but it does not produce sedation, motor impairment, or muscle relaxation (Goldberg and Finnerty 1979; Cohn and Wilcox 1986; Seppälä et al. 1982; Greenblatt et al. 1994). Buspirone lacks the potential for abuse or dependence in humans (Cole et al. 1982; Balster 1990; Sellers et al. 1992). Its most common adverse-effects are dizziness, drowsiness, gastrointestinal complaints, and headache (Newton et al. 1986).

It has been suggested that buspirone acts as a full or partial agonist for 5-$HT_{1A}$ receptors (Eison and Temple 1986; Taylor 1988). Like serotonin, buspirone inhibits spontaneous firing of serotonergic neurons in dorsal raphe nucleus and hippocampal slice preparations. In animal studies, the anticonflict activity of buspirone has been shown to be abolished when the serotonergic system is damaged. Unlike benzodiazepines, buspirone does not act by increasing binding of GABA on the GABA receptors, and it has only a modest effect on the dopaminergic system (Cimino et al. 1993; Taylor 1988). Buspirone can also increase the spontaneous firing of noradrenergic neurons in the locus coerulaeus (Eison and Temple 1986).

Buspirone is rapidly and completely absorbed after oral administration (Mayol et al. 1985). Time to reach the $C_{max}$ of buspirone is less than an hour (Gammans et al. 1986). Its systemic bioavailability is low: less than 5% of an oral dose reaches the systemic circulation; this fact, together with its good absorption, implies that it is subject to extensive first-pass metabolism (Mayol et al. 1985). Buspirone is a lipophilic drug with an apparent volume of distribution of 5.3 l/kg. It is highly, over 95%, bound to plasma proteins, both albumin and $\alpha_1$-acid

glycoprotein (Gammans et al. 1986). Its mean elimination $t_{½}$ is about 2.5 hours (Mayol et al. 1985). No statistically significant differences in the pharmacokinetics of buspirone have been found between any age- or sex groups (Gammans et al. 1986). Plasma concentrations of buspirone are higher in patients with renal impairment than in healthy volunteers (Barbhaiya et al. 1994), and patients with hepatic impairment have significantly increased AUC values and significantly prolonged elimination half-lives compared to those of normal subjects (Barbhaiya et al. 1994).

The major routes of biotransformation of buspirone include hydroxylation on the spiro and pyrimidine rings and N-dealkylation of the butyl-substituted side chain (Jajoo et al. 1989; Jajoo et al. 1990). Data concerning specific CYP enzymes involved in its biotransformation are scarce, but the *in vivo* interaction profile of buspirone indicates that CYP3A4 is the major CYP involved in its metabolism (Kivistö et al. 1997; Lamberg et al. 1998a). Plasma concentrations of two primary metabolites of buspirone, 5-OH-buspirone and 1-PP (1-pyrimidinylpiperazine), can exceed those of the parent drug (Gammans et al. 1986). Of the two metabolites, 5-OH-buspirone seems pharmacologically inactive, but 1-PP is about 20% as active as its parent drug.

Relatively few pharmacokinetic studies involve the interactions of buspirone with other drugs. In a study by Seppälä et al. (1982), alcohol does not interact significantly with buspirone. Food has been shown to increase the AUC of buspirone by 80%, and it appears that this is due to reduced first-pass metabolism, as the sum of buspirone and total (free and conjugated) 5-hydroxybuspirone remained unchanged (Gammans et al. 1986). Gammans et al. (1987) studied the effect of cimetidine on the pharmacokinetics of buspirone, finding no significant interaction between these two drugs. Alprazolam and buspirone have no significant effects on each other's pharmacokinetics (Buch et al. 1993). Buspirone does not markedly affect the pharmacokinetics of haloperidol (Huang et al. 1996). A 4-day pretreatment with itraconazole 200 mg/day or erythromycin 1.5 g/day increased the AUC values of buspirone about 19-fold and 6-fold (Kivistö et al. 1997). Lamberg and coworkers (1998a) found that a 3-day pretreatment with verapamil 80 mg/day or diltiazem 60 mg/day increased the AUC of buspirone 3.4-fold and 5.5-fold. Pretreatment with fluvoxamine moderately increased plasma buspirone concentrations (Lamberg et al. 1998b), whereas the antihistamine terfenadine had no significant effect on its pharmacokinetics (Lamberg et al. 1999). Rifampicin, a potent CYP-inducer, has reduced buspirone AUC by about 90% (Lamberg et al. 1998c).

### 3.2. Cisapride

Cisapride is a widely used gastrointestinal prokinetic agent indicated for the treatment of reflux esophagitis, the symptomatic management of dyspepsia, and the relief of gastric symptoms associated with diabetes mellitus, systemic sclerosis, and autonomic neuropathy (McCallum 1991). It is structurally a substituted piperidinyl benzamide that is chemically related to metoclopramide (McCallum 1991). It has been suggested that cisapride interacts with 5-hydroxytryptamine receptors. It differs from other prokinetic agents in that it has no antidopaminergic properties. It has been suggested that it exerts its effect by increasing the physiologic release of acetylcholine from postganglionic nerve-endings of the myenteric plexus, which leads to improved propulsive motor activity of the esophagus, stomach, small bowel, and large bowel (McCallum 1991).

Cisapride is well absorbed, and its peak plasma concentration is reached within 2 hours of oral dosing (Van Peer et al. 1986). However, its absolute bioavailability is estimated at 40 to 50%, indicating first-pass metabolism (Van Peer et al. 1986). Cisapride is 98% bound to plasma proteins, and its volume of distribution is about 2 l/kg. Its elimination $t_{½}$ has been reported to be 7 to 10 h after oral dosing in healthy volunteers (McCallum 1991). Cisapride is extensively metabolized, principally by N-dealkylation and aromatic hydroxylation (Van Peer et al. 1986): oxidative N-dealkylation yields norcisapride, and aromatic hydroxylation yields 3-fluoro-4-hydroxycisapride and 4-fluoro-2-hydroxycisapride (McCallum et al. 1988). *In vitro*, biotransformation of cisapride is catalyzed mainly by CYP3A4 (Desta et al. 1999; Gotschall et al. 1999; Bohets et al. 2000). Excretion of metabolites in urine and feces each represents 50% of the dose (Van Peer et al. 1986).

Cisapride is generally well tolerated, gastrointestinal complaints being the most common adverse effects (McCallum 1991). However, rare cases have been reported of serious ventricular arrhythmias or long QT syndrome (Lewin et al. 1996; Sekkarie et al. 1997; Wysowski and Bacsanyi 1996). A prolonged QT interval may result in torsades de pointes, a ventricular tachycardia that can lead to ventricular fibrillation and be a cause of syncope and sudden cardiac death. It has been demonstrated that cisapride can dose-dependently block potassium channels and thus cause prolongation of the QT interval (Drolet et al. 1998). In most of the cases of arrhythmia during treatment with cisapride, concomitant electrolyte

disturbances, cardiac abnormalities or comedications that increase cisapride concentrations appear to have contributed (Wysowski and Bacsanyi 1996).

In 20 healthy preoperative patients, oral cisapride 20 mg increased the $C_{max}$ of controlled-release morphine 20 mg by 56% (Rowbotham et al. 1991). Intravenous cisapride increased significantly the AUC(0-1) for diazepam without any significant effect on AUC(0-48) (Bateman 1986). In a study by Finet et al. (1991), cisapride increased the mean AUC(0-6) of oral cyclosporine by 38%. Kirch et al. (1989) has reported that cisapride reduces the AUC(0-24) of cimetidine and cimetidine increases the AUC(0-24) of cisapride. Ketoconazole, itraconazole, and erythromycin, which are known CYP3A4 inhibitors, have been reported to raise cisapride concentrations (Bedford and Rowbotham 1996). The manufacturer has warned against concomitant use of cisapride with potent CYP3A4 inhibitors. A combination of cisapride and clarithromycin has been reported to cause a 3-fold increase in cisapride concentration and an average increase of 25 ms in $QT_c$ interval above the pretreatment value in healthy volunteers (Van Haarst et al. 1998). In a study by Gross et al. (1999), 250 ml of normal-strength grapefruit juice increased cisapride AUC(0-25) 1.5-fold. Recently, cisapride was withdrawn from the market in the USA and in some other countries due to its arrhythmogenic potential.

### 3.3. Simvastatin

Simvastatin is a cholesterol-lowering agent which inhibits HMG-CoA reductase, an enzyme catalyzing a rate-limiting step in the biosynthesis of cholesterol in humans. It is a lipophilic drug, about 1000-fold more lipophilic than pravastatin (Hamelin and Turgeon 1998). Simvastatin is administered as an inactive lactone prodrug which is hydrolyzed by carboxyesterases and nonenzymatically to the active agent simvastatin acid, a competitive inhibitor of HMG-CoA reductase (Tang and Kalow 1995). It is extensively (61 to 85% in rats and dogs) absorbed from the gastrointestinal tract when administered orally (Vickers et al. 1990a). A low-fat meal does not impair its absorption. In a study by Pentikäinen et al. (1992) the peak inhibition of HMG-CoA reductase activity in healthy male volunteers occurred 2.5 h after a single oral dose. Its bioavailability is low, due to extensive first-pass metabolism. About 7% of an oral simvastatin dose in dogs reached the systemic circulation unchanged (Vickers et

al. 1990a). Its active metabolites are less lipophilic than the parent compound and tend to remain in the liver, the organ responsible for synthesis of most endogenous cholesterol (Vickers et al. 1990a). Both simvastatin and simvastatin acid are highly bound to plasma proteins, at about 98 and 94% (Vickers et al. 1990a).

Simvastatin has several metabolites identified in human microsomal studies, of which 6'-hydroxysimvastatin, 3''-hydroxysimvastatin, 6'-hydroxymethylsimvastatin, and 6'-hydroxycarbonylsimvastatin, when converted to their acid forms, are 50, 20, 90, and 40% as active as simvastatin acid, respectively (Vickers et al. 1990b). The inactive lactone form of simvastatin and its metabolites remain in a reversible equilibrium in plasma with their corresponding active β-hydroxyacid forms (Vickers et al. 1990a; Vickers et al. 1990b). In humans, biotransformation of simvastatin occurs mainly by the CYP3A4 expressed in the liver and small intestine (Prueksaritanont et al. 1997), but unlike simvastatin, simvastatin acid is not metabolized by mouse or rat liver microsomes. The elimination $t_{½}$ of simvastatin is about 2 hours (Lennernäs and Fager 1997). Unchanged simvastatin and its metabolites are eliminated via biliary excretion into the feces. In humans, 13% of a simvastatin dose has been collected in urine (Mauro 1993).

Simvastatin is usually well tolerated, gastrointestinal adverse effects being the most common reason for discontinuation of therapy. Mild transient elevations in serum transaminases are seen in about 3.5% and sustained elevations in about 1% of patients receiving simvastatin. Clinically symptomatic hepatitis or hepatic dysfunction is rare (Plosker and McTavish 1995). A potentially severe but rare adverse effect of simvastatin is myopathy, which can proceed to rhabdomyolysis. Increased risk for myopathy has been associated with concomitant administration of simvastatin and cyclosporine (Meier et al. 1995). In addition to the combination of simvastatin and cyclosporine, case reports exist of rhabdomyolysis being associated with concomitant use of simvastatin and itraconazole (Segaert et al. 1996) or mibefradil (Schmassmann-Suhijar et al. 1998). All the drugs used concomitantly with simvastatin in these cases are inhibitors of CYP3A4 (Pelkonen et al. 1998; Welker et al.1998; Wang et al. 1999). In kidney-transplant patients administered simvastatin, HMG-CoA reductase inhibitory activity was elevated when they also received cyclosporin (Arnadottir et al. 1993). A 4-day pretreatment with itraconazole 200 mg/day increased the $C_{max}$ and AUC of total simvastatin acid 17-fold and 19-fold, respectively (Neuvonen et al. 1998). In two different

studies, pretreatment with erythromycin 2.0 g per day for one week and 1.5 g per day for 2 days increased the AUC of simvastatin 6.5-fold and 6.2-fold (Donahue et al. 1998; Kantola et al. 1998b). The calcium-channel antagonists verapamil and diltiazem are known inhibitors of CYP3A4 (Renton 1985). A 2-day administration of verapamil increased the AUC of simvastatin 4.6-fold (Kantola et al. 1998b). After a two-week treatment with diltizem 120 mg twice a day, the AUC of simvastatin was increased 5-fold (Mousa et al. 2000).

### 3.4. Atorvastatin

Atorvastatin is a lipophilic inhibitor of HMG-CoA reductase administered as the calcium salt of the active hydroxy acid form. The $C_{max}$ value of atorvastatin is reached within 2 to 4 hours after oral administration (Lea and McTavish 1997). More than 40% of an oral dose of atorvastatin is absorbed from the gastrointestinal tract. Its systemic bioavailability is approximately 12% because of extensive metabolism during the first pass. Food decreases the rate of atorvastatin absorption significantly but influences little the extent of absorption (Radulovic et al. 1995). A greater than proportional increase in $C_{max}$ and AUC has been observed across the 5- to 80-mg dose-range (Whitfield et al. 1993). Atorvastatin is 98% bound to proteins in plasma (Lea and McTavish 1997). Its two active metabolites, 2-hydroxyatorvastatin and 4-hydroxyatorvastatin, are formed in reactions catalyzed by CYP3A4. These two metabolites are just as potent HMG-CoA reductase inhibitors as atorvastatin. About 70% of the AUC of inhibitory activity is accounted for by active metabolites. It has been assumed that the long-lasting HMG-CoA reductase inhibitory activity of atorvastatin may reflect the longer residence of atorvastatin or its active metabolites in the liver (Naoumova et al. 1997). Atorvastatin acid has shown an elimination $t_{½}$ of 11 to 24 hours, and an HMG-CoA reductase inhibitory activity that ranges from 20 to 30 hours (Cilla et al. 1996; Lea and McTavish 1997). Less than 2% of atorvastatin acid and its metabolites are excreted renally (Lea and McTavish 1997). It has been suggested that atorvastatin may undergo enterohepatic recycling (Gibson et al. 1996a). Age-related differences in atorvastatin pharmacokinetics have been observed, possibly due to changes in hepatic clearance. Mean AUC and elimination $t_{½}$ values have been about 26% greater and 36% longer in the elderly than in young adults (Gibson et al. 1996a). Furthermore, modest gender-related differences in atorvastatin pharmacokinetics have been reported, its mean AUC and elimination $t_{½}$ being approximately 11% lower and 20%

shorter in women than in men (Gibson et al. 1996a). Patients with hepatic impairment have significantly higher concentrations of atorvastatin than do healthy volunteers, whereas renal dysfunction does not alter the pharmacokinetics of atorvastatin (Gibson et al. 1996b; Stern et al. 1997).

Atorvastatin is usually well tolerated but can cause gastrointestinal discomfort. There is at least one reported case of rhabdomyolysis in association with concomitant use of atorvastatin and gemfibrozil (Duell et al. 1998).

Atorvastatin 40 mg twice daily was found not to affect the oxidative metabolism of antipyrine (Yang et al. 1996). Coadministration with atorvastatin 80 mg/day resulted in a 8% decrease and 35% increase in the $C_{max}$ and AUC of terfenadine, respectively, compared with values after ingestion of terfenadine alone (Stern et al. 1998a). A 10-day administration of atorvastatin 80 mg/day increased the $C_{max}$ and AUC of digoxin 20% and 15% (Boyd et al. 2000). During coadministration of atorvastatin 10 mg once daily with cimetidine 300 mg four times daily for 2 weeks, the AUC of atorvastatin (measured by REA) remained unchanged (Stern et al. 1998b). The $C_{max}$ and AUC of atorvastatin, measured as HMG-CoA reductase inhibitors, were increased 38% and 33%, respectively, by erythromycin 500 mg 4 times daily for 7 days (Siedlik et al. 1999). A 4-day pretreatment with itraconazole 200 mg/day increased the AUC of atorvastatin acid and of atorvastatin lactone about 3- and 4-fold, respectively. Furthermore, the mean AUC of 2-hydroxyatorvastatin and of 2-hydroxyatorvastatin lactone was decreased by 43% and 62% by itraconazole (Kantola et al. 1998c).

### 3.5. Pravastatin

Pravastatin is a hydrophilic HMG-CoA reductase inhibitor administered in its active open acid form. Following oral ingestion, it is absorbed rapidly, and $C_{max}$ is reached at approximately one hour (Pan 1991). After oral administration, about 34% of the dose is absorbed. The absolute bioavailability of pravastatin averages 18%, indicating that about 50% of the ingested dose undergoes presystemic metabolism (Singhvi et al. 1990). The $C_{max}$ and AUC values of pravastatin are dose-proportional (Pan et al. 1990a). In hypercholesterolemic patients, its bioavailability is reduced by about 30% when it is taken with food (Pan et al. 1993).

Approximately 50% of the circulating pravastatin is bound to plasma proteins. Its volume of distribution in steady-state conditions and during the elimination phase averages 0.46 and 0.88 l/kg (Singhvi et al. 1990). Total and renal clearance values average 13.5 and 6.5 ml/min/kg, respectively. Its elimination $t_{½}$ is about 2 hours (Pan 1991). After i.v. administration of radiolabeled pravastatin, 60% was recovered in urine and 34% in feces, indicating substantial biliary excretion (Singhvi et al. 1990). Following oral ingestion, recovery of radioactivity was 20% in urine and 71% in feces (Singhvi et al. 1990).

Pravastatin is extensively metabolized during the first pass and has a hepatic extraction ratio of 0.66 (Pan et al. 1991). It has two major metabolites, the 3α-hydroxy isomeric metabolite and 3α,5β,6β-trihydroxy isomeric metabolite (Everett et al. 1991). The former of these possesses 1/40 of the pharmacologic activity of the parent drug, whereas the latter is considered inactive. At least 15 other metabolites have been identified in urine, feces, and plasma (Everett et al. 1991). Pravastatin accounts for about 75% of the serum AUC for HMG-CoA reductase inhibitory activity (Pan et al. 1990b). *In vitro,* its biotransformation is partly inhibited by CYP3A4 inhibitors; some other CYP enzymes appear also to play a role in its metabolism (Jacobsen et al. 1999).

As with other HMG-CoA reductase inhibitors, gastrointestinal complaints are the most common adverse effects related to therapy with pravastatin. In a 2.5-year follow-up study in heart-transplant patients, pravastatin was discontinued in a few cases due to myopathy after its coadministration with cyclosporine (Park et al. 1998).

In renal-transplant patients receiving cyclosporine, considerably elevated values have been found for $C_{max}$ and AUC (Regazzi et al. 1994; Olbricht et al. 1997). In a study by Pan et al. (1991), propranolol reduced the mean AUC value of total inhibitors by 23% and that of pravastatin by 16%. Digoxin did not significantly alter steady-state pharmacokinetics of pravastatin (Triscari et al. 1993). A 7-day treatment with erythromycin 500 mg 4 times daily increased the $C_{max}$ and AUC of pravastatin 2.2-fold and 1.7-fold (Donahue et al. 1998). A 4-day administration of itraconazole 200 mg once daily resulted in a slight but statistically nonsignificant increase in the AUC of pravastatin and a 1.7-fold increased AUC value for total inhibitors (Neuvonen et al. 1998). A 2-week pretreatment with diltiazem 120 mg twice daily did not affect the $C_{max}$ or AUC of pravastatin (Azie et al. 1998).

### 3.6. Triazolam

Triazolam, structurally a triazolobenzodiazepine and closely related to alprazolam and midazolam, is a short-acting benzodiazepine hypnotic. It binds to benzodiazepine receptors and enhances GABAergic synaptic inhibition in the central nervous system. After oral administration, about 85% of a triazolam dose is absorbed. Its $t_{max}$ is from 1 to 2 hours. It has an oral bioavailability of 50 to 60%, indicating that it is subject to first-pass metabolism. It is about 90% bound to plasma proteins. Triazolam has an apparent volume of distribution of 1.1 l/kg. Its elimination $t_{½}$ ranges from 2 to 4 hours (Eberts et al. 1981).

Triazolam is extensively metabolized and has two major metabolites, α-hydroxy- and 4-hydroxytriazolam (Eberts et al. 1981). Of these, α-hydroxytriazolam, but not 4-hydroxytriazolam, shows pharmacological activity (Ziegler et al. 1983). Triazolam is primarily biotransformed by CYP3A4 (Kronbach et al. 1989; von Moltke et al. 1996), and its metabolites are subsequently glucuronized, with about 90% of the dose excreted renally. Apparent oral clearance of triazolam has been shown to be significantly reduced in elderly as compared with young subjects (Greenblatt et al. 1983). The pharmacokinetics of triazolam have not been found to differ between patients with hepatic cirrhosis and healthy volunteers (Robin et al. 1993). Its elimination was unaffected by renal disease (Kroboth et al. 1985).

Like other benzodiazepines, triazolam exhibits hypnotic, muscle relaxant, and anticonvulsant effects in animals; in humans it shortens the time to onset of sleep, increases total sleep duration, and decreases the frequency of nocturnal awakening. Large doses of triazolam can cause ventilatory suppression. It impairs cognitive and psychomotor functions like driving skills (Pakes et al. 1981). Its main adverse effects include amnesic episodes, rebound anxiety, and aggression (Dollery 1999).

Triazolam has been shown to be very susceptible to interactions with CYP3A4 inhibitors. Erythromycin pretreatment 333 mg t.i.d. for three days doubled triazolam AUC (Phillips et al. 1986). The azole antifungals itraconazole and ketoconazole increased the AUC of triazolam 27-fold and 22-fold and prolonged triazolam $t_{½}$ 7-fold and 6-fold, respectively (Varhe et al. 1994), markedly increasing the intensity and duration of triazolam effects. Its AUC was increased by fluconazole but not by terbinafine (Varhe et al. 1996a). The calcium-channel

antagonist diltiazem also increased triazolam concentrations (Varhe et al. 1996b; Kosuge et al. 1997). A single dose of grapefruit juice increased its AUC by about 50% and slightly increased its effects (Hukkinen et al. 1995).

## AIMS OF THE STUDY

The overall aim of this study was to discover the potential of grapefruit juice to affect the pharmacokinetics of drugs metabolized to varying degree during their first-pass and elimination phases by CYP3A4 and to characterize factors that determine grapefruit juice-drug interaction.

More specific aims of this study were:

To study the possible effects of multiple-dose grapefruit juice on the pharmacokinetics of buspirone, cisapride, simvastatin, atorvastatin, pravastatin, and triazolam (Studies I-V).

To investigate the effect of differing doses of grapefruit juice on the extent of interaction, with triazolam serving as a model of a CYP3A4 substrate (Study V).

To study the duration of effect of grapefruit juice on the pharmacokinetics of CYP3A4 substrate, with simvastatin serving as a model drug (Study VI).

## MATERIALS AND METHODS

### 1. Subjects

After receiving adequate written and oral information on the study protocols, all volunteers gave their written consent before entering the study. All studies were conducted according to the Declaration of Helsinki. The study protocols were approved by the Ethics Committee of the Department of Clinical Pharmacology, University of Helsinki. The Finnish National Agency for Medicines was informed about the studies.

Each study included 10 to 12 healthy volunteers (age range, 19 to 34 years; weight range, 50 to 101 kg); in total, 62 subjects (35 males, 27 females) participated. Ten subjects participated in more than one study: seven subjects two times, two subjects three times, and one subject four times. None was a regular smoker. The volunteers used no continuous medication except for 12 female subjects taking oral contraceptive steroids. All were considered to be healthy according to medical history, clinical examination, and routine laboratory tests (e.g., blood hemoglobin, serum alanine aminotransferase, creatinine, and creatine kinase). In addition, in Study II, a 12-lead electrocardiogram was taken before and during the study. None of the female subjects was pregnant or nursing, and they were instructed to avoid becoming pregnant during the study. Excessive alcohol consumption was an exclusion criterion. Subjects who had taken any kind of medication relevant to the study during the 4 weeks prior to the study, who had donated blood, or who had participated in other studies involving drugs during the 4 weeks prior to the study were excluded.

## 2. Study designs

All the study drugs were in current clinical use in Finland, and only oral formulations were used. Studies I, II, III, and IV were randomized, cross-over studies with two phases (one of which served as a control). Study IV comprised two different studies (a and b). The phases were separated by a 2-week washout period (3 weeks in Study IV). Study V was a randomized, cross-over study with four phases (one control phase) and washout periods of 2 weeks. Study VI was a nonrandomized cross-over study with five phases. Following ingestion of the drug, timed blood samples were taken for up 12 to 72 hours for the determination of the plasma or serum drug concentrations. Study designs are summarized below and in Table 1.

In each study, the volunteers ate a standard meal 3 to 4 hours following the administration of the drug and then a standard light meal 7 to 8 hours afterwards. Use of alcohol or, coffee and cola drinks or smoking during the study days was not allowed. Consumption of grapefruit products was forbidden during the pretreatment periods and study days except for intake according to the protocol. The same grapefruit juice brand was used in every study, Minute Maid frozen concentrated grapefruit juice (12 ounces [355 ml]), from Coca Cola Foods, Houston, Texas, USA. Before administration, the grapefruit juice was made at double strength with tap water in Studies I to VI (50/50, vol/vol). In addition, also normal-strength grapefruit juice was used in Study V (25/75, vol/vol).

**Table 1**. Structure of the studies. GFJ = grapefruit juice

| Study No. | Pretreatment | Washout period weeks | Study drug | Administration of study drug | |
|---|---|---|---|---|---|
| | | | | day | hours |
| I | GFJ (double strength) 200 ml t.i.d for 2 days (at 7.00-8.00, 12.00-13.00, and 20.00-21.00) plus on day 3 (at 9.00, 9.30, and 10.30)<br>Water | 2 | Buspirone 10 mg | 3 | 9.00-9.30 |
| II | As in Study I | 2 | Cisapride 10 mg | 3 | 9.00-9.30 |
| III | As in Study I | 2 | Simvastatin 60 mg | 3 | 9.00-9.30 |
| IVa | As in Study I | 3 | Atorvastatin 40 mg | 3 | 9.00-9.30 |
| IVb | As in Study I | 3 | Pravastatin 40 mg | 3 | 9.00-9.30 |
| V | Water | 2 | Triazolam 0.25 mg | 1 | 9.00-9.30 |
| | GFJ 200 ml (normal strength) | | | 1 | 9.00-9.30 |
| | GFJ 200 ml (double strength) | | | 1 | 9.00-9.30 |
| | GFJ as in Study I | | | 3 | 9.00-9.30 |
| VI | Water | See text | Simvastatin 40 mg | 1 | 9.00-9.30 |
| | GFJ as in Study I | | | 3 | 9.00-9.30 |
| | GFJ as in Study I | | | 6 | 9.00-9.30 |
| | GFJ as in Study I | | | 4 | 9.00-9.30 |
| | GFJ as in Study I | | | 10 | 9.00-9.30 |

**Study I**

Ten volunteers (4 males, 6 females, 2 OC-users) participated in a randomized cross-over study with two phases separated by a 2-week washout period. Pretreatments were double-strength grapefruit juice or water 200 ml t.i.d. for 2 days. On the third day, a single 10-mg dose of buspirone (Buspar, Bristol-Myers Squibb, Espoo, Finland) was ingested at 9 a.m. with 200 ml double-strength grapefruit juice or water. In addition, 200 ml grapefruit juice or water was ingested ½ and 1½ hours after the drug. Blood samples were obtained for 12 hours after administration for determination of plasma concentrations of buspirone. Psychomotor tests were performed for 8 hours to measure the effects of buspirone.

**Study II**

Ten healthy volunteers (all males) participated in this randomized cross-over study with a 2-week washout period. Pretreatments were identical to those in Study I. On the third day, the subjects were given 10 mg cisapride (one 10-mg Prepulsid tablet, Janssen-Cilag, Beerse, Belgium) at 9 a.m. with double-strength grapefruit juice or water 200 ml. In addition, 200 ml grapefruit juice or water was ingested ½ and 1½ hours after the drug. Blood samples were drawn for up to 36 hours after the drug for determination of plasma concentrations of cisapride. In addition, 12-lead ECGs were taken before and 2, 5, 8, and 12 hours after the drug.

**Study III**

Ten volunteers (5 males, 5 females, 3 OC-users) participated in a randomized cross-over study with two phases, separated by a 2-week washout period. Pretreatments were identical to those in Study I. On the third day, the volunteers received simvastatin 60 mg (3 Zocor 20 mg tablets, Merck Sharp & Dohme BV, Haarlem, The Netherlands) at 9 a.m. with double-strength grapefruit juice or water 200 ml. In addition, 200 ml grapefruit juice or water was ingested ½ and 1½ hours after the drug. Blood samples were drawn as in Study I - except that blood was not sampled at 1½ hours - for determination of serum concentrations of simvastatin, simvastatin acid, and HMG-CoA reductase inhibitors.

**Study IV**

This study included two separate investigations, IVa and IVb. Study design and pretreatments were in both investigations as in previous studies. A 3-week washout period was used. In Study IVa, 12 healthy volunteers (6 males and 6 females, 4 OC-users) received on the third day 40 mg atorvastatin (two 20-mg Lipitor tablets, Warner Lambert Nordic AB, Solna, Sweden) at 9 a.m. with double-strength grapefruit juice or water 200 ml. In addition, 200 ml grapefruit juice or water was ingested ½ and 1½ hours after the drug. After the third day, the subjects continued to drink double-strength grapefruit juice or water 200 ml t.i.d. for 2 more days. Blood samples were obtained for 72 hours after the drug administration for determination of serum concentrations of atorvastatin acid, its metabolites, and HMG-CoA reductase inhibitors. In Study IVb, the volunteers (3 males and 8 females, 1 OC-user) received 40 mg pravastatin (two 20-mg Pravachol tablets, Bristol-Myers Squibb, Bromma, Sweden) at 9 a.m. with double-strength grapefruit juice or water 200 ml. In addition, 200 ml grapefruit juice or water was ingested ½ and 1½ hours after the drug. Blood samples were obtained for 24 hours after the drug for determination of serum concentrations of pravastatin, pravastatin lactone, and HMG-CoA reductase inhibitors.

**Study V**

Twelve subjects (6 males, 6 females, 3 OC-users) participated in this randomized cross-over study that consisted of four phases with 2-week washout periods. Volunteers received 0.25 mg triazolam (Halcion, Upjohn, Kalamazoo, MI, USA) with 200 ml water, 200 ml normal-strength or double-strength grapefruit juice, or on the third day of repeated (t.i.d.) administration of double-strength grapefruit juice (as in previous studies). Blood samples were collected for 23 hours after the drug for determination of plasma concentrations of triazolam, and the effects of triazolam were measured by four psychomotor tests for up to 10 hours.

**Study VI**

This cross-over study consisted of three parts and 5 study days, during each study day 10 healthy volunteers (9 males and 1 female) received 40 mg simvastatin (two Zocor 20 mg tablets, Merck Sharp & Dohme BV, Haarlem, The Netherlands). The three parts of the study

were separated by an interval of 2 weeks to allow simvastatin-free days between the study days. In the first part of the study, each volunteer was administered 40 mg simvastatin at 9 a.m. with 200 ml water. In the second study part, volunteers received juice as pre-treatment exactly as in Study I. On day 3, each subject was given 40 mg simvastatin at 9 a.m. with 200 ml grapefruit juice. In addition, the subjects ingested juice ½ and 1½ hours after simvastatin intake. Three days after the last dose of grapefruit juice, 40 mg simvastatin was given with water at 9 a.m. In the third study part, the subjects ingested grapefruit juice t.i.d. for 3 days as in the second part of the study. Twenty-four hours after the last dose of grapefruit juice, each subject received 40 mg simvastatin with water at 9 a.m. Seven days after the last dose of grapefruit juice, each subject was given 40 mg simvastatin water at 9 a.m. Blood samples were collected for 12 hours after the drug for determination of serum concentrations of simvastatin and simvastatin acid.

**3. Blood sampling**

On the day of administration of the study drug, a forearm vein was cannulated with a plastic cannula and kept patent with an obturator. Timed blood samples were drawn before administration of buspirone and ½, 1, 1½, 2, 3, 4, 5, 6, 8, 10, and 12 hours afterwards in Study I. In Study II, blood samples were obtained before administration of cisapride and ½, 1, 1½, 2, 3, 4, 5, 6, 9, 12, 24, and 32 hours afterwards. In Study III, the blood samples were collected before administration of simvastatin and at ½, 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours. In Studies IVa and IVb, the time-points for blood sampling were before and ½, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, and 72 hours after administration of atorvastatin, and before and ½, 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours after administration of pravastatin. In Study V, blood samples were obtained before administration of triazolam and at ½, 1, 1½, 2, 3, 4, 5, 6, 8, 10, and 23 hours. In Study VI, the blood samples were drawn before and 1, 2, 3, 4, 6, 8, 10, and 12 hours after administration of simvastatin. The blood samples were drawn into tubes containing ethylenediaminetetra-acetic acid (EDTA) in Studies I, II, and V and into siliconized Venoject tubes in Studies III, IV, and VI. Plasma or serum was separated within 30 minutes after blood sampling and stored at -70°C.

## 4. Determination of drug concentrations

**Buspirone (Study I).** Plasma buspirone concentrations were measured by means of a gas chromatographic method that involved solid-phase extraction and nitrogen-phosphorus detection (Gaillard et al. 1993; Kivistö et al. 1999). Zolpidem served as an internal standard. The limit of quantification was 0.20 ng/ml. The between-day coefficient of variation (CV) was < 11%.

**Cisapride (Study II).** Plasma concentrations of cisapride were determined by use of reversed-phase high-performance liquid chromatography (HPLC) that involved solid phase extraction (Woestenborghs et al. 1988) and fluorescence detection (Preechagoon and Charles 1995). Metoclopramide served as an internal standard. The quantification limit for cisapride was 2.0 ng/ml. The between-day CV was < 13%.

**Simvastatin, simvastatin acid, and active and total HMG-CoA reductase inhibitors (Study III).** Serum concentrations of simvastatin and simvastatin acid were measured by turbo ionspray liquid chromatography-tandem mass spectrometry (LC-MS-MS) in the positive ion mode as described in the original paper (Study III). The limit of quantification was 0.5 ng/ml for simvastatin and simvastatin acid and the interassay CV < 8%.

In Studies III and IV, the serum concentrations of statins and their metabolites were determined by means of a nonspecific radioenzyme inhibition assay (REA) in addition to a specific method (LC-MS-MS). In REA, serum samples were assayed for inherent (active) HMG-CoA reductase inhibitory activity resulting from the β-hydroxyacid form of the parent drug and active metabolites, and after base hydrolysis which converts inactive lactones to active species (total inhibitory activity). This method is considered to be nonspecific because it quantitates the inhibitory activity against HMG-CoA reductase activity rather than actual concentrations of a drug or its metabolite(s).

Serum concentrations of active and total HMG-CoA reductase inhibitors were measured in Study III as described by Manning et al. (1989). Base hydrolysis was achieved by incubation of 0.1 ml serum with 0.01 N potassium hydroxide. Concentrations were reported as nanogram equivalents (ng-eq) of simvastatin acid per milliliter. The limits of quantification for active and

total HMG-CoA reductase inhibitors were 1.0 ng-eq/ml and 2.0 ng-eq/ml. For active HMG-CoA reductase inhibitors, the interassay CV was < 12 %. For total HMG-CoA reductase inhibitors, the interassay CV was 5% or less.

**Atorvastatin acid, its metabolites, pravastatin, pravastatin lactone, and active and total HMG-CoA reductase inhibitors (Study IV).** Atorvastatin and its metabolites were quantified by LC-MS-MS, as described in the original paper (Study IVa). The limit of quantification was 0.5 ng/ml for all analytes. The interassay CV was < 10% for all analytes at relevant concentrations.

In Study IVb, serum concentrations of pravastatin and pravastatin lactone were determined by liquid chromatography with electronspray tandem mass spectrometry in the positive ion mode (LC-MS-MS). The limit of quantification was 0.5 ng/ml for pravastatin and its lactone. The CV was ≤ 8% for both analytes at relevant concentrations.

Serum concentrations of active (no hydrolysis) and total (after hydrolysis of lactones) HMG-CoA reductase inhibitors were determined with the use of REA (Manning et al. 1989). Concentrations were reported as nanogram-equivalents (ng-eq) of atorvastatin acid per milliliter (Study IVa) and as nanogram-equivalents (ng-eq) of pravastatin sodium per milliliter (Study IVb). The quantification limit for active and total HMG-CoA reductase inhibitors was 0.5 and 1.0 ng-eq/ml, in Study IVa. In Study IVb, the quantification limit for active and total HMG-CoA reductase inhibitors was 2.0 and 5.0 ng-eq/ml. The CV was < 10% for all analytes at relevant concentrations.

**Triazolam (Study V).** Plasma triazolam concentrations were determined by use of capillary gas chromatography (Gaillard et al. 1993). This method involved solid phase extraction and electron-capture detection. The limit of quantification was 0.1 ng/ml. The between-day CV was < 16%.

**Simvastatin and simvastatin acid (Study VI).** Serum concentrations of simvastatin and simvastatin acid were assayed by use of turbo ionspray LC-MS-MS in the positive ion mode as described in the original paper (Study III). The quantification limit was 0.1 ng/ml for both

simvastatin and simvastatin acid. The between-day CV for simvastatin acid was < 12%. The between-day CV for simvastatin was < 15%.

## 5. Pharmacokinetic calculations

The pharmacokinetics of the study drugs were characterized by the following variables: the peak concentration in plasma or serum ($C_{max}$), time of peak concentration ($t_{max}$), the elimination half-life ($t_{½}$), and areas under the plasma or serum concentration-time curve [AUC(0-t) and AUC(0-∞)]. The $C_{max}$ and $t_{max}$ values were obtained directly from the original data. The terminal log-linear phase of the plasma or serum drug concentration-time curve was identified visually for each subject. The elimination coefficient ($k_{el}$) was determined from the log-linear phase of the drug concentration-time curve by linear regression analysis. The $t_{½}$ was calculated by the equation: $t_{½} = \ln 2/k_{el}$. The AUC(0-t) values were calculated by use of the trapezoidal rule. The AUC(0-∞) was obtained by adding to the AUC(0-t) the residual area AUC(t-∞) calculated as the last measurable concentration divided by the $k_{el}$. Pharmacokinetic calculations were performed with the pharmacokinetic program MK-Model, version 5.0 (Biosoft, Cambridge, UK) in all studies, except for Study III, in which the Top Fit version 2.0 program (Dr. Karl Thomae GmbH, Schering AG, Gödecke AG, Germany) was used.

## 6. Pharmacodynamic testing

The effects of buspirone and triazolam were tested in Studies I and V, respectively. Six different tests were used in Study I: subjective drug effect, subjective drowsiness, DSST, CFFT, and postural sway with eyes open and eyes closed. In Study V, the same tests were used as in Study I except for postural sway. Pharmacodynamic measurements were performed after each blood sampling for up to 8 hours in Study I and up to 10 hours in Study V. The volunteers were trained to perform the psychomotor tests properly before the commencement of each trial.

**Subjective drug effect.** Subjective drug effect was evaluated on a 100 mm-long horizontal visual analogue scale (VAS) with adjectives with opposite meanings at both ends. The subject

puts a mark somewhere on this scale as a self-rating of his or her feelings at the moment. In this test, the scale ranges from No drug effect to Maximal drug effect, expressed in Finnish.

**Subjective drowsiness.** The performance of this test is identical to that of the subjective drug effect, except that Alert and Drowsy are at the ends of the scale for self-evaluation of subjective drowsiness.

**DSST.** The Digit Symbol Substitution Test has been demonstrated to be sensitive for measuring both cognitive and motor effects of psychoactive drugs (Stone 1984). In this test, the volunteer substitutes digits (1-9) for simple coded symbols. The number of correctly substituted digits in 3 minutes was the test result in Study I. In Study V, the recording time was 2 minutes. To prevent subjects' learning the code, the symbols corresponding to the digits were different at the each time of recording.

**CFFT.** The results of this test are affected by the sensitivity of the visual cortex, the state of arousal of the CNS, and the integrative activity of the CNS (Smith and Misiak 1976). In this test, discrimination of the fusion of a flickering red light is measured. For the first measurement, the frequency of flickering constantly increases, and the subject indicates the point at which the flickering lights give the sensation of a steady light. This threshold frequency expressed in Hertz is the result of the measurement. After this, another measurement is performed during which lights flicker at a decreasing frequency, and the subject indicates the point at which flickering is distinguishable. The mean of these two measurements is the result of the test. The distance from the eyes of the volunteer to the flickering lights is standardized to 1 m. To control variations in pupillary diameter, special spectacles are worn, and illumination is kept constant during each study day. It has been shown that hypnotics like benzodiazepines reduce the test score and stimulants such as amphetamine increase it (Smith and Misiak 1976; Longbottom and Pleuvry 1984).

**Postural sway.** In this test, postural sway of the subject standing on a metal plate is recorded by means of a computer-controlled swaymeter (Erikois-Elektroniikka Ltd., Orimattila, Finland). The recorded parameter is the speed of the subject's mass center (mm/min). The measurement time is 30 seconds with the subject's eyes open and thereafter 30 seconds with the eyes closed. Body sway is an indicator of corrective mechanisms to maintain an upright

posture (Robin et al. 1991). The precision at which the automatic equilibration system of a subject maintains the center of gravity close to the mid-position depends on the subject's state of arousal (Patat and Foulhoux 1985). Benzodiazepines have been shown to increase body sway (Backman et al. 1996), whereas buspirone has had negligible effects (Lamberg et al. 1998a).

The incremental (body sway, VAS) or decremental (DSST, CFFT) area under the effect-against-time curve (i.e., areas above or below baseline) was calculated by the linear trapezoidal rule from 0 to 4 hours and from 0 to 8 hours in Study I and from 0 to 6 hours in Study V.

**ECG recording**

In Study II, 12-lead ECG recordings were taken before the administration of cisapride and 2, 5, 8, and 12 hours later. Heart rate and ECG intervals (PQ, QRS, and QT) were measured with automated software (Cardiovit AT-6, Schiller, Switzerland). The QT interval was corrected for heart rate by dividing the measured QT interval by the square root of the RR interval ($QT_c$ interval).

## 7. Statistical analysis

Data are expressed as mean values ± SEM (Studies I and III), mean values ± SD (Studies II, IV-VI), or median with range in the case of $t_{max}$. Results are presented in the figures as mean values ± SEM. Pharmacokinetic variables were log-transformed before analysis when appropriate. In Studies I to IV, continuous pharmacokinetic variables ($C_{max}$, $t_{½}$, AUC), pharmacodynamic variables (AUC values in Study I), and $QT_c$ data (Study II) between the phases were compared with use of the Student *t*-test (two-tailed). The $t_{max}$ values were compared with the Wilcoxon signed rank test. In Studies V and VI, the pharmacokinetic variables and the AUC values for pharmacodynamic tests (Study V) between the phases were compared with use of analysis of variance (ANOVA); a posteriori testing was done by the Tukey test. The $t_{max}$ values were compared with Friedman's two-way ANOVA, followed by the Wilcoxon test. Pearson's linear correlation coefficient was used to test linear correlations in Study II. In all studies, $p < 0.05$ was considered to be statistically significant. The statistical

analyses were performed with the statistical program Systat, version 5.0 (Systat, Evanston, IN, USA) in Studies I to IV, and version 6.0.1 (SPSS, Chicago, IL, USA) in Studies V and VI.

## RESULTS

### 1. Effects of grapefruit juice on the pharmacokinetics of buspirone (Study I)

Grapefruit juice had a considerable effect on plasma concentrations of buspirone (Table 2, Figs. 1 and 2). The mean $C_{max}$ of buspirone was increased by grapefruit juice about 4-fold ($p < 0.01$), and its mean AUC(0-∞) was affected even more, a mean 9-fold (range, 3-fold to 20-fold; $p < 0.01$). The $t_{max}$ of buspirone occurred significantly later in the grapefruit juice than in the water phase (3 hours versus 0.75 hour; $p < 0.01$). Its $t_{½}$ was 1.8 hours in the water phase and 2.7 hours in the grapefruit juice phase ($p < 0.01$).

The results of the pharmacodynamic tests were only modestly affected by grapefruit juice ingestion. The subjective overall drug effect was significantly ($p < 0.01$) increased, but no other differences appeared between the grapefruit juice and the water phase in the psychomotor tests. Mild side-effects were reported by 8 subjects during the grapefruit juice and 7 subjects during the water phase. These side-effects resolved spontaneously within 1 to 3 hours in all cases.

### 2. Effects of grapefruit juice on the pharmacokinetics of cisapride (Study II)

Grapefruit juice considerably increased cisapride concentrations (Table 2, Figs. 1 and 2). The mean $C_{max}$ and AUC(0-∞) of cisapride were increased by 81% and 144% ($p < 0.01$). The $t_{max}$ of cisapride occurred significantly later in the grapefruit juice than in the water phase (2.5 hours versus 1.5 hours; $p < 0.05$). Grapefruit juice increased the elimination $t_{½}$ of cisapride by about 24% ($p < 0.05$).

The mean $QT_c$ interval at 5 hours was slightly higher than the baseline value during both study phases ($p < 0.01$), with no significant difference in $QT_c$ interval found between the phases at any time-point.

### 3. Effects of grapefruit juice on the pharmacokinetics of simvastatin (Study III)

Grapefruit juice caused great changes in the pharmacokinetics of simvastatin and its active form, simvastatin acid (Table 2, Figs. 1 and 2). The mean $C_{max}$ and AUC(0-∞) values of simvastatin were increased approximately 9-fold ($p < 0.01$) and 16-fold ($p < 0.05$). During this phase, the $t_{max}$ of simvastatin was nonsignificantly delayed compared with the water phase ($p = 0.065$). The $t_{½}$ of simvastatin was not changed by grapefruit juice. The ratio of the $C_{max}$ of simvastatin in the grapefruit juice to that of the water phase ranged from 5.1 to 31.4; the corresponding ratio for the AUC(0-∞) ranged from 9.0 to 37.7. The $C_{max}$ and AUC(0-∞) of simvastatin acid were increased about 7-fold ($p < 0.01$) by grapefruit juice, but the $t_{max}$ and $t_{½}$ of simvastatin acid remained unaltered.

The mean AUC(0-∞) of active and total HMG-CoA reductase inhibitors were increased 2.4- and 3.6-fold ($p < 0.01$) by grapefruit juice. The individual changes ranged from 1.0- to 4.1-fold for the AUC(0-∞) of active inhibitors and from 2.2 to 7.7-fold for the AUC(0-∞) of total inhibitors.

### 4. Effects of grapefruit juice on the pharmacokinetics of atorvastatin and pravastatin (Study IV)

Multiple-dose grapefruit juice (ingested on a total of 5 consecutive days) significantly elevated serum concentrations of atorvastatin acid and atorvastatin lactone and reduced formation of 2-hydroxyatorvastatin (Table 2, Figs. 1 and 2). The $C_{max}$ of atorvastatin acid remained unchanged, whereas the AUC(0-72) was increased about 2.5-fold ($p < 0.01$) by grapefruit juice, with individual increases ranging from 1.7- to 5.7-fold. The median $t_{max}$ of atorvastatin acid was prolonged from 1 hour to 3 hours ($p < 0.01$). The elimination $t_{½}$ of atorvastatin acid increased from 7.8 hours in the water to 13.3 hours in the grapefruit-juice phase ($p < 0.01$). Grapefruit juice increased the mean $C_{max}$ and AUC(0-72) of atorvastatin lactone 2.6-fold and 3.3-fold ($p < 0.01$). The median $t_{max}$ of atorvastatin lactone was prolonged from 3 to 4 hours ($p < 0.05$), and the mean elimination $t_{½}$ from 8.3 to 12.6 hours ($p < 0.05$) by grapefruit juice.

After grapefruit juice ingestion, the mean $C_{max}$ and AUC(0-72) of 2-hydroxyatorvastatin acid were 25% ($p < 0.001$) and 58% ($p < 0.001$), of corresponding values in the water phase. The median $t_{max}$ of 2-hydroxyatorvastatin acid was prolonged from 1.5 to 10 hours ($p < 0.01$), and the mean elimination $t_{½}$ from 9.7 to 17.7 hours ($p < 0.001$) by grapefruit juice. Grapefruit juice decreased the mean $C_{max}$ and AUC(0-72) of 2-hydroxyatorvastatin lactone to 44% ($p < 0.001$) and 80% ($p < 0.05$), of the corresponding values in the water phase. The median $t_{max}$ was increased from 3.5 to 10 hours ($p < 0.01$) and the mean elimination $t_{½}$ from 10.3 to 15.0 hours ($p < 0.05$).

During the grapefruit-juice phase, the mean AUC(0-72) of active and total HMG-CoA reductase inhibitors were 28% ($p < 0.05$) and 50% ($p < 0.01$) greater than in the water phase. The mean elimination $t_{½}$ of total HMG-CoA reductase inhibitors averaged 13.4 hours with water and 17.0 hours with grapefruit juice ($p < 0.01$).

Grapefruit juice showed no significant effects on the pharmacokinetics of pravastatin, with the AUC(0-24), $C_{max}$, $t_{max}$, and elimination $t_{½}$ remaining unchanged (Table 2, Figs. 1 and 2). In addition, grapefruit juice did not alter the AUC(0-24), $C_{max}$, or $t_{max}$ of pravastatin lactone; the elimination $t_{½}$ of pravastatin lactone could not be calculated because of low serum concentrations. Grapefruit juice did not significantly affect the AUC(0-24), $C_{max}$, or $t_{½}$ of active or total HMG-CoA reductase inhibitors. The median $t_{max}$ of total HMG-CoA reductase inhibitors remained unaltered, whereas that of active HMG-CoA reductase inhibitors was increased from 1 to 2 hours ($p < 0.05$).

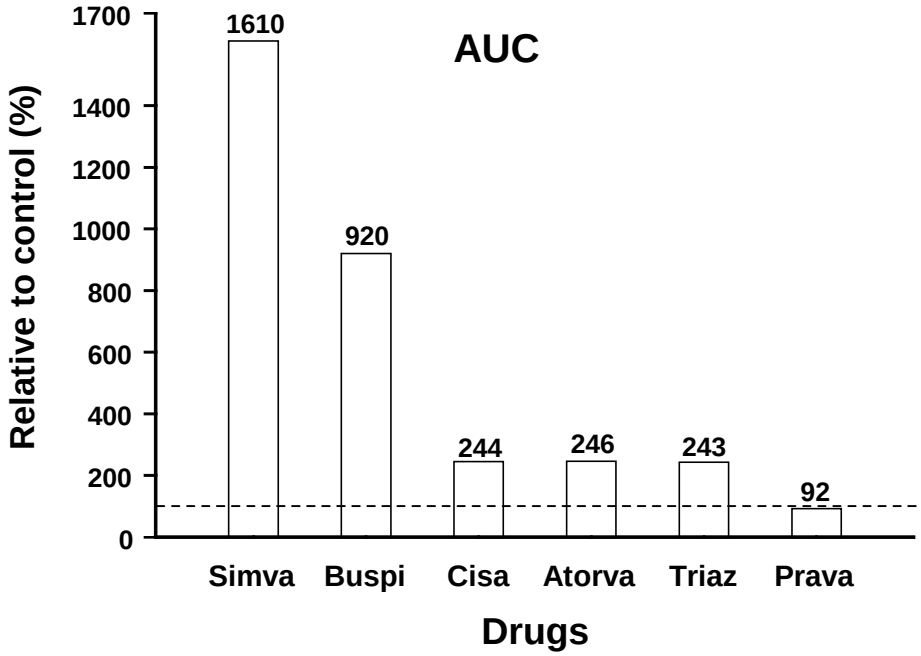
## 5. Effect of grapefruit juice dose on the interaction between grapefruit juice and triazolam (Study V)

The AUC(0-∞) of triazolam was increased by 53% ($p < 0.01$), 49% ($p < 0.0l$), and 143% ($p < 0.001$) by a single dose of normal-strength, by a single dose of double-strength, and by multiple-dose double-strength grapefruit juice, respectively (Table 2, Fig. 1). The $C_{max}$ of triazolam was increased by 40% by a single dose of normal-strength grapefruit juice ($p < 0.01$) and multiple-dose grapefruit juice ($p < 0.01$) and by 25% by a single dose of double-strength grapefruit juice ($p < 0.05$; Table 2, Fig. 2). The increase in the AUC(0-∞) of triazolam was

significantly ($p < 0.001$) greater after repeated consumption of grapefruit juice than after a single dose of normal- or double-strength grapefruit juice. Multiple-dose administration of grapefruit juice increased the $t_{½}$ of triazolam by 54% ($p < 0.001$), whereas it was not significantly changed by a single dose of either normal- or double-strength juice.

The effects of triazolam were significantly ($p < 0.05$) increased by multiple-dose administration of grapefruit juice according to the DSST and based on VAS tests for overall drug effect, and drowsiness.

**Figure 1.** Mean AUC values of study drugs after ingestion of grapefruit juice 200 ml t.i.d. for 3 days, expressed as percentages of corresponding values during the water phase (control). Simva = simvastatin; Buspi = buspirone; Cisa = cisapride; Atorva = atorvastatin; Triaz = triazolam; Prava = pravastatin.



## 6. Duration of effect of grapefruit juice on the pharmacokinetics of simvastatin (Study VI)

Concomitant administration with grapefruit juice increased the mean $C_{max}$ and AUC(0-∞) of simvastatin 12.0-fold ($p < 0.001$) and 13.5-fold ($p < 0.001$), compared with the water phase. When simvastatin was ingested 24 hours after the last dose of grapefruit juice, $C_{max}$ and AUC(0-∞) were increased 2.4-fold ($p < 0.01$) and 2.1-fold ($p < 0.001$) compared with the water phase. When simvastatin was administered 3 days after the final dose of grapefruit juice, the $C_{max}$ and and AUC(0-∞) were increased 1.5-fold ($p = 0.12$) and 1.4-fold ($p = 0.09$), respectively. Seven days after cessation of intake of grapefruit juice, no differences appeared in the $C_{max}$ and AUC(0-∞) between the phases. When simvastatin was administered 24 hours or 3 days after cessation of intake of grapefruit juice, the mean oral clearance ($Cl_{oral}$) of simvastatin was 50% ($p < 0.001$) and 63% ($p = 0.09$) of that during the water phase.

Concomitant administration of simvastatin with grapefruit juice increased the mean $C_{max}$ and AUC(0-∞) of simvastatin acid 5.0- and 4.5-fold, compared with the water phase ($p < 0.001$). When simvastatin was taken 24 hours after the last dose of grapefruit juice, the $C_{max}$ and AUC(0-∞) of simvastatin acid were increased 1.7-fold ($p < 0.01$). After an interval of 3 or 7 days between ingestion of grapefruit juice and simvastatin, the pharmacokinetic variables of simvastatin acid did not differ significantly from those in the water phase.

**Figure 2.** Mean $C_{max}$ values of study drugs after ingestion of grapefruit juice 200 ml t.i.d. for 3 days, expressed as percentages of corresponding values during the water phase (control). Simva = simvastatin; Buspi = buspirone; Cisa = cisapride; Atorva = atorvastatin; Triaz = triazolam; Prava = pravastatin.

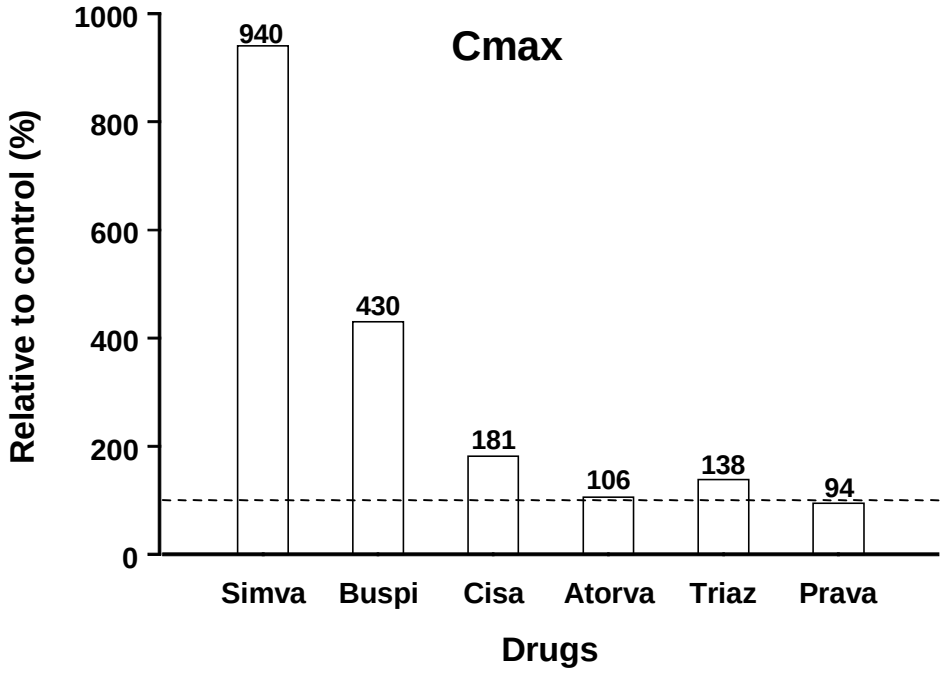


**Table 2.** Summary of effects of ingestion of grapefruit juice 200 ml t.i.d. for 3 days on selected pharmacokinetic parameters of study drugs.

| Study drug | AUC | $C_{max}$ | $t_{max}$ | $t_{½}$ |
|---|---|---|---|---|
| **buspirone** | ↑↑↑ | ↑↑↑ | ↑↑ | ↑ |
| **simvastatin** | ↑↑↑ | ↑↑↑ | ↔ | ↔ |
| **cisapride** | ↑↑ | ↑ | ↑ | ↑ |
| **atorvastatin** | ↑↑ | ↔ | ↑↑ | ↑ |
| **pravastatin** | ↔ | ↔ | ↔ | ↔ |
| **triazolam** | ↑↑ | ↑ | ↔ | ↑ |

Changes (increase or decrease) indicated as follows:

↑/↓ 15 to 100% change ($p < 0.05$)

↑↑/↓↓ 101 to 300% change ($p < 0.05$)

↑↑↑/↓↓↓ change > 300% ($p < 0.05$)

↔ no significant change ($p > 0.05$)

## DISCUSSION

### 1. Methodological considerations

All of the subjects participating in the present series of investigations were young and healthy. Cardiac, hepatic, and renal diseases may modify drug metabolism and excretion and therefore have a substantial effect on drug pharmacokinetics. Thus, results of these studies may not be directly extrapolated to subgroups with illnesses or who use concomitant medications. Every study was of cross-over design. In this design, each subject serves as his or her own control so that the large interindividual variation often associated with pharmacokinetic interaction studies can be minimized. The randomization and balancing used in most of the studies, together with adequately long wash-out periods, helped to avoid any possible carry-over effect. Based on the elimination half-lives of study drugs and the estimated duration of the grapefruit-juice effect, it was regarded that a 2- to 3-week washout period is sufficiently lengthy.

The number of subjects in these investigations was relatively small. However, based on previous interaction studies and information on pharmacokinetic properties of the study drugs, it was considered that, if in a sample with size of 10 to 12 volunteers a 30 to 50% difference in AUC is not statistically significant, then the probability of clinically important interaction is small. In each of the six studies, grapefruit juice was used (at least in one phase) according to one and the same scheme, to allow comparison of results between studies. The same brand of grapefruit juice was used in every study to minimize possible variability in the content of its active ingredients. Relatively large amounts of grapefruit juice were used to uncover any potential of the study drugs to interact with it. In some countries, quite a large portion of households purchase grapefruit juice, and it may be consumed individually in variable amounts on a daily basis. In these studies, the effect of grapefruit juice dose was also investigated. Among all the volunteers, grapefruit juice intake was associated in only one subject with gastrointestinal discomfort.

The volunteers fasted for at least one hour before administration of the study drugs to minimize any possible effect of food on their absorption; during each study day a standardized lunch and a light lunch were served.

The doses of the drugs used were recommended daily doses. All of the study drugs were administered only orally, as none were available as intravenous formulations. Use of intravenous formulations would have allowed a more direct means of investigating mechanisms by which grapefruit juice can alter pharmacokinetics, making it possible to estimate total clearance and bioavailability of the drugs during different study phases. However, on the basis of previous data, it seems likely that changes in the AUC of high-clearance drugs with low bioavailability, e.g., simvastatin and buspirone, are primarily due to alterations in their first-pass metabolism and to a lesser degree in their systemic clearance (Masica et al. 2000). On the other hand, the AUC of intermediate-clearance drugs is more sensitive to alterations in their systemic clearance during the hepatic elimination phase. A single dose of study drug was administered in each study. Previous studies, in which the CYP3A4 inhibitor has been given for a few days and then the CYP3A4 substrate in a single dose, have predicted the susceptibility of CYP3A4 substrates, e.g., lovastatin and simvastatin, to pharmacokinetic interactions with such drugs as itraconazole or erythromycin fairly reliably.

Both females and males were recruited as study volunteers. However, it was considered unnecessary to balance each study in respect to gender because of the cross-over design. Furthermore, in previous studies the menstrual cycle has not been found to affect the metabolism of CYP3A4 substrates (Kashuba et al. 1998; Kharasch et al. 1997). Some of the female subjects were using OCs that contained ethinyl estradiol, gestodene, desogestrel, or levonorgestrel, potent mechanism-based inhibitors of CYP3A4 (Guengerich 1988; Guengerich 1990; Back et al. 1991). However, as previous data do not show OCs to have a major effect on the pharmacokinetics of CYP3A4 substrates *in vivo*, probably because of the low amounts of steroids in these products, use of OCs was not an exclusion criterion (Scavone et al. 1988; Stoehr et al. 1984). In the present studies, OCs showed no consistent effect on pharmacokinetic results. Regular smokers were not recruited into any of these studies.

The psychomotor effects of buspirone and of triazolam were measured by six and by four tests validated and widely used for measurement of psychomotor effects of drugs. Before entering the study, the subjects were properly trained to perform these tests to avoid any learning effect. While the effects of benzodiazepines on the results of these tests are well known, buspirone itself has shown previously only minor or no effects on the results (Smith and Misiak 1976; Stone 1984; Seppälä et al. 1982; Greenblatt et al. 1994).

The plasma concentrations of buspirone and of triazolam were measured by a sensitive gas chromatographic method in Studies I and V, respectively; the between-day CV was satisfactory for this method. In Study II, plasma cisapride concentrations were measured by HPLC. Sensitivity and between-day CV were satisfactory. The serum concentrations of statins were assayed by sensitive LC-MS-MS in Studies III, IV, and VI. The between-day CVs of these methods were good or satisfactory. In addition, in Studies III and IV, serum HMG-CoA reductase inhibitory activity was measured by nonspecific REA. With this method, the between-day CVs were good or satisfactory.

**2. Effects of grapefruit juice on the pharmacokinetics of buspirone**

In this study, grapefruit juice increased the $C_{max}$ and AUC(0-∞) of buspirone about 4-fold and 9-fold. The $t_{max}$ of buspirone was prolonged by grapefruit juice and the elimination $t_{½}$ of buspirone increased by about 50% in the grapefruit juice phase compared to the water phase. The interaction of grapefruit juice with buspirone was subject to considerable interindividual variability, the individual increase in $C_{max}$ ranging from 2-fold to nearly 16-fold and that in AUC from 3-fold to 20-fold. Substantial changes in the pharmacokinetics of buspirone were associated with only modest alterations in the pharmacodynamic results.

Buspirone is almost completely absorbed from the gastrointestinal tract. However, due to its extensive first-pass metabolism, its bioavailability is only about 5% (Mayol et al. 1985). Buspirone is oxidatively metabolized in the liver, yet the specific CYP enzymes involved remain to be identified (Jajoo et al. 1990). However, the known CYP3A4 inhibitors itraconazole, erythromycin, diltiazem, and verapamil have all considerably increased buspirone plasma concentrations *in vivo* (Kivistö et al. 1997; Lamberg et al. 1998a). In the present study, the effect of grapefruit juice on the pharmacokinetics of buspirone was smaller than that of itraconazole but greater than that found for verapamil or diltiazem (Kivistö et al. 1997; Lamberg et al. 1998a). In humans, CYP3A4 is extensively expressed not only in the liver but also in the apical enterocytes of the small intestine (Kolars et al. 1992; Paine et al. 1997). Thus, a large portion of the metabolism of buspirone may occur in the gut wall during the first pass. Lown et al. have suggested that grapefruit juice inhibits CYP3A4-mediated first-pass metabolism by direct inactivation of small intestinal CYP3A4 (Lown et al. 1997). Therefore, it

is probable that the considerable increase in the mean AUC value of buspirone observed in this study is due to a reduced gut first-pass metabolism caused by grapefruit juice. Findings in this study give support to the assumption that the gut wall is an important site for the biotransformation of buspirone, as it may be for some other CYP3A4 substrates with extensive first-pass metabolism. The magnitude of change in the pharmacokinetics of buspirone caused by grapefruit juice was almost the same as that in the pharmacokinetics of lovastatin, another CYP3A4 substrate with low bioavailability (Kantola et al. 1998a).

The prolonged $t_{max}$ of buspirone during the grapefruit-juice phase may reflect a delayed gastric emptying caused by the juice. The $t_{max}$-increasing effect of grapefruit juice has been seen in some but not all studies (Fuhr 1998). Lengthening of the elimination $t_{½}$ of buspirone may also be caused by postponed $t_{max}$ or may represent a minor effect of grapefruit juice on the systemic clearance of buspirone. Neither itraconazole nor the calcium-channel blockers verapamil and diltiazem greatly prolonged the elimination $t_{½}$ of buspirone (Kivistö et al. 1997; Lamberg et al. 1998a).

The considerably increased plasma buspirone concentrations during the grapefruit-juice phase were associated with only slightly altered pharmacodynamic test results. The only significant change compared to the water phase was seen in the subjective overall drug effect. Relatively small changes in psychomotor test results in association with markedly increased buspirone concentrations have been seen also in other studies (Kivistö et al. 1997; Lamberg et al. 1998a). This can be in part explained by the logarithmic relationship between drug concentration and effect. In addition, it may be that the classic psychomotor tests reflect more sensitively the pharmacodynamic effects of benzodiazepines than those of buspirone. On the other hand, it seems that high concentrations of buspirone are associated with increased frequency of adverse effects (Kivistö et al. 1997; Lamberg et al. 1998a).

### 3. Effects of grapefruit juice on the pharmacokinetics of cisapride

This study demonstrated that repeated consumption of grapefruit juice considerably elevates plasma concentrations of cisapride. The AUC and $C_{max}$ were increased by about 140% and 80% after ingestion of grapefruit juice. In addition, the elimination $t_{½}$ of cisapride was significantly

increased by grapefruit juice. The grapefruit juice-cisapride interaction was, however, found to be subject to considerable interindividual variation.

The oral bioavailability of cisapride is about 50%, due to first-pass metabolism (Van Peer et al. 1986; McCallum 1991). Recently it has been demonstrated that cisapride is biotransformed mainly by CYP3A4 (Desta et al. 1999; Gotschall et al. 1999; Bohets et al. 2000). It is thus likely that the considerable increase observed in the AUC of cisapride in the grapefruit-juice phase of this study was caused by inhibition of CYP3A4. Inhibition of CYP3A4-mediated metabolism occurred probably to a large extent during the first pass in the small intestine, because the $C_{max}$ was increased by about 80%, whereas the increase in the elimination $t_{½}$, although significant, was only about 25%. Moreover, the changes in cisapride $C_{max}$ and in AUC(0-∞) caused by grapefruit juice were correlated, whereas no such correlation was evident between the elimination $t_{½}$ and AUC(0-∞) of cisapride. The magnitude of the grapefruit juice-cisapride interaction was clearly smaller than that of grapefruit juice-buspirone or of grapefruit juice-simvastatin (Studies I and III). This is conceivable in the light of the bioavailabilities of these drugs: 50% for cisapride but less than 5% for both buspirone and simvastatin; i.e., cisapride undergoes less extensive CYP3A4-mediated first-pass metabolism.

Cisapride has been a widely used drug and is generally well tolerated. However, concern has recently been raised as to its safety because of an increasing number of reported cases in which use of cisapride is associated with prolongation of the QT interval, ventricular tachycardia and even death. In the majority of these cases, cisapride has been used in high doses or patients have cardiac or renal disease or there has been concomitant use of CYP3A4 inhibitors like imidazole antifungals and macrolide antibiotics (Wysowski and Bacsanyi 1996). It seems that the increased risk for cisapride-induced arrhythmias is related to high cisapride concentrations. In a study by van Haarst et al. (1998) combined use of cisapride (10 mg given four times a day) and clarithromycin (500 mg twice a day) for 5 days caused 3-fold increases in cisapride concentrations and prolonged the $QT_c$ interval significantly from that during cisapride monotherapy. Furthermore, hypokalemia or administration of other $QT_c$ interval-prolonging drugs together with cisapride may precipitate its arrhythmogenic effect. *In vitro* cisapride can concentration-dependently block potassium channels in cardiac myocytes and thus prolong repolarization time and $QT_c$ interval (Puisieux et al. 1996; Drolet et al. 1998). In this study, the mean $QT_c$ interval was slightly but significantly prolonged from baseline during both treatment

periods. However, despite the considerable increase in plasma concentrations of cisapride caused by grapefruit juice, no difference was seen between the grapefruit juice and water phases in the $QT_c$ interval. It is possible that long-term coadministration of grapefruit juice and cisapride or use of a higher single dose of cisapride may have increased the effect of cisapride on the $QT_c$ interval. In a recent study by Gross et al., a single dose of grapefruit juice increased the AUC of cisapride by about 40% (1999) but had no $QT_c$ interval-increasing effect.

Based on such findings, the manufacturer of cisapride has recently warned against concomitant use of cisapride with potent CYP3A4 inhibitors including grapefruit juice, and sale of medicinal products containing cisapride was suspended in the USA, Canada, and Germany during the year 2000.

**4. Effects of grapefruit juice on the pharmacokinetics of simvastatin**

Grapefruit juice greatly altered the pharmacokinetics of simvastatin. The mean AUC and $C_{max}$ of simvastatin were 16- and 9-fold, respectively, in the grapefruit juice phase compared to control values, and simvastatin acid AUC was increased about 7-fold. Grapefruit juice increased the AUC of active and total HMG-CoA reductase inhibitors 2.4-fold and 3.6-fold.

Simvastatin is an inactive lactone prodrug converted to the pharmacologically active simvastatin acid by carboxyesterases and even non-enzymatically. Biotransformation of simvastatin to other metabolites occurs mainly in reactions catalyzed by CYP3A4. In plasma, simvastatin and its lactone metabolites are in dynamic equilibrium with their corresponding hydroxy acid forms (Cheng and Jusko 1993). Bioavailability of simvastatin is low, and it is probably extensively metabolized during the first pass not only in the liver but also in the gut wall, where CYP3A4 is abundantly expressed (Kolars et al. 1992; Kivistö et al. 1996; Paine et al. 1997). It is likely that the greatly increased $C_{max}$ and AUC values of simvastatin observed in the present study were caused by inhibition of the small intestinal CYP3A4 by grapefruit juice. This fits well the finding that grapefruit juice augments bioavailability of CYP3A4 substrates by reducing protein expression of CYP3A4 in the small intestine (Lown et al. 1997). As simvastatin acid is not prone to metabolism by CYP3A4, it is likely that its concentrations were increased together with simvastatin concentrations due to the dynamic equilibrium between the

two forms in the plasma. The changes observed in the AUC and $C_{max}$ values of active and total HMG-CoA reductase inhibitors measured by REA were smaller than those of simvastatin. This is probably caused in the grapefruit-juice phase by reduced production of metabolites of simvastatin which possess HMG-CoA reductase inhibitory activity. Unlike the $C_{max}$ and AUC, the elimination $t_{½}$ of simvastatin, simvastatin acid, and HMG-CoA reductase inhibitors remained essentially unaltered. This can be explained by the predominantly intestinal effect of grapefruit juice and also by the pharmacokinetic properties of simvastatin: it is a high-extraction drug metabolized to a large extent during the first pass, its systemic clearance insensitive to the inhibition of the CYP3A4 enzyme. The greatly increased AUC value of simvastatin in this study may also at least in part be explained by inhibition of the small intestinal P-glycoprotein. Simvastatin may be a substrate for P-gp, just as the structurally closely related lovastatin has been found to be (Kim et al. 1999). *In vitro*, components in grapefruit juice inhibit P-gp (Takanaga et al. 1998; Eagling et al. 1999), but to date it has remained unclear to what degree grapefruit juice affects P-gp activity *in vivo*. In the study by Lown et al. (1997), grapefruit juice did not alter protein expression of P-gp in the small intestine. However, it is possible that grapefruit juice can in other ways alter its function.

With the same brand of grapefruit juice and the identical dosing scheme of juice used in both studies, grapefruit juice increased the AUC and $C_{max}$ of simvastatin (Study III) somewhat more than those of buspirone (Study I). The estimated bioavailability of both of these drugs is less than 5%. Simvastatin and probably also buspirone are predominantly biotransformed by CYP3A4 (Prueksaritanont et al. 1997; Kivistö et al. 1997). The difference in the magnitude of change in pharmacokinetics of simvastatin and buspirone may be partially explained by the large interindividual variation in the amount of expressed CYP3A4 in the liver and small intestine and by the relatively small number of study subjects. Three subjects took part in both Study I and III. Interestingly, for one of these subjects the increase in the AUC of simvastatin was 38-fold and in the AUC of buspirone 20-fold. Thus, a relatively constant individual susceptibility even to drastic changes in drug metabolism due to CYP3A4 inhibition by grapefruit juice may exist.

Lovastatin, like simvastatin, is an HMG-CoA reductase inhibitor that has low bioavailability and undergoes CYP3A4-mediated metabolism (Jacobsen et al. 1999). Not unexpectedly, the magnitude of interaction of grapefruit juice with simvastatin and with lovastatin is very similar.

The same amount of grapefruit juice as in Study III increased the AUC of lovastatin 15-fold (Kantola et al. 1998a). In that study the effect of grapefruit juice on HMG-CoA reductase inhibitors was not determined. However, due to the very similar pharmacokinetic properties of simvastatin and lovastatin, it is probable that the results in that study and the present one would have resembled each other also in this respect.

The effect of grapefruit juice on the pharmacokinetics of simvastatin is nearly as great as that of itraconazole and greater than that of erythromycin or verapamil (Neuvonen et al. 1998; Kantola et al. 1998b). In reported cases of myopathy during administration of simvastatin, the patient had often also taken potent CYP3A4 inhibitors (Schmassmann-Suhijar et al. 1998; Jacobson et al. 1997). It seems that an increase in risk for rare but serious adverse effects of HMG-CoA reductase inhibitors such as myopathy and rhabdomyolysis is associated with high drug doses and high HMG-CoA reductase inhibitory activity (Tobert al. 1988). As the present studies demonstrate, grapefruit juice can drastically increase concentrations of certain orally ingested CYP3A4 substrates. For any given subject, prediction of the magnitude of the interaction is difficult, given the wide interindividual variation in expression and activity of CYP3A4. Thus, in some individuals repeated ingestion at least of high amounts of grapefruit juice may cause an elevation of serum simvastatin concentrations that can increase the risk for adverse-effects of simvastatin.

**5. Effects of grapefruit juice on the pharmacokinetics of atorvastatin and pravastatin**

Grapefruit juice increased the AUC of atorvastatin acid and atorvastatin lactone about 2.5-fold and 3.3-fold, and the AUC values of active and total HMG-CoA reductase inhibitors were higher during the grapefruit juice phase than during the water phase. The AUC of the principal active metabolite 2-hydroxyatorvastatin and its lactone form were reduced by grapefruit juice, and the elimination half-lives of both atorvastatin acid and 2-hydroxyatorvastatin and their respective lactones significantly prolonged. In contrast, grapefruit juice had no effect on the AUC, $C_{max}$, or elimination $t_{½}$ of pravastatin, whether measured by LC-MS-MS or REA.

Atorvastatin is an HMG-CoA reductase inhibitor administered as the calcium salt of the active hydroxy acid form of atorvastatin acid. Its bioavailability after oral administration is about

12%. Atorvastatin is extensively metabolized and has at least two active metabolites, 2-hydroxyatorvastatin and 4-hydroxyatorvastatin, which are formed by CYP3A4. It has been estimated that 70% of the HMG-CoA reductase inhibitory activity of atorvastatin is accounted for by its metabolites (Lea and McTavish 1997).

Grapefruit juice increased the concentration of atorvastatin considerably less than that of simvastatin in Study III. This most probably reflects the less extensive CYP3A4-mediated atorvastatin first-pass metabolism compared with that of simvastatin. However, that the magnitude of the effect of grapefruit juice on the pharmacokinetics of atorvastatin was nearly as great as that of itraconazole suggests the importance of the small intestine and CYP3A4 in atorvastatin first-pass metabolism (Kantola et al. 1998c). In addition to grapefruit juice and itraconazole, the known CYP3A4 inhibitor erythromycin has been found significantly to increase atorvastatin concentrations (Siedlik et al. 1999). During the grapefruit-juice phase, 2-hydroxyatorvastatin and 2-hydroxyatorvastatin lactone concentrations were significantly reduced. This most likely resulted from the reduced CYP3A4-mediated formation of 2-hydroxyatorvastatin from atorvastatin caused by grapefruit juice.

Pravastatin is a hydrophilic HMG-CoA reductase inhibitor administered as a sodium salt of the active pravastatin acid. About 34% of the pravastatin dose is absorbed after oral administration, and approximately 50% of this absorbed dose undergoes presystemic metabolism, the oral bioavailability of pravastatin being about 20%. Pravastatin is metabolized by several enzymes to at least 17 different metabolites that contribute less than does the parent pravastatin to HMG-CoA reductase inhibitory activity. In the present study, grapefruit juice very little affected the pharmacokinetics of parent pravastatin or HMG-CoA reductase inhibitors. Moreover, in previous studies the known CYP3A4 inhibitors itraconazole, erythromycin, and diltiazem did not much affect the pharmacokinetics of pravastatin (Neuvonen et al. 1998; Donahue et al. 1998; Azie et al. 1998). However, cyclosporine has been found to increase pravastatin concentrations significantly (Regazzi et al. 1994; Olbricht et al. 1997). It has been hypothesized that this interaction between pravastatin and cyclosporin may be caused at least in part by cyclosporine through the inhibition of membrane transporter proteins such as P-gp. For instance, inhibition of P-gp in the gut wall may increase serum pravastatin concentrations. In any case, it has been proposed that inhibition of CYP3A4 does not interfere with pravastatin metabolism to such a degree that this would be of clinical relevance (Jacobsen et al. 1999). It is

thus conceivable that grapefruit juice did not increase serum concentrations of pravastatin in this study because the predominant mechanism of action of grapefruit juice is CYP3A4 inhibition.

## 6. Effect of grapefruit juice dose on the interaction between grapefruit juice and triazolam

In the present study, in which the effect of grapefruit juice dose on the pharmacokinetics and pharmacodynamics of the orally administered CYP3A4 substrate triazolam was investigated by means of a randomized four-phase cross-over study, a single 200-ml dose of normal- or double-strength grapefruit juice increased the AUC of triazolam by about half, and repeated ingestion of double-strength grapefruit juice led to an increase of 150% in the AUC of triazolam. The $C_{max}$ of triazolam was increased 40 to 50% during the three grapefruit-juice phases compared with that of the water phase. The elimination $t_{½}$ of triazolam was increased by 54% during the repeated ingestion of grapefruit juice, but remained unaffected by single-dose grapefruit juice. Pharmacodynamic effects of triazolam were significantly greater during the multiple-dose grapefruit juice phase than the water phase.

Only a few studies have addressed the role of grapefruit juice dose in grapefruit juice-drug interactions. In a study by Edgar et al. (1992) 200 ml of normal-strength grapefruit juice affected the pharmacokinetics of felodipine as much as did double- strength juice at 200 ml. Thus, our results concerning the effects of a single dose of normal- and double-strength grapefruit juice on the $C_{max}$ and AUC of triazolam are in accordance with the findings of Edgar et al. The effect of repeated ingestion of grapefruit juice on felodipine pharmacokinetics was investigated by Lown et al. (1997), who found that a single dose (250 ml) of grapefruit juice increased the AUC and $C_{max}$ of felodipine by 116% and 225%, respectively; grapefruit juice ingested three times daily for 5 days increased felodipine AUC and $C_{max}$ by 211% and 335%. Our findings are in line with theirs, demonstrating that repeated ingestion of grapefruit juice has a greater effect on the AUC of CYP3A4 substrates than does one single dose. Lown et al. found that multiple-dose administration of grapefruit juice inactivated small intestinal CYP3A4 significantly more than did a single dose of juice. Thus it appears that inhibition of CYP3A4-

mediated metabolism accumulates when grapefruit juice is ingested repeatedly, probably because the rate of regeneration of the active enzyme is exceeded by its rate of inactivation.

Lown et al. did not report their elimination $t_{½}$ of felodipine, but the activity of their hepatic CYP3A4 was unaffected by multiple-dose grapefruit juice, as measured by erythromycin breath test. On the other hand, in our study the elimination $t_{½}$ of triazolam after multiple-dose grapefruit juice was significantly prolonged. Assuming that the volume of distribution of triazolam was unchanged, prolongation of the elimination $t_{½}$ of triazolam suggests that chronic administration of grapefruit juice can reduce systemic clearance of triazolam, probably by inhibiting the hepatic CYP3A4. It appears that the increase in triazolam AUC during the multiple-dose grapefruit juice phase compared to the single-dose phases can be explained by decreased hepatic elimination of triazolam, because the $t_{½}$ but not $C_{max}$ of triazolam was increased by the repeated ingestion of grapefruit juice. In previous studies, grapefruit juice has increased the $C_{max}$ and AUC of orally administered CYP3A4 substrates, but when administered intravenously has not affected their pharmacokinetics (Ducharme et al. 1995b; Kupferschmidt et al. 1995; Lundahl et al. 1997). However, in those studies a single dose of grapefruit juice was ingested once or twice in association with drug administration.

Coadministration of cisapride with a single dose of grapefruit juice has increased cisapride AUC by about 50% without altering the elimination $t_{½}$ of cisapride (Gross et al. 1999). In our Study III, both the AUC and elimination $t_{½}$ of cisapride were increased significantly after repeated ingestion of grapefruit juice. In a recent preliminary report of Veronese et al. (1999), ingestion of grapefruit juice three times daily for 3 days increased the AUC of intravenously administered midazolam 5.9-fold compared with that of their control, and activity of the hepatic CYP3A4 was inhibited by grapefruit juice as measured by erythromycin breath test. Thus, it appears that during repeated ingestion, active ingredients in grapefruit juice, e.g., the lipophilic compounds furanocoumarins, may reach the systemic circulation in concentrations sufficient to inhibit both the small intestinal and the hepatic CYP3A4.

## 7. Duration of effect of grapefruit juice on the pharmacokinetics of simvastatin

In this study the aim was to investigate how long after ingestion of grapefruit juice its effect on the pharmacokinetics of simvastatin lasts. Simvastatin, a substrate for CYP3A4, was used as the model drug, as it has proven to be very sensitive to CYP3A4 inhibition (Study III; Neuvonen et al. 1998; Kantola et al. 1998b). Grapefruit juice was ingested on 3 consecutive days to reveal its maximal duration of effect on the pharmacokinetics of the CYP3A4 substrate.

Administration of multiple-dose grapefruit juice with simvastatin increased the AUC of simvastatin 13.5-fold compared with the water phase. After an interval of 24 hours between ingestion of the final dose of grapefruit juice and intake of simvastatin, the effect of grapefruit juice was reduced to such a degree that the AUC of simvastatin was 2-fold that of the water phase. When simvastatin was given 3 days after cessation of grapefruit juice intake, the AUC of simvastatin was less than 50% greater than for the water phase. After an interval of 7 days between cessation of grapefruit juice intake and intake of simvastatin, no change occurred in the AUC of simvastatin. The mean change in the AUC value of simvastatin acid induced by grapefruit juice was smaller than the change in the AUC value of simvastatin, this increase vanishing in 3 days. This is in line with the previous observation that simvastatin acid is not readily metabolized by CYP3A4.

A previous study has shown that a single dose of grapefruit juice may increase felodipine $C_{max}$ and AUC for up to 24 and 10 hours (Lundahl et al. 1995). In another study, volunteers were given a single oral dose of nisoldipine with water, with grapefruit juice, or 14 to 96 hours after a 7-day period of thrice daily ingestion of grapefruit juice (Takanaga et al. 2000a). Grapefruit juice affected nisoldipine pharmacokinetics for at least 3 days after the last intake of juice. Taking into consideration previous results of the accumulating effect of repeated intake of grapefruit juice, our present findings seem to be in line with those of these two studies. Recently, Rogers et al. (1999) published a study in which volunteers ingested grapefruit juice for 3 consecutive days during breakfast and received 40 mg lovastatin in the evening. The AUC of lovastatin was increased about 1.9-fold by the grapefruit juice, thus demonstrating the significance of time-interval between the grapefruit juice and CYP3A4 substrate ingestion in the extent of grapefruit juice-drug interaction.

According to Lown et al. (1997) grapefruit juice alters the pharmacokinetics of CYP3A4 substrates by inactivating the small intestinal CYP3A4. Thus, duration of grapefruit-juice effect depends on the rate of de novo synthesis of the small intestinal CYP3A4. In the present study it was roughly estimated that the $t_{½}$ of effect of grapefruit juice on the AUC of simvastatin was shorter during the first day after grapefruit juice ingestion, 7 to 8 hours, than during the following days, 30 to 40 hours. Takanaga et al. constructed a pharmacokinetic model based on the results of two previous studies (Lundahl et al. 1995; Lundahl et al. 1998). According to this model, the terminal $t_{½}$ of CYP3A4 was 8 hours (Takanaga et al. 2000b). In the subsequent study with nisoldipine, the terminal $t_{½}$ of CYP3A4 was calculated to be 30 hours, i. e., at 3-fold to 4-fold greater than the initial value (8 hours). The authors speculate that one explanation for this divergence of results might be the different length of times of data collection between these two studies (Lundahl et al. 1995; Takanaga et al. 2000a). The intake scheme for grapefruit juice was also different between the two studies. On the basis of these studies it appears that a large portion of the small intestinal CYP3A4 is regenerated, and most of the effect of both single- and multiple-dose grapefruit juice disappears within 24 hours after juice intake.

Previous studies have assumed that the predominant mechanism of action of grapefruit juice is inhibition of CYP3A4-mediated first-pass metabolism mainly in the intestinal wall. On the other hand, one can speculate as to the contribution of this possible inhibition of the hepatic CYP3A4 or P-gp to duration of the grapefruit juice effect. In particular, it appears that the intestinal and hepatic CYP3A4 are not coregulated (Lown et al. 1994). Differences in the catalytic activity and relative amounts of CYP3A4 in the liver and intestine may constitute one cause of the interindividual variation observed in duration of the grapefruit juice effect. However, in one recent study, intravenous diltiazem did not affect the AUC of lovastatin, thus supporting previous proposals that the small intestine is the main site of the first-pass metabolism of lovastatin and thus probably also of simvastatin (Masica et al. 2000). The smaller the AUC initially, the larger the increase due to CYP3A4 inhibition caused by grapefruit juice (Lown et al. 1997).

There are studies suggesting that by inhibiting P-gp, grapefruit juice may alter significantly the pharmacokinetics of orally administered drugs (Takanaga et al. 1998; Edwards et al. 1999). However, Eagling et al. (1999) concluded that the *in vivo* effects of grapefruit juice are only to a minor extent caused by modulation of P-gp function. Furthermore, our results with

simvastatin are in line with those of Lundahl et al. (1995) and Takanaga et al. (2000a), who used dihydropyridine calcium-channel blockers as the model drugs, which are not good substrates for P-gp. Thus, in the present study, relationships between time after intake of grapefruit juice and its effects can probably be explained by inhibition of CYP3A4 of different degrees.

## 8. Clinical implications

Grapefruit juice can substantially interfere with the pharmacokinetics of some concomitantly ingested CYP3A4 substrates. CYP3A4 is a major drug-metabolizing enzyme in humans, participating in the metabolism of about half the drugs used therapeutically. Of the drugs studied in this series of investigations, simvastatin and cisapride seem to have the potential for clinically relevant pharmacokinetic interactions with grapefruit juice. Both drugs undergo considerable CYP3A4-mediated first-pass metabolism and produce dose-dependent and, even serious adverse effects. High serum concentrations of simvastatin increase risk for myopathy, and cisapride prolongs the QT interval in a concentration-dependent manner. Further, at least in high doses, grapefruit juice may increase risk of adverse effects of buspirone and atorvastatin.

That the pharmacokinetics of CYP3A4 substrates are subject to considerable interindividual variation makes it difficult to predict the magnitude of the grapefruit juice effect for any individual patient. The unpredictability of the effect can be further increased by variation in the content of the active components in grapefruit juice. It is recommended to refrain from simultaneous use of grapefruit juice with CYP3A4 substrates that have a substantial first-pass metabolism and a narrow therapeutic range. If the dose-response curve is flat, reduction of drug dose can be applied. It has been suggested that if an interaction between grapefruit juice and a CYP3A4 substrate is not excluded in a pharmacokinetic study, and this drug is contraindicated for use with itraconazole and erythromycin, then it should not be taken along with grapefruit juice (Kane and Lipsky 2000). Because of the additive CYP3A4 inhibitory effect, caution should be exercised if the patient is already using drugs that are considered moderate CYP3A4 inhibitors, e.g., diltiazem or verapamil.

The oral bioavailability of CYP3A4 substrates with extensive intestinal first-pass metabolism may be enhanced by their coingestion with grapefruit juice (Fuhr 1998; Ameer and Weintraub 1997). In the case of cyclosporine, for example, it may thus be possible to reduce doses and achieve significant cost savings. However, because of the unpredictability of the grapefruit juice effect, appropriate dose adjustments may necessitate more frequent cyclosporine blood-concentration monitoring, and this can reduce any savings gained.

## CONCLUSIONS

From the six studies the following conclusions can be drawn:

1. Grapefruit juice, particularly in multiple doses, may increase plasma concentrations of some CYP3A4 substrates with low oral bioavailability almost as strongly as does the potent CYP3A4 inhibitor itraconazole. Grapefruit juice probably acts mainly by inhibiting CYP3A4-mediated first-pass metabolism in the small intestine. However, some effects of grapefruit juice may result in part from modulation of the function of P-glycoprotein.

2. The magnitude of the effect of grapefruit juice on the pharmacokinetics of a CYP3A4 substrate is related to the extent of its CYP3A4-mediated first-pass metabolism.

3. Multiple-dose ingestion of grapefruit juice affects CYP3A4 substrate pharmacokinetics significantly more than does a single dose, and may also inhibit hepatic CYP3A4 during the elimination phase.

4. The effect of multiple-dose grapefruit juice on the pharmacokinetics of CYP3A4 substrate with extensive first-pass metabolism subsides markedly within 24 hours and disappears completely within 3 to 7 days after termination of ingestion of grapefruit juice.

5. Concomitant use of grapefruit juice with CYP3A4 substrates should be avoided if these are subject to extensive first-pass metabolism and have a narrow therapeutic range; alternatively, the dose of the CYP3A4 substrate drugs should be reduced accordingly.

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## REFERENCES


Ameer B, Weintraub RA. Drug interactions with grapefruit juice. *Clin Pharmacokinet* 1997;33:103-21

Ameer B, Weintraub RA, Johnson JV, Yost RA, Rouseff RL. Flavanone absorption after naringin, hesperidin, and citrus administration. *Clin Pharmacol Ther* 1996;60:34-40

Arnadottir M, Eriksson LO, Thysell H, Karkas JD. Plasma concentration profiles of simvastatin 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitory activity in kidney transplant recipients with and without ciclosporin. *Nephron* 1993;65:410-3

Azie NE, Brater DC, Becker PA, Jones DR, Hall SD. The interaction of diltiazem with lovastatin and pravastatin. *Clin Pharmacol Ther* 1998;64:369-77

Back DJ, Houlgrave R, Tna JF, Ward S, Orme LE. Effect of progestogens, gestodene, 3-ketodesogestrel, levonorgestrel, norethinsterone and norgestimate on the oxidation of ethinylestradiol and other substrates by human liver microsomes. *J Steroid Biochem Mol Biol* 1991;38:219-25

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

Back DJ, Tjia JF, Karbwang J, Colbert J. In vitro inhibition studies of tolbutamide hydroxylase activity of human liver microsomes by azoles, sulphonamides and quinolines. *Br J Clin Pharmacol* 1988;26:23-9

Backman JT, Olkkola KT, Aranko K, Himberg J-J, Neuvonen PJ. Dose of midazolam should be reduced during diltiazem and verapamil treatments. *Br J Clin Pharmacol* 1994;37:221-5

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

Bailey DG, Arnold JMO, Bend JR, Tran LT, Spence JD. Grapefruit juice-felodipine interaction: reproducibility and characterization with the extended release drug formulation. *Br J Clin Pharmacol* 1995;40:135-40

Bailey DG, Arnold JMO, Munoz C, Spence JD. Grapefruit juice-felodipine interaction: mechanism, predictability, and effect of naringin. *Clin Pharmacol Ther* 1993a;53:637-42

Bailey DG, Arnold JMO, Strong HA, Munoz, Spence JD. Effect of grapefruit juice and naringin on nisoldipine pharmacokinetics. *Clin Pharmacol Ther* 1993b;54:589-94

Bailey DG, Kreeft JH, Munoz C, Freeman DJ, Bend JR. Grapefruit juice-felodipine interaction: Effect of naringin and 6',7'-dihydroxybergamottin in humans. *Clin Pharmacol Ther* 1998;64:248-56

Bailey DG, Spence JD, Munoz C, Arnold JMO. Interaction of citrus juices with felodipine and nifedipine. *Lancet* 1991;337:268-9

Balster RL. Abuse potential of buspirone and related drugs. *J Clin Psychopharmacol* 1990;10:31S-37S

Barbhaiya RH, Shukla UA, Pfeffer M, Pittman KA, Shrotriya R, Laroudie C, Gammans RE. Disposition kinetics of buspirone in patients with renal or hepatic impairment after administration of single and multiple doses. *Eur J Clin Pharmacol* 1994;46:41-7

Bateman DN. The action of cisapride on gastric emptying and the pharmacokinetics of oral diazepam. *Eur J Clin Pharmacol* 1986;30:205-8

Bedford TA, Rowbotham DJ. Cisapride. Drug interactions of clinical significance. *Drug Safety* 1996;15:167-75

Benton RE, Honig PK, Zamani K, Cantilena LR, Woosley RL. Grapefruit juice alters terfenadine pharmacokinetics, resulting in prolongation of repolarization on the electrocardiogram. *Clin Pharmacol Ther* 1996;59:383-8

Bertilsson L. Geographical/interracial differences in polymorphic drug oxidation. Current state of knowledge of cytochromes P450(CYP)2D6 and 2C19. *Clin Pharmacokinet* 1995;29:192-209

Bertilsson L, Henthorn TK, Sanz E, Tybring G, Säwe J, Villén T. Importance of genetic factors in the regulation of diazepam metabolism: Relationship to S-mephenytoin, but not debrisoquin, hydroxylation phenotype. *Clin Pharmacol Ther* 1989;45:348-55

Bertilsson L, Tybring G, Widén J, Chang M, Tomson T. Carbamazepine treatment induces the CYP3A4 catalysed sulphoxidation of omeprazole, but has less or no effect on hydroxylation via CYP2C19. *Br J Clin Pharmacol* 1997;44:186-9

Bohets H, Lavrijsen K, Hendrickx J, van Houdt J, van Genechten V, Verboven P, Meuldermans W, Heykants J. Identification of the cytochrome P450 enzymes involved in the metabolism of cisapride: in vitro studies of potential co-medication interactions. *Br J Pharmacol* 2000;129:1655-67

Boyd RA, Stern RH, Stewart BH, Wu X, Reyner EL, Zegarac EA, Randinitis EJ, Whitfield L. Atorvastatin coadministration may increase digoxin concentrations by inhibition of intestinal P-glycoprotein-mediated secretion. *J Clin Pharmacol* 2000;40:91-8

Breyer-Pfaff U, Pfandl B, Nill K, Nusser E, Monney C, Jonzier-Perey M, Baettig D, Baumann P. Enantionselective amitriptyline metabolism in patients phenotyped for two cytochrome P450 isozymes. *Clin Pharmacol Ther* 1992;52:350-8

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

Buch AB, van Harken DR, Seidehamel RJ, Barbhaiya RH. A study of pharmacokinetic interaction between buspirone and alprazolam at steady state. *J Clin Pharmacol* 1993;33:1104-9

Butler MA, Iwasaki M, Guengerich FP, Kadlupar FF. Human cytochrome P-450PA (P-450IA2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines. *Proc Natl Acad Sci U S A* 1989;86:7696-700

Capewell S, Freestone S, Critchley JAJH, Pottage A, Prescott LF. Reduced felodipine bioavailability in patients taking anticonvulsants. *Lancet* 1988;2:480-2

Cheng H, Jusko WJ. Pharmacokinetics of reversible metabolic systems. *Biopharm Drug Dispos* 1993;14:721-66

Cholerton S, Daly AK, Idle JR. The role of individual human cytochromes P450 in drug metabolism and clinical response. *Trends Pharmacol Sci* 1992;13:434-9

Cilla DD, Whitfield LR, Gibson DM, Sedman AJ, Posvar EL. Multiple-dose pharmacokinetics, pharmacodynamics, and safety of atorvastatin, an inhibitor of HMG-CoA reductase, in healthy subjects. *Clin Pharmacol Ther* 1996;60:687-95

Cimino M, Ponzio F, Achilli G, Vantini G, Perego C, Algeri S, Garattini S. Dopaminergic effects of buspirone, a novel anxiolytic agent. *Biochem Pharmacol* 1983;32:1069-74

Cohn JB, Wilcox CS. Low-sedation potential of buspirone compared with alprazolam and lorazepam in the treatment of anxious patients: a double-blind study. *J Clin Psychiatry* 1986;47:409-12

Cole JO, Orzack MH, Beake B, Bird M, Bar-Tal Y. Assessment of the abuse liability of buspirone in recreational sedative users. *J Clin Psychiatry* 1982;43 (12 sec 2):69-74

Conney AH, Pantuck EJ, Kuntzman R, Kappas A, Anderson KE, Alvares AP. Nutrition and chemical biotransformations in man. *Clin Pharmacol Ther* 1977;22:707-19

Decker CJ, Rashed MS, Baillie TA, Maltby D, Correia MA. Oxidative metabolism of spironolactone: evidence for the involvement of electrophilic thiosteroid species in drug-mediated destruction of rat hepatic cytochrome P450. *Biochem* 1989;28:5128-36

Desta Z, Thacker D, Soukhova N, Shin JG, Flockhart DA. Identification and characterization of human cytochrome P450 (CYP) isoforms interacting with cisapride [abstract]. *Clin Pharmacol Ther* 1999;65:126

Dollery C, ed. Therapeutic drugs, vol 2. Edinburgh, Great Britain: Churchill Livingstone, 1999:T171-T174

Donahue S, Chen L, Wason S, Norton J, Franc J. Effect of erythromycin on the pharmacokinetics of pravastatin and simvastatin. European Atherosclerosis Society 70.th congress, Geneva, Switzerland (Abstract book) 1998:50

Drolet B, Khalifa M, Daleau P, Hamelin BA, Turgeon J. Block of the rapid component of the delayed rectifier potassium current by the prokinetic agent cisapride underlies drug-related lengthening of the QT interval. *Circulation* 1998;97:204-10

Ducharme MP, Slaughter RL, Warbasse LH, Chandrasekar PH, Van de Velde V, Mannens G, Edwards DJ. Itraconazole and hydroxyitraconazole serum concentrations are reduced more than tenfold by phenytoin. *Clin Pharmacol Ther* 1995a;58:617-24

Ducharme MP, Warbasse LH, Edwards DJ. Disposition of intravenous and oral cyclosporine after administration with grapefruit juice. *Clin Pharmacol Ther* 1995b;57:485-91

Duell PB, Connor WE, Illingworth DR. Rhabdomyolysis after taking atorvastatin with gemfibrozil. *Am J Cardiol* 1998;81:368-9

Eagling VA, Back DJ, Barry MG. Differential inhibition of cytochrome P450 isoforms by the protease inhibitors, ritonavir, saquinavir and indinavir. *Br J Clin Pharmacol* 1997;44:190-4

Eagling VA, Profit L, Back DJ. Inhibition of the CYP3A4-mediated metabolism and P-glycoprotein-mediated transport of the HIV-1 protease inhibitor saquinavir by grapefruit juice components. *Br J Clin Pharmacol* 1999;48:543-52

Eberts FS, Philopoulos Y, Reineke LM, Vliek RW. Triazolam disposition. *Clin Pharmacol Ther* 1981;29:81-93

Edgar B, Bailey D, Bergstrand R, Johnsson G, Regårdh CG. Acute effects of drinking grapefruit juice on the pharmacokinetics and dynamics of felodipine in healthy subjects. *Eur J Clin Pharmacol* 1992;42:313-7

Edgar B, Regårdh CG, Johnsson G, Johansson L, Lundborg P, Löfberg I, Rönn O. Felodipine kinetics in healthy man. *Clin Pharmacol Ther* 1985;38:205-11

Edwards DJ, Bellevue FH, Woster PM. Identification of 6',7'-dihydroxybergamottin, a cytochrome P450 inhibitor, in grapefruit juice. *Drug Metab Dispos* 1996;24:1287-90

Edwards DJ, Fitzsimmons ME, Schuetz EG, Yasuda K, Ducharme MP, Warbasse LH, Woster PM, Schuetz JD, Watkins P. 6'7'-Dihydroxybergamottin in grapefruit juice and Seville orange juice: effects on cyclosporine disposition, enterocyte CYP3A4, and P-glycoprotein. *Clin Pharmacol Ther* 1999;65:237-44

Eison AS, Temple DL. Buspirone: review of its pharmacology and current perspectives on its mechanism of action. *Am J Med* 1986;80 (suppl 3b):1-9

Everett DW, Chando TJ, Didonato GC, Singhvi SM, Pan HY, Weinstein SH. Biotransformation of pravastatin sodium in humans. *Drug Metab Dispos* 1991;19:740-8

Finet L, Westeel PF, Hary L, Maurel G, Andrejak M, Dupas JL. Effects of cisapride on the intestinal absorption of cyclosporine in renal transplant recipients. *Gastroenterology* 1991;100 (5 Pt2):A209

Fitzsimmons ME, Collins JM. Selective biotransformation of the human immunodeficiency virus protease inhibitor saquinavir by human small-intestinal cytochrome P4503A4: potential contribution to high first-pass metabolism. *Drug Metab Dispos* 1997;25:256-66

Fuhr U. Drug interactions with grapefruit juice. Extent, probable mechanism and clinical relevance. *Drug Saf* 1998;18:251-72

Fuhr U, Klittich K, Staib AH. Inhibitory effect of grapefruit juice and its bitter principal, naringenin, on CYP1A2 dependent metabolism of caffeine in man. *Br J Clin Pharmacol* 1993;35:431-6

Fuhr U, Kummert AL. The fate of naringin in humans: a key to grapefruit juice-drug interactions? *Clin Pharmacol Ther* 1995;58:365-73

Fuhr U, Maier-Brüggemann A, Blume H, Mück W, Unger S, Kuhlmann J, Huschka C, Zaigler M, Rietbrock S, Staib AH. Grapefruit juice increases oral nimodipine bioavailability. *Int J Clin Pharmacol Ther* 1998;36:126-32

Fuhr U, Maier A, Keller A, Steinijans VW, Sauter R, Staib AH. Lacking effect of grapefruit juice on theophylline pharmacokinetics. *Int J Clin Pharmacol Ther* 1995;33:311-4

Fukuda K, Ohta T, Oshima Y, Ohashi N, Yoshikawa M, Yamazoe Y. Specific CYP3A4 inhibitors in grapefruit juice: furocoumarin dimers as components of drug interaction. *Pharmacogenetics* 1997;7:391-6

Gaillard Y, Gay-Montchamp JP, Ollagnier M. Simultaneous screening and quantitation of alpidem, buspirone and benzodiazepines by dual-channel gas chromatography using electron-capture and nitrogen-phosphorus detection after solid-phase extraction. *J Chromatogr* 1993;622:197-208

Gammans RE, Mayol RF, LaBudde JA. Metabolism and disposition of buspirone. *Am J Med* 1986;80 (suppl3b):41-51

Gammans RE, Pfeffer M, Westrick ML, Faulkner HC, Rehm KD, Goodson PJ. Lack of interaction between cimetidine and buspirone. *Pharmacotherapy* 1987;7:72-9

Garg SK, Kumar N, Bhargava VK, Prabhakar SK. Effect of grapefruit juice on carbamazepine bioavailability in patients with epilepsy. *Clin Pharmacol Ther* 1998;64:286-8

Gibson DM, Bron NJ, Richens A, Hounslow NJ, Sedman AJ, Whitfield LR. Effect of age and gender on pharmacokinetics of atorvastatin in humans. *J Clin Pharmacol* 1996a;36:242-6

Gibson DM, Yang BB, Abel RB, Blum RA, Horton M, Stern RH, Sedman JA, Whitfield LR. Effect of hepatic and renal impairment on pharmacokinetics and pharmacodynamics of atorvastatin [abstract]. *Pharm Res* 1996b;13 (suppl):S428

Goldberg HL, Finnerty RL. The comparitive efficacy of buspirone and diazepam in the treatment of anxiety. *Am J Psychiatry* 1979;136:1184-7

Gorski JC, Hall SD, Jones DR, VandenBranden, Wrighton SA. Regioselective biotransformation of midazolam by members of the human cytochrome P450 3A (CYP) subfamily. *Biochem Pharmacol* 1994;47:1643-53

Gotschall RR, Marcucci K, Leeder JS, Kearns GL. Cisapride biotransformation: not all CYP3As are created equal [abstract]. *Clin Pharmacol Ther* 1999;65:127

Greenblatt DJ, Divoll M, Abernethy DR, Moschitto LJ, Smith RB, Shader RI. Reduced clearance of triazolam in old age: relation to antipyrine oxidicing capacity. *Br J Clin Pharmacol* 1983;15:303-9

Greenblatt DJ, Harmatz JS, Gouthro TA, Locke J, Shader RI. Distinguishing a benzodiazepine agonist (triazolam) from a nonagonist anxiolytic (buspirone) by electroencephalography: kinetic-dynamic studies. *Clin Pharmacol Ther* 1994;56:100-11

Gross AS, Goh YD, Addison RS, Shenfield GM. Influence of grapefruit juice on cisapride pharmacokinetics. *Clin Pharmacol Ther* 1999;65:395-401

Grygiel JJ, Birkett DJ. Cigarette smoking and theophylline clearance and metabolism. *Clin Pharmacol Ther* 1981;30:491-6

Guengerich FP. Oxidation of 17 alpha-ethynylestradiol by human liver cytochrome P-450. *Mol Pharmacol* 1988;33:500-8

Guengerich FP. Mechanism-based inactivation of human liver microsomal cytochrome P-450 IIIA4 by gestodene. *Chem Res Toxicol* 1990a;3:363-71

Guengerich FP, Brian WR, Iwasaki M, Sari MA, Baarnhielm C, Berntsson P. Oxidation of dihydropyridine calcium channel blockers and analogues by human liver cytochrome P-450 IIIA4. *J Med Chem* 1991;34:1838-44

Guengerich FP, Kim D-H. In vitro inhibition of dihydropyridine oxidation and aflatoxin $B_1$ activation in human liver microsomes by naringenin and other flavonoids. *Carcinogenesis* 1990b;11:2275-9

Guo L-Q, Fukuda K, Ohta T, Yamazoe Y. Role of furanocoumarin derivatives on grapefruit juice-mediated inhibition of human CYP3A activity. *Drug Metab Dispos* 2000;28:766-71

Hagen RE, Dunlap WJ, Mizelle JW, Wender SH, Lime BJ, Albach RF, Griffiths FP. A chromatographic-fluorometric method for determination of naringenin rutinoside, and related flavanone blycosides in grapefruit juice and in juice sacs. *Anal Biochem* 1965;12:472-82

Hakkola J, Tanaka E, Pelkonen O. Developmental expression of cytochrome P450 enzymes in human liver. *Pharmacol Toxicol* 1998;82:209-17

Hamelin BA, Turgeon J. Hydrophilicity/lipophilicity: relevance for the pharmacology and clinical effects of HMG-CoA reductase inhibitors. *Trends Pharmacol Sci* 1998;19:26-37

He K, Iyer KR, Hayes RN, Sinz MW, Woolf TF, Hollenberg PF. Inactivation of cytochrome P450 3A4 by bergamottin, a component of grapefruit juice. *Chem Res Toxicol* 1998;11:252-9

Hollander AAMJ, van Rooij J, Lentjes EGWM, Arbouw F, van Bree JB, Schoemaker RC, van Es LA, van der Woude FJ, Cohen AF. The effect of grapefruit juice on cyclosporine and prednisone metabolism in transplant patients. *Clin Pharmacol Ther* 1995;57:318-24

Honig PK, Wortham DC, A Lazarev, Cantilena LR. Grapefruit juice alters the systemic bioavailability and cardiac repolarization of terfenadine in poor metabolizers of terfenadine. *J Clin Pharmacol* 1996;36:345-51

Huang HF, Jann MW, Wei F-C, Chang T-P, Chen J-S, Juang D-J, Lin S-K, Lam YWF, Chien C-P, Chang W-H. Lack of pharmacokinetic interaction between buspirone and haloperidol in patients with schizophrenia. *J Clin Pharmacol* 1996;36:963-9

Hukkinen SK, Varhe A, Olkkola KT, Neuvonen PJ. Plasma concentrations of triazolam are increased by concomitant ingestion of grapefruit juice. *Clin Pharmacol Ther* 1995;58:127-31

Hunt CM, Watkins PB, Saenger P, Stave GM, Barlascini N, Watlington CO, Wright JT Jr, Guzelian PS. Heterogeneity of CYP3A isoforms metabolizing erythromycin and cortisol. *Clin Pharmacol Ther* 1992;51:18-23

Inaba T, Tyndale RE, Mahon WA. Quinidine: potent inhibition of sparteine and debrisoquine oxidation in vivo. *Br J Clin Pharmacol* 1986;22:199-200

Ioannides-Demos LL, Christophidis N, Ryan P, Angelis P, Liolios L, McLean AJ. Dosing implications of a clinical interaction between grapefruit juice and cyclosporine and metabolite concentrations in patients with autoimmune diseases. *J Rheumatol* 1997;24:49-54

Jacobsen W, Kirchner G, Hallensleben K, Mancinelli L, Deters M, Hackbarth I, Benet LZ, Sewing K-F, Christians U. Comparison of cytochrome P-450-dependent metabolism and drug interactions of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors lovastatin and pravastatin in the liver. *Drug Metab Dispos* 1999;27:173-9

Jacobson RH, Wang P, Glueck CJ. Myositis and rhabdomyolysis associated with concurrent use of simvastatin and nefazodone. *JAMA* 1997;277:296-7

Jajoo HK, Blair IA, Klunk LJ, Mayol RF. In vitro metabolism of the antianxiety drug buspirone as a predictor of its metabolism in vivo. *Xenobiotica* 1990;20:779-86

Jajoo HK, Mayol RF, LaBudde JA, Blair IA. Metabolism of the antianxiety drug buspirone in human subjects. *Drug Metab Dispos* 1989;17:634-40

Jeppesen U, Gram LF, Vistisen K, Loft S, Poulsen HE, Brøsen K. Dose-dependent inhibition of CYP1A2, CYP2C19 and CYP2D6 by citalopram, fluoxetine, fluvoxamine and paroxetine. *Eur J Clin Pharmacol* 1996;51:73-8

Josefsson M, Zackrisson A-L, Ahlner J. Effect of grapefruit juice on the pharmacokinetics of amlodipine in healthy volunteers. *Eur J Clin Pharmacol* 1996;51:189-93

Kamimori GH, Sirisuth N, Greenblatt DJ, Eddington ND. The influence of the menstrual cycle on triazolam and indocyanine green pharmacokinetics. *J Clin Pharmacol* 2000;40:739-44

Kane GC, Lipsky JJ. Drug-grapefruit juice interactions. *Mayo Clin Proc* 2000;75:933-42

Kantola T, Kivistö KT, Neuvonen PJ. Grapefruit juice greatly increases serum concentrations of lovastatin and lovastatin acid. *Clin Pharmacol Ther* 1998a;63:397-402

Kantola T, Kivistö KT, Neuvonen PJ. Erythromycin and verapamil considerably increase serum simvastatin and simvastatin acid concentrations. *Clin Pharmacol Ther* 1998b;64:177-82

Kantola T, Kivistö KT, Neuvonen PJ. Effect of itraconazole on the pharmacokinetics of atorvastatin. *Clin Pharmacol Ther* 1998c;64:58-65

Kashuba AD, Bertino JS Jr, Rocci ML Jr, Kulawy RW, Beck DJ, Nafziger AN. Quantification of 3-month intraindividual variability and the influence of sex and menstrual cycle phase on CYP3A activity as measured by phenotyping with intravenous midazolam. *Clin Pharmacol Ther* 1998;64:269-77

Kharasch ED, Russell M, Garton K, Lentz G, Bowdle TA, Cox K. Assessment of cytochrome P450 3A4 activity during the menstrual cycle using alfentanil as a noninvasive probe. *Anesthesiology* 1997;87:26-35

Kim RB, Wandel C, Leake B, Cvetkovic M, Fromm MF, Dempsey PJ, Roden MM, Belas F, Chaudhary AK, Roden DM, Wood AJJ, Wilkinson GR. Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. *Pharm Res* 1999;16:408-14

Kirch W, Janisch HD, Ohnhaus EE, van Peer A. Cisapride-cimetidine interaction: enhanced cisapride bioavailability and accelerated cimetidine absorption. *Ther Drug Monit* 1989;11:411-4

Kivistö KT, Bookjans G, Fromm MF, Griese E-U, Munzel P, Kroemer HK. Expression of CYP3A4, CYP3A5 and CYP3A7 in human duodenal tissue. *Br J Clin Pharmacol* 1996;42:387-9

Kivistö KT, Laitila J, Mårtensson K, Neuvonen PJ. Determination of buspirone and 1-(2-pyrimidinyl)-piperazine (1-PP) in human plasma by capillary gas chromatography. *Ther Drug Monit* 1999;21:317-21

Kivistö KT, Lamberg TS, Kantola T, Neuvonen PJ. Plasma buspirone concentrations are greatly increased by erythromycin and itraconazole. *Clin Pharmacol Ther* 1997;62:348-54

Knodell RG, Hall SD, Wilkinson GR, Guengerich FP. Hepatic metabolism of tolbutamide: characterization of the form of cytochrome P-450 involved in methyl hydroxylation and relationship to *in vivo* disposition. *J Pharmacol Exp Ther* 1987;241:1112-9

Kolars JC, Lown KS, Schmiedlin-Ren P, Ghosh M, Fang C, Wrighton SA, Merion RM, Watkins PB. CYP3A gene expression in human gut epithelium. *Pharmacogenetics* 1994;4:247-59

Kolars JC, Schmiedlin-Ren P, Schuetz JD, Fang C, Watkins PB. Identification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes. *J Clin Invest* 1992;90:1871-8

Kosuge K, Nishimoto M, Kimura M, Umemura K, Nakashima M, Ohashi K. Enhanced effect of triazolam with diltiazem. *Br J Clin Pharmacol* 1997;43:367-72

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

Kroboth PD, Smith RB, Silver MR, Rault R, Sorkin MI, Puschett JB, Juhl RP. Effects of end stage renal disease and aluminium hydroxide on triazolam pharmacokinetics. *Br J Clin Pharmacol* 1985;19:839-42

Kronbach T, Fischer V, Meyer UA. Cyclosporine metabolism in human liver: identification of a cytochrome P-450III gene family as the major cyclosporine-metabolizing enzyme explains interactions of cyclosporine with other drugs. *Clin Pharmacol Ther* 1988;43:630-5

Kronbach T, Mathys D, Umeno M, Gonzalez FJ, Meyer UA. Oxidation of midazolam and triazolam by human liver cytochrome P450IIIA4. *Mol Pharmacol* 1989;36:89-96

Kunze KL, Trager WF. Isoform-selective mechanism-based inhibition of human cytochrome P450 1A2 by furafylline. *Chem Res Toxicol* 1993;6:649-56

Kupferschmidt HHT, Fattinger KE, Ha HR, Follath F, Krähenbühl S. Grapefruit juice enhances the bioavailability of the HIV protease inhibitor saquinavir in man. *Br J Clin Pharmacol* 1998;45:355-9

Kupferschmidt HHT, Ha HR, Ziegler WH, Meier PJ, Krähenbühl S. Interaction between grapefruit juice and midazolam in humans. *Clin Pharmacol Ther* 1995;58:20-8

Lamberg TS, Kivistö KT, Laitila J, Mårtensson K, Neuvonen PJ. The effect of fluvoxamine on the pharmacokinetics and pharmacodynamics of buspirone. *Eur J Clin Pharmacol* 1998b;54:761-6

Lamberg TS, Kivistö KT, Neuvonen PJ. Effects of verapamil and diltiazem on the pharmacokinetics and pharmacodynamics of buspirone. *Clin Pharmacol Ther* 1998a;63:640-5

Lamberg TS, Kivistö KT, Neuvonen PJ. No significant effect of terfenadine on the pharmacokinetics of the CYP3A4 substrate buspirone. *Pharmacol Ther* 1999;84:165-9

Lamberg TS, Kivistö KT, Neuvonen PJ. Concentrations and effects of buspirone are considerably reduced by rifampicin. *Br J Clin Pharmacol* 1998c;45:381-5

Lea AP, McTavish D. Atorvastatin. A review of its pharmacology and therapeutic potential in the management of hyperlipidaemias. *Drugs* 1997;53:828-47

Leemann T, Transon C, Dayer P. Cytochrome P450TB (CYP2C): a major monooxygenase catalyzing diclofenac 4'-hydroxylation in human liver. *Life Sci* 1993;52:29-34

Lennernäs H, Fager G. Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. *Clin Pharmacokinet* 1997;32:403-25

Leo MA, Lasker JM, Raucy JL, Kim CI, Black M, Lieber CS. Metabolism of retinol and retinoic acid by human liver cytochrome P450IIC8. *Arch Biochem Biophys* 1989;269:305-12

Lewin MB, Bryant RM, Fenrich AL, Grifka RG. Cisapride induced long QT interval. *J Pediatr* 1996;128:279-81

Longbottom RT, Pleuvry BJ. Respiratory and sedative effects of triazolam in volunteers. *Br J Anaesth* 1984;56:179-85

Lown KS, Bailey DG, Fontana RJ, Janardan SK, Adair CH, Fortlage LA, Brown MB, Guo W, Watkins PB. Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression. *J Clin Invest* 1997;99:2545-53

Lown KS, Ghosh M, Watkins PB. Sequences of intestinal and hepatic cytochrome P450 3A4 cDNAs are identical. *Drug Metab Dispos* 1998;26:185-7

Lown KS, Kolars JC, Thummel KE, Barnett JL, Kunze KL, Wrighton SA, Watkins PB. Interpatient heterogeneity in expression of CYP3A4 and CYP3A5 in small bowel. Lack of prediction by the erythromycin breath test. *Drug Metab Dispos* 1994;22:947-55

Lum BL, Gosland MP. MDR expression in normal tissues: pharmacologic implications for the clinical use of P-glycoprotein inhibitors. *Hematol Oncol Clin North Am* 1995;9:319-36

Lundahl J, Regårdh CG, Edgar B, Johnsson G. Relationship between time of intake of grapefruit juice and its effect on pharmacokinetics and pharmacodynamics of felodipine in healthy subjects. *Eur J Clin Pharmacol* 1995;49:61-7

Lundahl J, Regårdh CG, Edgar B, Johnsson G. Effects of grapefruit juice ingestion - pharmacokinetics and haemodynamics of intravenously and orally administered felodipine in healthy men. *Eur J Clin Pharmacol* 1997;52:139-45

Lundahl JU, Regårdh CG, Edgar B, Johnsson G. The interaction effect of grapefruit juice is maximal after the first glass. *Eur J Clin Pharmacol* 1998;54:75-81

Manning JA, Swanson BN, Emaus NA, Fry J. A semiautomated, radioenzymatic assay for pravastatin and lovastatin in human serum [abstract]. *Pharm Res* 1989;6:S237

Masica AL, Azie NE, Brater DC, Hall SD, Jones DR. Intravenous diltiazem and CYP3A-mediated metabolism. *Br J Clin Pharmacol* 2000;50:273-6

Mauro VF. Clinical pharmacokinetics and practical applications of simvastatin. *Clin Pharmacokinet* 1993;24:195-202

Mayol RF, Adamson DS, Gammans RE, LaBudde JA. Pharmacokinetics and disposition of $^{14}$C-buspirone HCl after intravenous and oral dosing in man [abstract]. *Clin Pharmacol Ther* 1985;37:210

McCallum RW. Cisapride: a new class of prokinetic agent. *Am J Gastroenterol* 1991;86:135-49

McCallum RW, Prakash C, Campoli-Richards DM, Goa KL. Cisapride. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use as a prokinetic agent in gastrointestinal motility disorders. *Drugs* 1988;36:652-81

McLemore TL, Adelberg S, Liu MC, McMahon NA, Yu SJ, Hubbard WC, Czerwinski M, Wood TG, Storeng R, Lubet RA, Eggleston JC, Boyd MR, Hines RN. Expression of CYP1A1 gene in patients with lung cancer: evidence for cigarette smoke-induced gene expression in normal lung tissue and for altered gene regulation in primary pulmonary carcinomas. *J Natl Cancer Inst* 1990;82:1333-9

Meier C, Stey C, Brack T, Maggiorini M, Risti B, Krähenbühl S. Rhabdomyolysis in patients treated with simvastatin and cyclosporin: role of the hepatic cytochrome P450 enzyme system activity. *Schweiz Med Wochenschr* 1995;125:1342-6

Miniscalco A, Lundahl J, Regårdh CG, Edgar B, Eriksson UG. Inhibition of dihydropyridine metabolism in rat and human liver microsomes by flavonoids found in grapefruit juice. *J Pharmacol Exp Ther* 1992;261:1195-9

Morselli PL. Role of drug metabolism in drug disposition and effects. In: Pacifici GM, Fracchia GN, eds. Advances in drug metabolism in man. Luxenbourg: European Commission, Office for Official Publications of the European Communities, 1995:3-34

Mousa O, Brater DC, Sundblad KJ, Hall SD. The interaction of diltiazem with simvastatin. *Clin Pharmacol Ther* 2000;67:267-74

Murray M. Mechanisms and significance of inhibitory drug interactions involving cytochrome P450 enzymes (Review). *Int J Mol Med* 1999;3:227-38

Nakamura K, Goto F, Ray WA, McAllister CB, Jacqz E, Wilkinson GR, Branch RA. Interethnic differences in genetic polymorphism of debrisoquin and mephynytoin hydroxylation between Japanese and Caucasian populations. *Clin Pharmacol Ther* 1985;38:402-8

Naoumova RP, Dunn S, Rallidis L, Abu-Muhana O, Neuwirth C, Rendell NB, Taylor GW, Thompson GR. Prolonged inhibition of cholesterol synthesis explains the efficacy of atorvastatin. *J Lipid Res* 1997;38:1496-500

Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus IC, Nebert DW. P450 superfamily: update on new sequences, gene mapping, accession numbers and recommended nomenclature. *Pharmacogenetics* 1996;6:1-42

Neuvonen PJ, Jalava K-M. Itraconazole drastically increases plasma concentrations of lovastatin and lovastatin acid. *Clin Pharmnacol Ther* 1996;60:54-61

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

Newton RE, Marunycz JD, Alderdice MT, Napoliello MJ. Review of the side-effect profile of buspirone. *Am J Med* 1986;80 (suppl 3b):17-21

Olbricht C, Wanner C, Eisenhauer T, Kliem V, Doll R, Boddaert M, O'Grady P, Krekler M, Mangold B, Christians U. Accumulation of lovastatin, but not pravastatin, in the blood of cyclosporine-treated kidney graft patients after multiple doses. *Clin Pharmacol Ther* 1997;62:311-21

Olkkola KT, Aranko K, Luurila H, Hiller A, Saarnivaara L, Himberg J-J, Neuvonen PJ. A potentially hazardous interaction between erythromycin and midazolam. *Clin Pharmacol Ther* 1993;53:298-305

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

Paine MF, Khalighi M, Fisher JM, Shen DD, Kunze KL, Marsh CL, Perkins JD, Thummel KE. Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. *J Pharmacol Exp Ther* 1997;283:1552-62

Paine MF, Shen DD, Kunze KL, Perkins JD, Marsh CL, McVicar JP, Barr DM, Gillies BS, Thummel KE. First-pass metabolism of midazolam by the human intestine. *Clin Pharmacol Ther* 1996;60:14-24

Pakes GE, Brogden RN, Heel RC, Speight TM, Avery GS. Triazolam. A review of its pharmacological properties and therapeutic efficacy in patients with insomnia. *Drugs* 1981;22:81-110

Pan HY. Clinical pharmacology of pravastatin, selective inhibitor of HMG-CoA reductase. *Eur J Clin Pharmacol* 1991;40 (suppl 1):S15-S18

Pan HY, DeVault AR, Swites BJ, Whigan D, Ivashkiv E, Willard DA, Brescia D. Pharmacokinetics and pharmacodynamics of pravastatin alone and with cholestyramine in hypercholesterolemia. *Clin Pharmacol Ther* 1990a;48:201-7

Pan HY, DeVault AR, Wang-Iverson D, Ivashkiv E, Swanson BN, Sugerman AA. Comparative pharmacokinetics and pharmacodynamics of pravastatin and lovastatin. *J Clin Pharmacol* 1990b;30:1128-35

Pan HY, DeVault AR, Brescia D, Willard DA, McGovern ME, Whigan DB, Ivashkiv E. Effect of food on pravastatin pharmacokinetics and pharmacodynamics. *Int J Clin Pharmacol Ther Toxicol* 1993;31:291-4

Park JW, Merz M, Garcis D, Schüler S. Safety of long-term pravastatin treatment in heart transplant patients receiving cyclosporine. 2.5-years follow-up. European Atherosclerosis Society 70.th congress, Geneva, Switzerland (Abstract book) 1998:54

Pasanen M, Stenbäck F, Park SS, Gelboin HV, Pelkonen O. Immunohistochemical detection of human placental cytochrome P-450-associated mono-oxygenase system inducuble by maternal cigarette smoking. *Placenta* 1988;9:267-75

Patat A, Foulhoux P. Effect on postural sway of various benzodiazepine tranquillers. *Br J Clin Pharmacol* 1985;20:9-16

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

Pentikäinen PJ, Saraheimo M, Schwartz JI, Amin RD, Schwartz MS, Brunner-Ferber F, Rogers JD. Comparative pharmacokinetics of lovastatin, simvastatin and pravastatin in humans. *J Clin Pharmacol* 1992;32:136-40

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

Phillips JP, Antal EJ, Smith RB. A pharmacokinetic interaction between erythromycin and triazolam. *J Clin Psychopharmacol* 1986;6:297-9

Pichard L, Gillet G, Fabre I, Dalet-Beluche I, Bonfils C, Thenot J-P. Identification of the rabbit and human cytochromes P-450IIIA as the major enzymes involved in the N-demethylation of diltiazem. *Drug Metab Dispos* 1990;18:711-9

Plosker GL, McTavish D. Simvastatin. A reappraisal of its pharmacology and therapeutic efficacy in hypercholesterolaemia. *Drugs* 1995;50:334-63

Preechagoon Y, Charles BG. Analysis of cisapride in neonatal plasma using high-performance liquid chromatography with a base-stable column and fluorescence detection. *J Chromatogr B Biomed Sci Appl* 1995;670:139-43

Preiss R. P-glycoprotein and related transporters. *Int J Clin Pharmacol Ther* 1998;36:3-8

Proppe DG, Hoch OD, McLean JA, Visser KE. Influence of chronic ingestion of grapefruit juice on steady-state blood concentrations of cyclosporine A in renal transplant patients with stable graft function (letter). *Br J Clin Pharmacol* 1995;39:337-8

Prueksaritanont T, Gorham LM, Ma B, Liu L, Yu X, Zhao JJ, Slaughter DE, Arison BH, Vyas KP. In vitro metabolism of simvastatin in humans - identification of metabolizing enzymes and effect of the drug on hepatic P450s. *Drug Metab Dispos* 1997;25:1191-9

Puisieux FL, Adamantidis MM, Dumotier BM, Dupuis BA. Cisapride-induced prolongation of cardiac action potential and early afterdepolarizations in rabbit Purkinje fibres. *Br J Pharmacol* 1996;117:1377-9

Radulovic LL, Cilla DD, Posvar EL, Sedman AJ, Whitfield LR. Effect of food on the bioavailability of atorvastatin, an HMG-CoA reductase inhibitor. *J Clin Pharmacol* 1995;35:990-4

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

Rashid J, McKinstry C, Renwick AG, Dirnhuber M, Waller DG, George CF. Quercetin, an *in vitro* inhibitor of CYP3A, does not contribute to the interaction between nifedipine and grapefruit juice. *Br J Clin Pharmacol* 1993;36:460-3

Rau SE, Bend JR, Arnold JMO, Tran LT, Spence JD, Bailey DG. Grapefruit juice-terfenadine single-dose interaction: magnitude, mechanism, and relevance. *Clin Pharmacol Ther* 1997;61:401-9

Regazzi MB, Iacona I, Campana C, Gavazzi A, Viganò M, Perani G. Clinical efficacy and pharmacokinetics of HMG-CoA reductase inhibitors in heart transplant patients treated with cyclosporin A. *Transplant Proc* 1994;26:2644-45

Relling MV. Are the major effects of P-glycoprotein modulators due to the altered pharmacokinetics of anticancer drugs? *Ther Drug Monit* 1996;18:350-6

Renton KW. Inhibition of hepatic microsomal drug metabolism by the calcium channel blockers diltiazem and verapamil. *Biochem Pharmacol* 1985;34:2549-53

Rettie AE, Korzekwa KR, Kunze KL, Lawrence RF, Eddy AC, Aoyama T, Gelboin HV, Gonzalez FJ, Trager WF. Hydroxylation of warfarin by human cDNA-expressed cytochrome P-450: a role for P-4502C9 in the etiology of (S)-warfarin-drug interactions. *Chem Res Toxicol* 1992;5:54-9

Robin DW, Hasan SS, Lichtenstein MJ, Shiavi RG, Wood AJJ. Dose-related effect of triazolam on postural sway. *Clin Pharmacol Ther* 1991;49:581-8

Robin DW, Lee M, Hasan SS, Wood AJJ. Triazolam in cirrhosis: Pharmacokinetics and pharmacodynamics. *Clin Pharmacol and Ther* 1993;54:630-7

Rogers JD, Zhao J, Liu L, Amin RD, Gagliano KD, Porras AG, Blum RA, Wilson MF, Stepanavage M, Vega JM. Grapefruit juice has minimal effects on plasma concentrations of lovastatin-derived 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Clin Pharmacol Ther* 1999;66:358-66

Rowbotham DJ, Milligan K, McHugh P. Effect of cisapride on morphine absorption after oral administration of sustained-release morphine. *Br J Anaesth* 1991;67:421-5

Scavone JM, Greenblatt DJ, Locniskar A, Shader RI. Alprazolam pharmacokinetics in women on low-dose oral contraceptives. *J Clin Pharmacol* 1988;28:454-7

Schmassmann-Suhijar D, Bullingham R, Gasser R, Schmutz J, Haefeli WE. Rhabdomyolysis due to interaction of simvastatin with mibefradil. *Lancet* 1998;351:1929-30

Schmiedlin-Ren P, Edwards DJ, Fitzsimmons ME, He K, Lown KS, Woster PM, Rahman A, Thummel KE, Fisher JM, Hollenberg PF, Watkins PB. Mechanisms of enhanced oral availability of CYP3A4 substrates by grapefruit juice constituents. Decreased enterocyte CYP3A4 concentration and mechanism-based inactivation by furanocoumarins. *Drug Metab Dispos* 1997;25:1228-33

Schuetz EG, Beck WT, Schuetz JD. Modulators and substrates of P-glycoprotein and cytochrome P4503A coordianately up-regulate these proteins in human colon carcinoma cells. *Mol Pharmacol* 1996;49:311-8

Schubert W, Cullberg G, Edgar B, Hedner T. Inhibition of 17β-estradiol metabolism by grapefruit juice in ovariectomized women. *Maturitas* 1994;20:155-63

Segaert MF, De Soete C, Vandewiele I, Verbanck J. Drug-interaction-induced rhabdomyolysis. *Nephrol Dial Transplant* 1996;11:1846-7

Sekkarie MA. Torsades de pointes in two chronic renal failure patients treated with cisapride and clarithromycin. *Am J Kidney Dis* 1997;30:437-9

Sellers EM, Schneiderman JF, Romach MK, Kaplan HL, Somer GR. Comparative drug effects and abuse liability of lorazepam, buspirone, and secobarbital in nondependent subjects. *J Clin Psychopharmacol* 1992;12:79-85

Seppälä T, Aranko K, Mattila MJ, Shrotriya RC. Effects of alcohol on buspirone and lorazepam actions. *Clin Pharmacol Ther* 1982;32:201-7

Sesardic D, Pasanen M, Pelkonen O, Boobis AR. Differential expression and regulation of members of the cytochrome P450IA gene subfamily in human tissues. *Carcinogenesis* 1990;11:1183-8

Shimada T, Martin MV, Pruess-Schwartz D, Marnett LJ, Guengerich FP. Roles of individual human cytochrome P-450 enzymes in the bioactivation of benzo(a)pyrene, 7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene, and other dihydrodiol derivatives of polycyclic aromatic hydrocarbons. *Cancer Res* 1989;49:6304-12

Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* 1994;270:414-23

Shimada T, Yun CH, Yamazaki H, Gautier JC, Beaune PH, Guengerich FP. Characterization of human lung microsomal cytochrome P-450 1A1 and its role in the oxidation of chemical carcinogens. *Mol Pharmacol* 1992;41:856-64

Siedlik PH, Olson SC, Yang BB, Stern RH. Erythromycin coadministration increases plasma atorvastatin concentrations. *J Clin Pharmacol* 1999;39:501-4

Sigusch H, Henschel L, Kraul H, Merkel U, Hoffmann A. Lack of effect of grapefruit juice on diltiazem bioavailability in normal subjects. *Pharmazie* 1994;49:675-9

Singh BN. Effects of food on clinical pharmacokinetics. *Clin Pharmacokinet* 1999;37:213-55

Singhvi SM, Pan HY, Morrison RA, Willard DA. Disposition of pravastatin sodium, a tissue-selective HMG-CoA reductase inhibitor, in healthy subjects. *Br J Clin Pharmacol* 1990;29:239-43

Skjelbo E, Gram LF, Brøsen K. The N-demethylation of imipramine correlates with the oxidation of S-mephenytoin (S/R-ration). A population study. *Br J Clin Pharmacol* 1993;35:331-4.

Smith JM, Misiak H. Critical flicker frequency (CFF) and psychotropic drugs in normal human subjects - a review. *Psychopharmacology* 1976;47:175-82

Soons PA, Vogels BAPM, Roosemalen MCM, Schoemaker HC, Uchida E, Edgar B, Lundahl J, Cohen AF, Breimer DD. Grapefruit juice and cimetidine inhibit stereoselective metabolism of nitrendipine in humans. *Clin Pharmacol Ther* 1991;50:394-403

Stern RH, Smithers JA, Olson SC. Atorvastatin does not produce a clinically significant effect on the pharmacokinetics of terfenadine. *J Clin Pharmacol* 1998a;38:753-7

Stern RH, Gibson DM, Whitfield LR. Cimetidine does not alter atorvastatin pharmacokinetics or LDL-cholesterol reduction. *Eur J Clin Pharmacol* 1998b;53:475-8

Stern RH, Yang BB, Horton M, Moore S, Abel RB, Olson SC. Renal dysfunction does not alter the pharmacokinetics or LDL reduction of atorvastatin. *J Clin Pharmacol* 1997;37:816-9

Stoehr GP, Kroboth PD, Juhl RP, Wender DB, Phillips JP, Smith RB. Effect of oral contraceptives on triazolam, temazepam, alprazolam, and lorazepam kinetics. *Clin Pharmacol Ther* 1984;36:683-90

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

Takanaga H, Ohnishi A, Matsuo H, Murakami H, Sata H, Kuroda K, Urae A, Higuchi S, Sawada Y. Pharmacokinetic analysis of felodipine-grapefruit juice interaction based on an irreversible enzyme inhibition model. *Br J Clin Pharmacol* 2000b;49:49-58

Takanaga H, Ohnishi A, Matsuo H, Sawada Y. Inhibition of vinblastine efflux mediated by P-glycoprotein by grapefruit juice components in caco-2 cells. *Biol Pharm Bull* 1998;21:1062-6

Takanaga H, Ohnishi A, Murakami H, Matsuo H, Higuchi S, Urae A, Irie S, Furuie H, Matsukuma K, Kimura M, Kawano K, Orii Y, Tanaka T, Sawada Y. Relationship between time after intake of grapefruit juice and the effect on pharmacokinetics and pharmacodynamics of nisoldipine in healthy subjects. *Clin Pharmacol Ther* 2000a;67:201-14

Tang BK, Kalow W. Variable activation of lovastatin by hydrolytic enzymes in human plasma and liver. *Eur J Clin Pharmacol* 1995;47:449-51

Tassaneeyakul W, Birkett DJ, Veronese ME, McManus ME, Tukey RH, Quattrochi LC, Gelboin HV, Miners JO. Specificity of substrate and inhibitor probes for human cytochrome P450 1A1 and 1A2. *J Pharmacol Exp Ther* 1993;265:401-7

Taylor DP. Buspirone, a new approach to the treatment of anxiety. *FASEB J* 1988;2:2445-52

Thummel KE, O'Shea D, Paine MF, Shen DD, Kunze KL, Perkins JD, Wilkinson GR. Oral first-pass elimination of midazolam involves both gastrointestinal and hepatic CYP3A-mediated metabolism. *Clin Pharmacol Ther* 1996;59:491-502

Tinel M, Belghiti J, Descatoire V, Amouyal G, Letteron P, Geneve J, Larrey D, Pessayre D. Inactivation of human liver cytochrome P-450 by the drug methoxsalen and other psoralen derivatives. *Biochem Pharmacol* 1987;36:951-5

Tobert JA. Efficacy and long-term adverse effect pattern of lovastatin. *Am J Cardiol* 1988;62:28J-34J

Triscari J, Swanson BN, Willard DA, Cohen AI, Devault A, Pan HY. Steady state serum concnetrations of pravastatin and digoxin when given in combination. *Br J Clin Pharmacol* 1993;36:263-5

Vanakoski J, Mattila MJ, Seppälä T. Grapefruit juice does not enhance the effects of midazolam and triazolam in man. *Eur J Clin Pharmacol* 1996;50:501-8

Van Haarst AD, van't Klooster GAE, van Gerven JMA, Schoemaker RC, van Oene JC, Burggraaf J, Coene MC, Cohen AF. The influence of cisapride and clarithromycin on QT intervals in healthy volunteers. *Clin Pharmacol Ther* 1998;64:542-6

Van Peer A, Woestenborghs R, Verlinden M, Meuldermans W, Heykants J. Pharmacokinetics of cisapride in healthy volunteers [abstract]. *Digestion* 1986;43:138

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

Varhe A, Olkkola KT, Neuvonen PJ. Fluconazole, but not terbinafine, enhances the effects of triazolam by inhibiting its metabolism. *Br J Clin Pharmacol* 1996a;41:319-23

Varhe A, Olkkola KT, Neuvonen PJ. Diltiazem enhances the effects of triazolam by inhibiting its metabolism. *Clin Pharmacol Ther* 1996b;59:369-75

Varis T, Kivistö KT, Neuvonen PJ. Grapefruit juice can increase the plasma concentrations of oral methylprednisolone. *Eur J Clin Pharmacol Ther* 2000;56:489-93

Veronese ME, Mackenzie PI, Doecke CJ, McManus ME, Miners JO, Birkett DJ. Tolbutamide and phenytoin hydroxylations by cDNA-expressed human liver cytochrome P450 2C9. *Biochem Biophys Res Commun* 1991;175:1112-9

Veronese ML, Gillen LP, Dorval EP, Pequignot EC, Waldman SA, Greenberg HE. Inhibition of hepatic and intestinal CYP3A4 by "high dose" grapefruit juice (GFJ) [abstract]. *J Clin Pharmacol* 1999;39:980

Vickers S, Duncan CA, Chen IW, Rosegay A, Duggan DE. Metabolic disposition studies on simvastatin, a cholesterol-lowering prodrug. *Drug Metab Dispos* 1990a;18:138-45

Vickers S, Duncan CA, Vyas KP, Kari PH, Arison B, Prakash SR, Ramjit HG, Pitzenberger SM, Stokker G, Duggan DE. In vitro and vivo biotransformation of simvastatin, an inhibitor of HMG CoA reductase. *Drug Metab Dispos* 1990b;18:476-83

Von Moltke LL, Greenblatt DJ, Harmatz JS, Duan SX, Harrel LM, Cotreau-Bibbo MM, Pritchard GA, Wright CE, Shader RI. Triazolam biotransformation by human liver microsomes in vitro: effects of metabolic inhibitors and clinical confirmation of a predicted interaction with ketoconazole. *J Pharmacol Exp Ther* 1996;276:370-9

Wacher VJ, Wu C-J, Benet LZ. Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: Implications for drug delivery and activity in cancer chemotherapy. *Mol Carcinogen* 1995;13:129-34

Wang J-S, Wen X, Backman JT, Taavitsainen P, Neuvonen PJ, Kivistö KT. Midazolam α-hydroxylation by human liver microsomes *in vitro*: inhibition by calcium channel blockers, itraconazole and ketoconazole. *Pharmacol Toxicol* 1999;85:157-61

Wang RW, Kari PH, Lu AYH, Thomas PE, Guengerich FP, Vyas KP. Biotransformation of lovastatin. IV. Identification of cytochrome P450 3A proteins as the major enzymes responsible for the oxidative metabolism of lovastatin in rat and human liver microsomes. *Arch Biochem Biophys* 1991;290:355-61

Watkins PB, Murray SA, Winkelman LG, Heuman DM, Wrighton SA, Guzelian PS. Erythromycin breath test as an assay of glucocorticoid-inducible liver cytochromes P-450. Studies in rats and patients. *J Clin Invest* 1989;83:688-97

Weber A, Jäger R, Börner A, Klinger G, Vollanth R, Matthey K, Balogh A. Can grapefruit juice influence ethinylestradiol bioavailability? *Contraception* 1996;53:41-7

Wedlund PH, Aslanian WS, McAllister CB, Wilkinson GR, Branch RA. Mephenytoin hydroxylation deficiency in Caucasians: frequency of a new oxidative drug metabolism polymorphism. *Clin Pharmacol Ther* 1984;36:773-80

Welker HA, Wiltshire H, Bullingham R. Clinical pharmacokinetics of mibefradil. *Clin Pharmacokinet* 1998;35:405-23

Whitfield LR, Cilla DD, Posvar EL, Sedman AJ, Gibson DM. Single- and multiple-dose pharmacokinetics of CI-981, a potent HMG-CoA reductase inhibitor. *Pharm Res* 1993; 10 Suppl.:S340

Woestenborghs R, Lorreyne W, van Rompaye F, Heykants J. Determination of cisapride in plasma and animal tissues by high-performance liquid chromatography. *J Chromatogr* 1988;424:195-200

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

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

Wysowski DK, Bacsanyi J. Cisapride and fatal arrhythmia. *N Engl J Med* 1996;335:290-1

Yang BB, Hounslow NJ, Sedman AJ, Forgue ST. Effects of atorvastatin, an HMG-CoA reductase inhibitor, on hepatic oxidative metabolism of antipyrine. *J Clin Pharmacol* 1996;36:356-60

Yee GC, Stanley DL, Pessa LJ, Dalla Costa T, Beltz SE, Ruiz J, Lowenthal DT. Effect of grapefruit juice on blood cyclosporin concentration. *Lancet* 1995;345:955-6

Zaidenstein R, Dishi V, Gips M, Soback S, Cohen N, Weissgarten J, Blatt A, Golik A. The effect of grapefruit juice on the pharmacokinetics of orally administered verapamil. *Eur J Clin Pharmacol* 1998;54:337-40

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

Ziegler WH, Schalch E, Leishman B, Eckert M. Comparison of the effects of intravenously administered midazolam, triazolam, and their hydroxy metabolites. *Br J Clin Pharmacol* 1983;16 (suppl):63S-9S