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EFFECTS OF CYTOCHROME P450 ENZYME INHIBITORS ON THE PHARMACOKINETICS OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND VENLAFAXINE

by

Ville-Veikko Hynninen

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From the Department of Anesthesiology, Intensive Care, Emergency Care and Pain Medicine, and the Department of Pharmacology, Drug Development and Therapeutics, and Clinical Drug Research Graduate School

Supervised by

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

and

Professor Klaus Olkkola
Department of Anesthesiology, Intensive Care, Emergency Care and Pain Medicine
University of Turku, Turku, Finland

Reviewed by

Professor Hannu Raunio
Department of Pharmacology and Toxicology
University of Kuopio, Kuopio, Finland

and

Professor Arja Rautio
Centre for Arctic Medicine
University of Oulu, Oulu, Finland

Opponent

Professor Kari Kivistö
Clinical Pharmacology and Toxicology
University of Tampere
Tampere, Finland

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To Johanna, Vilma, Aapo and Anna

ABSTRACT

Ville-Veikko Hynninen

Effects of cytochrome P450 enzyme inhibitors on the pharmacokinetics of nonsteroidal anti-inflammatory drugs and venlafaxine

From the Department of Pharmacology, Drug Development and Therapeutics and from the Department of Anesthesiology, Intensive Care, Emergency Care and Pain Medicine, University of Turku, Turku, Finland
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Cytochrome P450 (CYP) enzymes play a pivotal role in the metabolism of many drugs. Inhibition of CYP enzymes usually increases the plasma concentrations of their substrate drugs and can thus alter the safety and efficacy of these drugs. The metabolism of many widely used nonsteroidal anti-inflammatory drugs (NSAIDs) as well as the metabolism of the antidepressant venlafaxine is known to be catalyzed by CYP enzymes. In the present studies, the effect of CYP inhibition on the pharmacokinetics and pharmacodynamics of NSAIDs and venlafaxine was studied in clinical trials with healthy volunteers and with a cross-over design, by using different antifungal agents as CYP inhibitors.

The results of these studies demonstrate that the inhibition of CYP enzymes leads to increased concentrations of NSAIDs. In most cases, the exposure to ibuprofen, diclofenac, etoricoxib, and meloxicam was increased 1.5- to 2-fold when they were used concomitantly with antifungal agents. CYP2D6 inhibitor, terbinafine, substantially increased the concentration of parent venlafaxine, whereas the concentration of active moiety of venlafaxine (parent drug plus active metabolite) was only slightly increased. Voriconazole, an inhibitor of the minor metabolic pathway of venlafaxine, produced only minor changes in the pharmacokinetics of venlafaxine.

These studies show that an evident increase in the concentrations of NSAIDs may be expected, if they are used concomitantly with CYP inhibitors. However, as NSAIDs are generally well tolerated, use of single doses of NSAIDs concomitantly with CYP inhibitors is not likely to adversely affect patient safety, whereas clinical relevance of long-term concomitant use of NSAIDs with CYP inhibitors needs further investigation. CYP2D6 inhibitors considerably affect the pharmacokinetics of venlafaxine, but the clinical significance of this interaction remains unclear.

Keywords: pharmacokinetics, drug interactions, CYP, nonsteroidal anti-inflammatory drugs, venlafaxine, antifungals

TIIVISTELMÄ

Ville-Veikko Hynninen

Sytokromi P450 entsyymien estäjien vaikutukset tulehduskipulääkkeiden ja venlafaksiinin farmakokinetiikkaan

Farmakologia, lääkekehitys ja lääkehoito sekä Anestesiologia, tehohoito, ensihoito ja kivunhoito, Turun yliopisto, Turun yliopistollinen keskussairaala, Turku
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Sytokromi P450 (CYP)-entsyymit ovat tärkeimpiä lääkeaineiden metaboliaa katalysoivista entsyymeistä. CYP-ensyymien toiminnan estyminen (inhibitio) tyypillisesti nostaa niiden välityksellä hajoavien lääkeaineiden pitoisuuutta plasmassa ja saattaa täten lisätä kyseisten lääkeaineiden vaikutusta tai lisätä niiden aiheuttamien haittavaikutusten määrää. Useiden tulehduskipulääkkeiden, kuten myös masennuslääke venlafaksiinin, tiedetään metaboloituvan CYP-entsyymien välityksellä. Tässä tutkimussarjassa selvitettiin sienilääkkeiden aiheuttaman CYP-entsyymien inhibition vaikutusta tulehduskipulääkkeiden ja venlafaksiinin farmakokinetiikkaan ja farmakodynamiikkaan. Kliiniset lääketutkimukset tehtiin terveillä vapaaehtoisilla koehenkilöillä käyttäen vaihtovuoroista koejärjestelyä.

Tämän tutkimussarjan tulokset osoittavat, että CYP-entsyymien inhibitio nostaa tulehduskipulääkkeiden plasmapitoisuuksia. Altistus ibuprofeenille, diklofenaakille, etorikoksibille ja meloksikaamille kasvoi useimmiten 1.5- 2 -kertaiseksi, kun niitä annosteltiin samanaikaisesti sienilääkkeiden kanssa. Venlafaksiinin CYP2D6-välitteisen metabolian inhibitio terbinafiinilla nosti voimakkaasti venlafaksiinin plasmapitoisuuksia, mutta venlafaksiinin ja sen ekvipotentin aktiivisen metaboliitin yhteenlaskettu plasmapitoisuus kasvoi vain hieman. Venlafaksiinin vaihtoehtoisen metaboliareitin inhibitio muutti venlafaksiinin farmakokinetiikkaa marginaalisesti.

Tutkimus osoittaa, että CYP entsyymien toimintaa estävät lääkkeet nostavat tulehduskipulääkkeiden plasmapitoisuuksia. Tulehduskipulääkket ovat yleensä hyvin siedettyjä lääkkeitä ja siten yksittäiset tulehduskipulääkeannokset yhdessä CYP entsyymien toimintaa estävien lääkkeiden kanssa tuskin vaarantavat potilasturvallisuutta. Sen sijaan pitkään jatkuvan yhteiskäytön riskit vaativat lisäselvityksiä. CYP2D6 entsyymin toimintaa estävät lääkkeet aiheuttavat suuria muutoksia venlafaksiinin farmakokinetiikkaan, mutta yhteisvaikutuksen kliininen merkitys on epäselvä.

Avainsanat: farmakokinetiikka, lääkeyhteisvaikutus, CYP, tulehduskipulääkkeet, venlafaksiini, sienilääkkeet

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ABBREVIATIONS

ANOVA	analysis of variance
AUC(0-t)	area under plasma concentration-time curve from zero to t hours
BMI	body mass index
CI	confidence interval
CL	plasma clearance
C_{max}	peak plasma concentration
C_{trough}	trough concentration of a drug
CV	coefficient of variation
CYP	cytochrome P450
DDI	drug-drug interaction
EM	extensive metabolizer
HPLC	high performance liquid chromatography
k_{el}	elimination rate constant
MRP	multidrug resistance-associated protein
NADPH	nicotinamide adenine dinucleotide phosphate
NSAID	nonsteroidal anti-inflammatory drug
OATP	organic anion transporting polypeptide
ODV	O-desmethylvenlafaxine
P-gp	P-glycoprotein
PM	poor metabolizer
SD	standard deviation
SEM	standard error of mean
$t_{½}$	terminal elimination half-life
UM	ultrarapid metabolizer
t_{max}	time to peak concentration
V_d	volume of distribution
VAS	visual analogue scale

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications.

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

II Hynninen VV, Olkkola KT, Leino K, Lundgren S, Neuvonen PJ, Rane A, Valtonen M, Laine K. Effect of voriconazole on the pharmacokinetics of diclofenac. Fundam Clin Pharmacol 2007; 21: 651-6.

III Hynninen VV, Olkkola KT, Neuvonen PJ, Laine K. Oral voriconazole and miconazole oral gel produce comparable effects on the pharmacokinetics and pharmacodynamics of etoricoxib. Eur J Clin Pharmacol (in press).

IV Hynninen VV, Olkkola KT, Bertilsson L, Korhonen T, Neuvonen PJ, Laine K. Opposite interactions of meloxicam with two azole antimycycotics; voriconazole increases while itraconazole decreases meloxicam plasma concentrations. Submitted.

V Hynninen VV, Olkkola KT, Bertilsson L, Kurkinen K, Neuvonen PJ, Laine K. Effect of terbinafine and voriconazole on the pharmacokinetics of the antidepressant venlafaxine. Clin Pharmacol Ther 2008; 83: 342-8

The articles are referred to with their Roman numerals in the text. Original communications have been reproduced with the permission of the copyright holders.

1 INTRODUCTION

The desirable and undesirable effects of a drug are usually related to its concentration at the sites of action, which in turn depends on the amount of a drug administered, and on the pharmacokinetic behavior of a drug. Pharmacokinetics refers to the movement of drug into, through, and out of the body and is divided into several processes including absorption, distribution, metabolism, and excretion. Pharmacokinetics of a drug depends on the drug's chemical properties as well as on patient-related factors, such as genetic factors, sex, age, weight, and diseases. In addition, whenever two drugs are co-administered, a drug-drug interaction (DDI) may occur and affect drug concentration by influencing drug absorption, distribution, metabolism, or excretion. Inhibition of cytochrome P-450 (CYP) mediated metabolism of a drug, leading to increased concentration of drug, is one of the most common causes of harmful DDIs and has led to the removal of several drugs from the market during the past years (Friedman et al. 1999, Lasser et al. 2002, Pelkonen et al. 2008).

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most frequently prescribed medications worldwide, used in the treatment of pain, fever, and inflammation. The widespread use of NSAIDs has meant that the adverse effects of these relatively safe drugs have also become increasingly prevalent, especially when they are used in high doses for prolonged periods of time (Henry et al. 1996, Solomon et al. 2006, Cannon et al. 2006). *In vitro* studies have revealed that CYP enzymes play an important role in the metabolism of many NSAIDs (Rodrigues 2005). Venlafaxine is an antidepressant that is also used in the treatment of neuropathic pain. Due to its mechanism of action, it has many dose-related serotonergic adverse effects. Also, the metabolism of venlafaxine is shown to be catalyzed by CYP enzymes.

Antifungal agents are well known inhibitors of CYP enzymes and are involved in many clinically significant interactions with drugs that are metabolized by CYP enzymes (Venkatakrishnan et al. 2000, Huang et al. 2007, Pelkonen et al. 2008). They are widely used in pharmacokinetic interaction studies when investigating the effect of CYP inhibition on the pharmacokinetics of drugs.

As NSAIDs are commonly used drugs, it is likely that they are sometimes concomitantly used with CYP inhibitors. However, the effect of CYP inhibition on the pharmacokinetics of NSAIDs has not been investigated systematically. Therefore, it was considered important to explore the effect of CYP inhibition on the pharmacokinetics and pharmacodynamics of NSAIDs, using voriconazole and some other antifungal agents as CYP inhibitors. In addition, because of the wide use of both venlafaxine and the non-azole antifungal agent, terbinafine, it is likely that these drugs are coadministered in clinical practice. Thus, it was considered important to evaluate the effect of terbinafine on pharmacokinetics and pharmacodynamics of venlafaxine and to compare this effect with that of voriconazole.

2 REVIEW OF THE LITERATURE

2.1 Drug metabolism

The elimination of drugs from the body involves the processes of metabolism and excretion. The kidney is the primary organ for drug excretion. However, renal excretion of unchanged drug plays only a modest role in the overall elimination of most drugs, since lipophilic drugs filtered through a kidney glomerulus are largely reabsorbed back into the circulation from renal tubules. Therefore, the metabolism of drugs into less lipophilic metabolites is essential for the elimination of these drugs from the body. In general, drug metabolism leads to chemical alteration of the drug, resulting in more polar and hydrophilic metabolites, which are more easily excreted from the body (Rowland & Tozer 1995). In addition to metabolism, active transport across biological membranes represents a critical step in the elimination of many drugs. It is well-established that different efflux and uptake transporters such as P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs), or organic anion transporting polypeptides (OATPs) are involved in the overall elimination and efficacy of numerous drugs. These proteins are mainly expressed at physiological sites of drug absorption and elimination, thus mainly leading to diminished absorption and/or increased transporter-facilitated excretion (Oswald et al. 2007, Choi et al. 2008, Zhang et al. 2008).

Biotransformation or metabolic reactions can be classified as either phase I functionalization or phase II conjugation reactions. Phase I reactions add or expose a functional group on the parent drug, and these reactions include oxidation, reduction and hydrolysis. The formed metabolite can be excreted into urine or can undergo a subsequent phase II reaction. In phase II reactions, the drug or metabolite is conjugated with endogenous molecules. The typical conjugation reactions are glucuronidation, sulfation, and acetylation. Usually, a drug first undergoes phase I reactions followed by phase II reactions, but sometimes it can be conjugated without a prior phase I reaction (Benedetti et al. 2007, Iyanagi 2007).

Metabolism most commonly leads to the inactivation of drug, but sometimes also the metabolites are pharmacologically active, and then the therapeutic effect consist of activity of both parent drug and metabolite. Some drugs, e.g. losartan (Mufano et al. 1992) and tramadol (Poulsen et al. 1996), are prodrugs, which are inactive compounds and need to be activated by metabolism in order to attain a therapeutically active form. Sometimes the metabolism leads to formation of toxic metabolite, as in the case of paracetamol (Dahlin et al. 1984, James et al. 2003).

Most of the enzymes catalyzing the metabolism of drugs are located in the liver, but are also found e.g. in the intestine, skin, lungs and kidneys (de Waziers et al. 1990, Kivistö et al. 1996, Pelkonen et al. 2008). Thus, orally administered drug is exposed to metabolism already during its absorption when the drug passes through the intestinal wall. In addition, from intestine the drug enters the liver through the portal vein and

can thus become metabolized both in the liver and in the intestine before entering the systemic circulation. This is called first pass metabolism, which can greatly reduce the amount of parent drug reaching systemic circulation. In that case, the drug is said to have low oral bioavailability.

2.2 Cytochrome P450 (CYP) enzyme system

CYP enzymes are heme containing proteins, which are involved in the metabolism of numerous chemically diverse endogenous and exogenous compounds, including e.g. drugs and other xenobiotics. They are the most important group of enzymes involved in phase I reactions and are capable of catalyzing many oxidative as well as reductive reactions. A typical CYP catalyzed oxidative reaction requires substrate (R), CYP enzyme, molecular oxygen, NADPH, and NADPH-P450 reductase and can be shown as follows:

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + NADP^+ + H_2O$$

The mechanism involves many electron-transfer steps, where electrons are supplied from NADPH via NADPH-P450 reductase. The overall effect of the reaction is the addition of one atom of oxygen to the substrate (drug) to form a hydroxyl group, the other atom of oxygen being converted to water. The role of CYP enzyme is to function as a terminal oxidase that introduces molecular oxygen to the substrate (Brown et al. 2008, Guengerich 2008).

CYP enzymes are divided into families and subfamilies according to their amino acid sequence similarity. Enzymes that have over 40% amino acid sequence homology belong to the same family and are identified by Arabic numerals (e.g. CYP1, CYP2). Within the family, enzymes having over 55% sequence homology are in the same subfamily, identified by a letter (e.g. CYP2C, CYP2D). Furthermore, individual CYP isoforms within the subfamily are identified by an additional Arabic numeral (e.g. CYP2C9, CYP2C19) (Nelson et al. 1996). In humans, there are 57 different CYP enzymes arranged in 18 families and 42 subfamilies and they catalyse the metabolism of numerous endogenous substrates and xenobiotics (Nebert & Russell 2002, Pelkonen et al. 2008). However, only CYPs belonging to families 1, 2, and 3 are important in the metabolism of drugs in humans. Each individual CYP isoform has characteristic substrate specifity based on structure of the substrate, but also considerable overlapping exists. As a result, more than one CYP isoform might be involved in a overall metabolism of a drug, which can lead to the formation of many primary and secondary metabolites (Brown et al. 2008, Pelkonen et al. 2008).

2.3 CYP enzymes

The most important CYP isoforms in drug metabolism are CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 (Wienker & Heath 2005, Guengerich 2008) and they are discussed in the following chapters. CYP2C8 contributes to the metabolism of ibuprofen and is therefore also discussed. With the exception of CYP3A4, each of

these CYP enzymes displays genetic polymorphism producing substantial variation in their enzyme activity. In addition, several drugs change the activity of CYP enzymes by acting as an inhibitor or an inducer of CYP enzyme.

2.3.1 CYP2C8

CYP2C8 is expressed mainly in the liver and together with other CYP2C enzymes it accounts about 20% of the hepatic CYP content (Shimada et al. 1994, Rostami-Hodjegan & Tucker 2007, Pelkonen et al. 2008). CYP2C8 was earlier thought to play a minor role in drug metabolism, but today it is known to metabolize many important drugs such as antidiabetic drugs; rapaglinide, rosiglitazone, pioglitazone (Kirchheiner et al. 2005b), anticancer drug; paclitaxel (Rahman et al. 1994), and the *R*-(-) enantiomer of ibuprofen (Hamman et al. 1997). The activity of CYP2C8 can be inhibited e.g. by gemfibrozil (Backman et al. 2002) and trimethoprim (Wen et al. 2002) and induced by rifampicin (Rae et al. 2001).

CYP2C8 carries 3 major variant alleles. *CYP2C8*2* is present mainly in Africans, whereas *CYP2C8*3* and *CYP2C8*4* have an allelic frequency of 15% and 7.5% in Caucasians, respectively (Bahadur et al. 2002). However, *in vivo* the effect of *CYP2C8*3* mutation is unclear and depends on the substrate drug used, resulting in either reduced or increased rate of metabolism (Martinez et al. 2005, Kirchheiner et al. 2006). Estimation of the effect of different CYP2C8 alleles is difficult, because there is no selective probe drug for CYP2C8 activity. In addition, *CYP2C8*3* is linked with the *CYP2C9*2* allele, and therefore, the evaluation of the individual effects of these variant alleles is complicated (Yasar et al. 2002).

2.3.2 CYP2C9

CYP2C9 is a predominant CYP2C form and is mainly expressed in the liver (Rostami-Hodjegan & Tucker 2007, Pelkonen et al. 2008). It is responsible for the metabolism of many clinically important drugs including warfarin (Rettie et al. 1992), losartan (Kaukonen et al. 1998), phenytoin (Veronese et al. 1991) and many NSAIDs (Rodrigues 2005). Fluconazole and metronidazole are typical inhibitors of CYP2C9 (O'Reilly 1976, Kunze et al. 1996), whereas inducers include e.g. rifampicin (Zilly et al. 1975, Pelkonen et al. 2008).

The polymorphic behaviour of CYP2C9 is determined mainly by two variant alleles, *CYP2C9*2* and *CYP2C9*3*. The allelic frequencies of *CYP2C9*2* and *CYP2C9*3* range from 8% to 19% and from 3.3% to 16.2% in Caucasians, respectively (Xie et al. 2002). The *CYP2C9*3* allele has stronger pharmacokinetic effects than *CYP2C9*2*. For most CYP2C9 substrates, heterozygous *CYP2C9*3* individuals have approximately 40-50% decreased clearance and homozygous *CYP2C9*3* individuals about 75-85% decreased clearance compared with wild-type individuals (Kircheiner et al. 2005a). The importance of the CYP2C9 polymorphism is shown especially with warfarin, which is metabolized mainly by CYP2C9 and possesses a narrow therapeutic window with fatal side effect profile (Kirchheiner & Brockmöller 2005a).

2.3.4 CYP2C19

CYP2C19 and CYP2C9 show over 90% similarity in amino acid sequence, and thus many drugs are substrates for both of these enzymes. CYP2C19 contributes to metabolism of several drugs, e.g. omeprazole (Andersson et al. 1993), diazepam (Andersson et al. 1994), phenytoin (Bajbai et al. 1996), and amitriptyline (Venkatakrishnan et a. 1998). Besides being a substrate for CYP2C19, omeprazole also inhibits the action of CYP2C19 (Funck-Brentano et al. 1997). In addition, CYP2C9 inhibitor, fluconazole, has a similar effect on CYP2C19 (Kang et al. 2002). The inducers of CYP2C19 include e.g. rifampicin (Feng et al. 1998) and the herbal product St. John`s wort (Wang et al. 2004).

The most important variant alleles of *CYP2C19* are **2* and **3,* which both result in non-functional enzyme. Poor metabolizers (PMs) carrying two 2 defective *CYP2C19* genes are present at a frequency of approximately 2-3% in Caucasians and approximately 20% in Asians (Xie et al. 1999, Desta et al. 2002, Ingelman-Sundberg 2007). CYP2C19 gene has also a promoter variant, termed *CYP2C19*17*, which has a frequency of about 18% in Caucasians and causes increased activity of CYP2C19 due to an increase in CYP2C19 transcription (Sim et al. 2006).

2.2.5 CYP2D6

Although CYP2D6 accounts only for 1.5% of total hepatic CYP content, it is responsible for the metabolism of about 25% of all drugs on the market (Shimada et al. 1994, Ingelman-Sundberg et al. 2007, Rostami-Hodjegan & Tucker 2007, Pelkonen et al. 2008). CYP2D6 is partially or entirely responsible for the metabolism of a variety of psychopharmacological and cardiovascular drugs, including venlafaxine (Otton et al. 1996), fluoxetine, paroxetine (Hiemke & Härtter 2000), and metoprolol (Otton et al. 1988). In addition, CYP2D6 is needed for the bioactivation of prodrugs codeine (Dayer et al. 1988) and tramadol (Poulsen et al. 1996). CYP2D6 is the only drug metabolizing CYP, which is not inducible by other drugs. Inhibitors of CYP2D6 include its own substrates such as fluoxetine, paroxetine (Hiemke & Härtter 2000), as well as terbinafine, which seems to be the only CYP2D6 inhibitor among antifungal drugs (Venkatakrishnan et al. 2000).

CYP2D6 is the most studied CYP with regard to genetic polymorphism. More than 60 different functional CYP2D6 gene variants, which cause abolished, decreased, normal, and ultrarapid enzyme activity, have been described (www.cypalleles.ki.se). Frequency of PMs, carrying two inactive alleles (e.g. **3, *4, *5*), is about 7% in Caucasians and the frequency of ultrarapid metabolizers (UMs), which result from duplication or multiduplications of active CYP2D6 genes, is about 1-2% in Scandinavians (Dahl et al. 1992, Dahl et al. 1995, Sachse et al. 1997). CYP2D6 polymorphism is of great clinical importance, and therefore, CYP2D6 genotype-based dosage recommendations have been published for some CYP2D6 substrates (Kirchheiner et al. 2001, Eichelbaum et al. 2006)

2.3.6 CYP3A4/5

CYP3A subfamily accounts for over 30% of total liver CYP content (Shimada et al. 1994, Rostami-Hodjegan & Tucker 2007, Pelkonen et al. 2008). The contribution of CYP3A5 to total liver CYP3A levels is estimated to be 3%-30%, with the remaining levels being composed of CYP3A4 (Westlind-Johnsson et al. 2003, Ingelman-Sundberg et al. 2007). CYP3A enzymes are also extensively expressed in intestinal wall (de Wazier et al. 1990, Pelkonen et al. 2008). CYP3A4 and CYP3A5 share approximately 90% amino-acid sequence identity and they thus share most of their substrates, inducers and inhibitors. It has been estimated that CYP3A enzymes facilitate the metabolism of 50% of all therapeutic drugs (Bertz & Granneman 1997). Typical CYP3A substrates include midazolam, triazolam, alfentanil, quinidine, nifedipine, and felodipine (Rendic 2002). All azole antifungals are inhibitors of CYP3A4, albeit with different potencies (Venkatakrishnan et al. 2000). Other CYP3A4 inhibitors include e.g. antibacterials erythromycin (Olkkola et al. 1993) and clarithromycin (Yeates et al. 1996), and grapefruit juice (Kupferschmidt et al. 1995). CYP3A activity can be induced typically by carbamazepine (Bertilsson et al. 1997).

Up to date, 20 different CYP3A4 variant alleles have been described (www.cypalleles.ki.se). However, their low frequencies rule them out as aetiology for the 4-6-fold interindividual differences in CYP3A activity (Floyd et al. 2003, Ingelman-Sundberg et al. 2007). CYP3A5 is highly polymorphic, and there are many mutations that greatly decrease the activity of CYP3A5 enzyme (Kuehl et al. 2001). CYP3A5 polymorphism seems to explain the interindividual variability in the metabolism of some CYP3A substrates, e.g. tacrolimus (Hesselink et al. 2003, Zheng et al. 2004), whereas studies with midazolam have conflicting results (Shih & Huang 2002, Wong et al. 2004). Thus, the possible genetic cause for the variability in the metabolism of CYP3A substrates is somewhat unclear.

2.4 Mechanism of CYP inhibition

Inhibition of CYP enzymes is most often classified into reversible and irreversible inhibition. Reversible inhibition is the most common mechanism in DDIs and can be further divided into competitive, noncompetitive, uncompetitive, and mixed-type inhibition. Reversible inhibition usually occurs as a direct competition at the active site on CYP enzyme between the substrate and inhibitor. The competition can be either for the heme prosthetic group or other regions of the active site of CYP enzyme. Binding to the CYP enzymes happens with weak bonds, which are both formed and broken down easily. The inhibitory effect depends on the strength of the bond between drugs (substrate and inhibitor) and CYP enzyme and concentrations of inhibitor and substrate. This type of inhibition might occur every time two substrates of the same CYP enzyme are present. Reversible inhibitors act rapidly, but do not permanently destroy CYP enzyme and the metabolic function of CYP enzyme normalizes following the elimination of the inhibitor (Lin & Lu 1998, Hollenberg 2002, Johnson 2008, Pelkonen et al. 2008).

In competitive form of reversible inhibition, competition of binding sites between the substrate and inhibitor takes place at the same position on the active site of CYP enzyme, whereas in the noncompetitive mode of reversible inhibition, the active binding site of the substrate and inhibitor is different from each other. In uncompetitive inhibition, the inhibitor binds to the enzyme-substrate complex, instead of the free CYP enzyme. Many times, reversible inhibition displays elements of both competitive and noncompetitive inhibition and then it is called mixed-type inhibition (Lin & Lu 1998, Hollenberg 2002, Pelkonen et al. 2008).

Irreversible inhibition is also called mechanism-based inhibition. Mechanism based inhibitors are CYP substrates that are converted to reactive intermediates via oxidative catalysis by CYPs. These intermediates can inactivate CYP enzyme by three different mechanisms; covalent adduction to an amino acid residue within the enzyme active site, arylation or alkylation of prosthetic heme moiety, and destruction of the heme group. Irreversible inhibition is usually long-lasting, because it is reversed only by synthesis of new, catalytically active enzymes (Lin & Lu 1998, Hollenberg 2002, Johnson 2008, Pelkonen et al. 2008).

2.5 Mechanism of CYP induction

Drugs or enviromental agents can induce the CYP enzyme by enhancing the rate of its synthesis or by reducing its rate of degradation, but mainly the increase in synthesis is seen. Increased synthesis of CYPs is mediated by a group of ligand-activated transcription factors, which include e.g. intracellular aryl hydrocarbon receptor (AhR) and nuclear receptors, pregnane X receptor (PXR) and constitutive androstane receptor (CAR). The inducer binds to and activates one or more of these receptors, which leads to increased transcription of respective CYPs in order to adjust the organism to the requirements of the chemical environment. Induction is a slow process; maximum induction is usually reached after 4-14 days and needs multiple dosing of inducing compaund to occur. Correspondingly, after withdrawing the inducer, the CYP enzyme activity returns to the original level in 1-3 weeks. Typical inducers of CYP enzymes include rifampicin, phenobarbital, phenytoin and carbamazepine (Dickins 2004, Hewitt et al. 2007).

2.6 Pharmacokinetic drug-drug interactions involving CYP enzymes

Harmful drug-drug interactions (DDIs) are one of the major concerns in pharmacotherapy. According to epidemiological studies, between 2.4-6.5% of all hospital admissions may be attributed to the adverse effects caused by drugs (Schneeweiss et al. 2002, Pirmohamed et al. 2004), and about 7% of already hospitalized patients may experience a serious adverse drug effect (Lazarou et al. 1998). The annual cost of these adverse drug effects is estimated to be hundreds of millions of euros. There is considerable uncertainty about the frequency of DDIs as a cause of clinically significant adverse drug effects, and estimates vary from 12% to 26% depending on the study population (Kelly 2001, McDonnell & Jacobs 2002, Pirmohamed et al. 2004). In any case, concomitant use of multiple drugs is increasingly common, especially among older people, and the risk of receiving

interacting drugs strongly correlates with the number of drugs taken (Åstrand et al. 2007). In Finnish pharmacies, 9.8% of all prescriptions included at least one potential interaction with drugs in the currently or previously dispensed prescriptions (Heikkilä et al. 2006). With deeper understanding and with the help of computerized surveillance programs, harmful DDIs could usually be predicted and avoided beforehand (McDonnell & Jacobs 2002, Pirmohamed et al. 2004, Heikkilä et al. 2006).

Drug-drug interactions may be a direct chemical interaction (pharmaceutical interaction), they may affect drug concentrations by influencing the processes underlying drug absorption, distribution, metabolism and/or elimination (pharmacokinetic interactions), or a more direct augmentation or attenuation of the effects may be observed (pharmacodynamic interaction). Most of the adverse drug effects found in hospitalized patients are dose-dependent or concentration-dependent (Lazarou et al. 1998, McDonnell & Jacobs 2002) and therefore, pharmacokinetic interactions leading to increased drug exposure might have serious consequences. As CYP mediated metabolism represents a major route of elimination for many drugs and as many drugs are metabolized by the same CYP enzyme, they have a crucial role in pharmacokinetic DDIs. A consequence of CYP inhibition is an increase in the plasma concentration of parent drug and a reduction in that of metabolite. If a drug is metabolized solely by one CYP enzyme, inhibition leads to prolonged pharmacological effect, and depending on the therapeutic index of a drug, to an increased likelihood of adverse drug effects. However, if a drug has many metabolic pathways, the inhibition of CYP mediated pathway can many times be compensated by unaffected pathways and so the increase in the plasma concentration of the parent drug remains small. Inhibition of cytochrome P-450 (CYP) mediated metabolism of a drug has led to the removal of several drugs from the market during the past years (Friedman et al. 1999, Lasser et al. 2002). By contrast, CYP induction may attenuate the pharmacological effect of a drug as plasma concentrations of the drug remain at subtherapeutic levels. In the case of prodrug, which needs CYP catalyzed transformation to become an active metabolite, the CYP inhibition might cause a decreased and induction an increased clinical drug effect. In a Finnish study, 0.9% of all hospitalized patients were found to be exposed to potentially harmful, CYP-mediated, DDIs (Laine et al. 2000).

2.6.1 Investigation of CYP mediated DDIs

Drug-drug interactions can be studied both *in vitro* and *in vivo*. Preliminary information of the interaction potential of a certain drug is usually obtained from *in vitro* studies. Different *in vitro* techniques can be used to identify CYP-mediated metabolic pathways of a drug and its ability to inhibit or induce different CYP enzymes, which is an essential piece of information, especially during the process of drug development. Based on the results of these *in vitro* studies, an appropriate CYP inducer and inhibitor and a probe substrate can be selected for the following *in vivo* interaction studies. Furthermore, if *in vitro* studies indicate that the drug investigated has no significant CYP mediated metabolism and does not inhibit or induce any CYP enzymes, no further *in vivo* interaction studies are needed (Huang et al. 2007, Fuhr 2008). In addition, in recent years, *in vitro* data have been increasingly used for

quantitative prediction of *in vivo* drug interactions. However, the extrapolation of *in vitro* data to clinical situations is still problematic for many reasons: e.g., the true free inhibitor concentration at the site of action (adjacent to CYP enzyme) is unknown *in vivo* situations and it can be notable different from that of plasma; besides liver, many other tissues (e.g. intestinal mucosa, skin, lungs) contribute to drug metabolism *in vivo*; and the results obtained from in vitro studies are highly dependent on several tecnical aspects (Venkatakrishnan et al. 2000, Wienkers et al. 2005, Pelkonen et al. 2008). Therefore, the precise extent of interaction between two drugs can still be derived from *in vivo* studies only.

When investigating the effect of CYP inhibition or induction on the metabolism of an investigational drug *in vivo*, the selection of the inhibitor or inducer should be based on *in vitro* or *in vivo* studies identifying the CYP enzymes that metabolize the investigational drug. In that case, the strongest inhibitor or/and inducer of the CYP enzyme in question should be used with highest recommended doses and with shortest dosing interval to make it possible to study the effect of maximum inhibition or induction on the metabolism of the investigational drug. For example, if the investigational drug is metabolized by CYP2C9, the appropriate choice of inhibitor could be fluconazole, and the choice of inducer could be rifampicin (Pelkonen et al. 2008). The knowledge of the effect of the maximum inhibition and induction on the pharmacokinetics of the investigational drug then allows the prediction of expected pharmacokinetic interactions between investigational drug and other CYP inhibitors and inducers as well. However, it is often still advisable to conduct interaction studies in which the selection of CYP inhibitor or inducer used is based on the likelihood of coadministration of CYP inhibitor and investigational drug in the clinical setting. In addition, the definitive study design depends on many factors for both the substrate and interacting drug (inhibitor/inducer). These include, for example, whether the use of the substrate and interacting drug is acute or chronic, the therapeutic index of the investigational drug, and the pharmacokinetics and pharmacodynamics of the drugs investigated (Huang et al. 2007, Fuhr 2008).

2.7 Nonsteroidal anti-inflammatory drugs (NSAIDs)

2.7.1 Mechanism of action

Since the introduction of acetylsalicylic acid (aspirin) as the first NSAID in 1897, NSAIDs have been widely used in the treatment of pain, inflammation, and fever. Today, they are among the most widely used medicines in the world. The primary mechanism of action of all NSAIDs is the inhibition of cyclooxygenase (COX), a hemeprotein that exists in two isoforms (COX-1 and COX-2) and converts arachidonic acid (AA) to prostanoids such as prostaglandin (PG) E_2, $PGF_{2\alpha}$, PGD_2, prostacyclin (PGI_2), and thromboxane A_2 (TxA_2) (Vane 1971, Warner & Mitchell 2004, Capone et al. 2007). In addition, a variant of the COX-1 enzyme, termed COX-3 has been described, but it seems to be without any COX-activity in humans (Kis et al. 2005). All NSAIDs, apart from aspirin, cause reversible COX inhibition by competing with arachidonic acid for a common binding site of COX enzyme, whereas aspirin

irreversibly modifies the catalytic activity of COX enzyme. COX-1 is constitutively expressed in most tissues, where it produces prostanoids involved homeostatic functions such as gastric cytoprotection, maintaining renal blood flow, and platelet activation. COX-2 is mainly regarded as an inducible enzyme. Its induction at the sites of inflammation by stimuli such as growth factors, cytokines, and lipopolysaccharides generates prostanoids involved in transmission of inflammation, pain, and fever (O´banion et al. 1992, Masferrer et al. 1990, Capone et al. 2007). Therefore, COX-1 inhibition by NSAIDs is thought to be principally responsible for their gastrointestinal and bleeding complications, whereas COX-2 inhibition is though to be responsible for their therapeutic anti-inflammatory, analgesic, and antipyretic efficacy (Mitchell et al. 1993, Warner & Mitchell 2004). However, this division is simplified, because COX-2 is also constitutively expressed in several tissues e.g. in the brain and kidney (Harris et al. 1994, Breder et al. 1995).

NSAIDs can be classified into traditional NSAIDs (tNSAIDs) and coxibs. A more accurate division can be made according to the ability of NSAIDs to inhibit COX enzymes (Figure 1). Nonselective COX inhibitors such as ibuprofen, ketoprofen, diclofenac, and naproxen have balanced inhibitory effect towards both COX isoforms. Selective COX-2 inhibitors are NSAIDs that inhibit COX-2 more potently than COX-1. These include coxibs (e.g. etoricoxib, celecoxib, lumiracoxib) and also meloxicam, nimesulide, and etodolac, which are sometimes also classified as preferential COX-2 inhibitors (Capone et al. 2007). There is very little difference in clinical efficacy between the NSAIDs when used at equivalent doses (Van Tulder et al. 2006, Ong et al. 2007). Rather, differences between compounds arise from dosing, pharmacokinetics, and tolerability profile (Ong et al. 2007).

2.7.2 Adverse effects

Gastrointestinal (GI) disturbances are well known unwanted adverse effects of NSAIDs. The most common of these is dyspepsia that occurs in 5-30% of regular tNSAID users (Larkai et al. 1987, Ofman et al. 2003). However, about half of tNSAID users have gastric erosions and 10% to 30% have peptic ulcers at endoscopy. The majority of erosions and ulcers are asymptomatic, but 7.3-13 of every 1000 patients who take tNSAID for one year, develop a serious GI complication, such as perforation and bleeding (Singh & Triadafilopoulos 1999). The risk of GI adverse effects is increased with older age, a history of peptic ulcer, and with high doses of tNSAIDs (Garcia Rodriguez & Jick 1994, Henry et al. 1996, Ofman et al. 2003). GI adverse effects are believed to result from direct and indirect irritation of the gastrointestinal tract. Most NSAIDs are weak acids, which directly irritate the gastric mucosa, whereas the systemic effect is mainly a result of inhibition of COX-1. COX-2 selective coxibs are shown to cause fewer GI adverse effects compared with nonselective NSAIDs (Bombardier et al. 2000, Silverstein et al. 2000, Laine et al. 2007, Rostom et al. 2007). In addition, the risk of GI complications in NSAID users can be reduced by concomitant use of proton pump inhibitors or misoprostol with NSAIDs (Targownik et al. 2008).

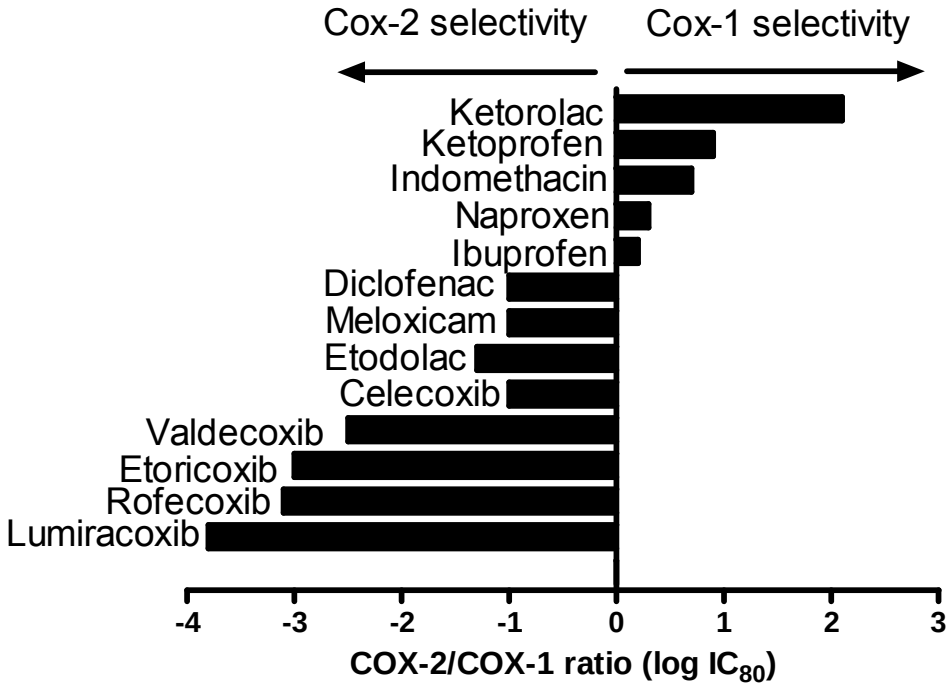


Figure 1. Relative COX selectivity of NSAIDs displayed as ratio of IC_{80} concentrations. IC_{80} ratios are shown logarithmically so that 0 represents similar activity against COX-1 and COX-2 (modified from Warner & Mitchell 2004).

Soon after entering the market, the use of coxibs was associated with an increased risk of cardiovascular (CV) adverse effects (e.g. myocardial infarctions, cardiovascular deaths, strokes) (Bombardier et al. 2000, Bresalier et al. 2005, Nussmeier et al. 2005). This led to the withdrawal of two coxibs (rofecoxib, valdecoxib) from the market in 2004 and 2005 and also to the re-evaluation of cardiovascular safety of other NSAIDs. Today, epidemiological data suggest that coxibs and other NSAIDs as a class all carry some variable potential risk for CV adverse effects (with the exception of aspirin and perhaps naproxen), particularly when taken at high doses for prolonged periods of time (Solomon et al. 2006, Cannon et al. 2006, Helin-Salmivaara et al. 2006, Warner & Mitchell 2008).

NSAIDs inhibit the synthesis of renal prostaglandins, which play an important role in kidney function via their effects on solute homeostasis, glomerular filtration, and vascular tone. The dependence of renal physiology on actions of prostaglandins is minimal under normal conditions. However, in situations of reduced renal perfusion and decreased circulating blood volume, renal function becomes increasingly dependent on renal prostaglandin synthesis (Brater 1999, Bennet et al. 1996). Accordingly, therapeutic doses of NSAIDs in healthy individuals cause little threat to kidney function, but in susceptible patients (e.g. elderly patients) they can produce

renal adverse effects. Clinical manifestations of renal adverse effects include e.g. acute renal insufficiency, hypertension, peripheral edema, hyperkalemia, congestive heart failure, and papillary necrosis. These occur in 1-5% of NSAID users and they are found to be dose-dependent but independent of COX selectivity of NSAIDs (Whelton & Hamilton, 1991, Brater 2001, Schwartz et al. 2002).

Nonselective COX inhibitors impair the platelet function by preventing the formation of thromboxane A_2, thus increasing the bleeding time (Cronberg et al. 1984, Capone et al. 2007). This might also contribute to NSAID induced GI bleeding. Other, much less common NSAID related adverse effects include e.g. central nervous system effects, liver disorders, intolerance, and skin reactions (Meyler's Side Effects of Drugs 2006).

2.7.3 Ibuprofen

Ibuprofen is a traditional NSAID of 2-arylpropionic acid class. Ibuprofen is mostly administered as a racemic preparation, which contains both *S*-(+)- and *R*-(-)-ibuprofen. Almost all of the pharmacological activity of ibuprofen comes from the *S*-(+)-ibuprofen, which shows much higher potency than *R*-(-)-ibuprofen in inhibiting prostaglandin synthesis (Villanueva et al. 1993, Neupert et al. 1997). However, after administration of racemic ibuprofen, about 60% of *R*-(-)-ibuprofen is unidirectionally converted to *S*-(+)-ibuprofen via formation of ibuprofenyl-CoA, followed by its epimerisation and hydrolysis (Lee et al. 1985, Tracy et al. 1993). Accordingly, *R*-(-)-ibuprofen acts as a prodrug and thus both enantiomers contribute to the pharmacological activity of ibuprofen. *S*-(+)-ibuprofen inhibits the activity of COX-1 and COX-2 at equal concentrations *ex vivo* and therefore, ibuprofen can be classified as nonselective COX inhibitor (Neupert et al. 1997). A positive correlation has been demonstrated between plasma ibuprofen concentrations and analgesic effect as well as between ibuprofen concentrations and improvement in disability in patients with rheumatoid arthritis or with hip or knee osteoarthritis (Grennan et al. 1983, Laska et al. 1986, Bradley et al. 1992).

Both ibuprofen enantiomers are extensively metabolized to inactive hydroxy and carboxy metabolites, 2- hydroxyibuprofen and carboxyibuprofen being the major metabolites (Mills et al. 1973, Tan et al. 2002). All phase I metabolites as well as intact enantiomers can be further conjugated with glucuronic acid to form phase II metabolites (Kepp et al. 1997). Total recovery of ibuprofen and its metabolites in urine is 70-90% with less than 1% of ibuprofen eliminated unchanged in urine (Geisslinger et al. 1993, Tan et al. 2002). *In vitro* studies have indicated that both enantiomers are metabolized by CYP2C9, but there also exists stereoselectivity on ibuprofen metabolism indicating that CYP2C9 is the main enzyme catalyzing the metabolism of *S*-(+)-ibuprofen, whereas CYP2C8 is the main enzyme catalyzing the metabolism of *R*-(-)-ibuprofen (Leemann et al. 1993, Hamman et al. 1997). Carriers of *CYP2C9*3* allele have a lower clearance of *S*-ibuprofen than individuals homozygous for the wild type allele (Kirchheiner et al. 2002). On the other hand, *CYP2C8*3* allele is associated with a slow elimination of both *R*-(-)- and *S*-(+)-ibuprofen (Garcia-Martin et al. 2004, Martinez et al. 2005).

2.7.4 Diclofenac

Diclofenac, a phenyl acetic acid, has typically been classified as a nonselective COX inhibitor, which equipotently inhibits the activity of COX-1 and COX-2 (Mitchell et al. 1993, Warner et al. 1999). However, there are also studies suggesting that diclofenac is 10- to 20-fold more potent towards COX-2 and can thus be placed into the cluster of selective COX-2 inhibitors (Patrignani et al. 1997, Hinz et al. 2003). Analgesic efficacy of diclofenac is shown to be dose-dependent in the treatment of postoperative pain (Collins et al. 1998, Handel et al. 2004).

Diclofenac is metabolised via hydroxylation and glururonidation, with less than 1% of the diclofenac dose excreted unchanged into urine (Geiger et al. 1975). The major hydroxy metabolite of diclofenac is 4'-hydroxy (OH)-diclofenac, with 3'-OH-, 5'-OH-, 4',5'-diOH-diclofenac being minor metabolites (Stierlin et al. 1979, Faigle et al. 1988). *In vitro*, the main enzyme responsible for the 4'-hydroxylation and 3'-hydroxylation of diclofenac is cytochrome CYP2C9 enzyme (Leemann et al. 1993, Bort et al. 1999), whereas the 5'-hydroxylation appears to be mediated by other CYP2C and CYP3A enzymes (Tang et al. 1999, Shen et al. 1999). Both intact diclofenac and its hydroxy metabolites can be converted to glucuronide conjugates by 5'-diphosphoglucuronosyl transferase (UGT) 2B7 (King et al. 2001). In addition, it has been shown that diclofenac glucuronide is subject to further 4-hydroxylation catalyzed by CYP2C8 (Kumar et al. 2002). Despite extensive CYP2C9-dependent 4-hydroxylation of diclofenac *in vitro,* many studies have revealed that *CYP2C9 *2* and *3* alleles have no influence on diclofenac pharmacokinetics *in vivo* (Yasar et al. 2001, Kirchheiner et al. 2003, Brenner et al. 2003).

2.7.5 Etoricoxib

Etoricoxib is a selective COX-2 inhibitor indicated for the treatment of acute pain, osteoarthritis, rheumatoid arthritis, chronic low back pain, and acute gouty arthritis (Riendeau et al. 2001, Dallob et al. 2003). Etoricoxib has been shown to have similar analgesic efficacy compared with tNSAIDs (Shi & Klotz 2008), and a linear relationship exists between its plasma concentrations and pain relief (Malmstrom et al. 2004).

The elimination of etoricoxib is characterized by extensive metabolism (Rodrigues et al. 2003). 6`-methyl hydroxylation is the major primary oxidative metabolic pathway of etoricoxib, whereas 1`-N-oxidation is a relatively minor pathway. 6-Hydroxymethyl-etoricoxib is further converted to 6-carboxy-etoricoxib (Kassahun et al. 2001). Sixty to seventy per cent of etoricoxib metabolites are excreted into urine and 20% into faeces. Less than 1% of an oral dose is detected as unchanged drug in urine (Rodrigues et al. 2003). The formation of inactive 6´-hydroxymethyl-etoricoxib is mainly catalyzed by CYP3A4 (60%), with CYP2C9, CYP2D6, CYP1A2, and CYP2C19 each contributing about 10% of etoricoxib metabolism (Kassahun et al. 2001).

Table 1. The pharmacokinetics of NSAIDs and venlafaxine after single oral dose.

Drug	Oral bioavailability (%)	Plasma protein binding (%)	t_{max}	V_d (l/kg)	$t_{½}$ (h)
Ibuprofen	100	98	2-3	0.15	2
Diclofenac	50-60	99	2	0.5	2
Etoricoxib	100	90	1	1.6 *	21
Meloxicam	89	99	4-11	0.17	13-20
Venlafaxine	45	30	2	4.5	4

T_{max} = time to peak concentration, V_d = volume of distribution, $t_{½}$ = terminal elimination half-life, * = the original value has been divided by 70 kg to unify the units.
Ibuprofen: Davies 1997, Aarons et al. 1983, Paliwal et al. 1993; Diclofenac: Willis et al. 1979, John 1979, Davies & Anderson 1997, Fowler et al. 1983; Etoricoxib: Agrawal et al. 2003, Agrawal et al. 2004; Meloxicam: Turck et al.1997, Gates et al. 2005, Schmid et al. 1995; Venlafaxine: Klamerus et al. 1992, Holliday & Benfield 1995, Patat et al. 1998

2.7.6 Meloxicam

Meloxicam, an oxicam derivative, belongs to the enol-acid group of NSAIDs. Meloxicam inhibits COX-2 activity 10- to 20-fold more potently than COX-1 activity and is classified as a preferential or selective COX-2 inhibitor (Patrignani et al. 1997, Panara et al. 1999). However, the COX-1 sparing effect of meloxicam depends on the dose used, and with high doses, meloxicam inhibits the activity of COX-1 up to 66% (Panara et al. 1999, De Meijer et al. 1999). Both 7.5 mg and 15 mg have been shown to be effective in the treatment of e.g. osteoarthritis and rheumatoid arthritis, but no clear difference in the efficacy between these doses has been detected (Lund et al. 1998, Yocum et al. 2000, Reginster et al. 1996, Lemmel et al. 1997).

Meloxicam is extensively metabolized in the liver to four pharmacologically inactive metabolites. The major metabolite is 5`-hydroxymethyl meloxicam, which is further oxidized to 5`-carboxy meloxicam. The oxidative cleavage of the benzothiazine ring of meloxicam creates two additional metabolites (Schmid et al. 1995, Chesne et al. 1998). Almost the entire meloxicam dose is detected as hydroxy and carboxy metabolites in urine and faeces, and only negligible amounts of parent drug are found in urine and faeces (Schmid et al. 1995). Based on *in vitro* studies, 80% of the formation of 5`-hydoxymethyl metabolite is catalyzed by CYP2C9 and remaining 20% by CYP3A4

(Chesne et al. 1998). The effect of different CYP2C9 genotypes on the metabolism of meloxicam has not been studied.

Table 2. CYP enzymes responsible for the oxidative metabolism of NSAIDs.

NSAID	Major CYP enzyme	Minor CYP enzymes	Reference
Ibuprofen			
S-(+)-ibuprofen	CYP2C9	CYP2C8	Leemann et al. 1993, Hamman et al. 1997
R-(-)-ibuprofen	CYP2C8	CYP2C9	
Diclofenac	CYP2C9	CYP2C8, CYP3A4	Tang et al. 1999, Shen et al. 1999, Kumar et al. 2002
Etoricoxib	CYP3A4	CYP2C9, CYP2D6, CYP1A2, CYP2C19	Kassahun et al. 2001
Meloxicam	CYP2C9	CYP3A4	Chesne et al. 1998

2.8 Venlafaxine

Venlafaxine is a phenylethylamine derivative antidepressant that strongly inhibits the presynaptic reuptake of serotonin and norepinephrine and weakly inhibits the reuptake of dopamine (Muth et al. 1986, Holliday & Benfield 1995). Venlafaxine is a chiral drug, and studies have supposed that *S*-(+)-venlafaxine inhibits the reuptake of noradrenaline and serotonin, whereas *R*-(-)-venlafaxine primarily inhibits the reuptake of serotonin (Holliday & Benfield 1995). The major metabolite of venlafaxine is O-desmethylvenlafaxine (ODV), which possesses a similar receptor affinity profile to the parent drug and therefore, the pharmacological activity of venlafaxine is a sum of activity of venlafaxine plus ODV (active moiety) (Holliday & Benfield 1995). Venlafaxine has been shown to be as effective as selective serotonin reuptake inhibitors in the treatment of depression (Weinmann et al. 2008). In addition, due to its favourable effects on serotonergic and noradrenergic transmission, venlafaxine is effective and increasingly used in the treatment of neuropathic pain (Saarto & Wiffen 2008). The most common adverse effect of venlafaxine is nausea, others include such as malaise, headache, dizziness, elevated blood pressure, palpitations, and diarrhoea. The adverse effects often occur with the initiation of venlafaxine therapy and seem to be dose-related (Holliday & Benfield 1995, Scott et al. 1996, Mackay et al. 1999).

Venlafaxine is eliminated mainly by hepatic metabolism to its major active metabolite O-desmethylvenlafaxine (ODV) and to its minor inactive metabolite, N-desmethylvenlafaxine (NDV), which are further metabolized to N,O-didesmethylvenlafaxine (NODV) (Figure 2) (Holliday & Benfield 1995). *In vitro* studies have demonstrated that the formation of ODV is catalyzed by CYP2D6, whereas the formation of NDV is catalyzed mainly by CYP3A4 and to a lesser extent by CYP2C9 and CYP2C19 (Otton et al. 1996, Fogelman et al. 1999). CYP2D6

catalyzes the O-demetylation of both enantiomers of venlafaxine, but it has also been suggested that it displays a stereoselectivity towards *R*-(-)-venlafaxine (Eap et al. 2003).

Patients with PM genotype for CYP2D6 have increased concentrations of venlafaxine and decreased concentrations of ODV, but the concentration of venlafaxine active moiety is unchanged (Lessard et al. 1999, Shams et al. 2006). In spite of unchanged venlafaxine active moiety concentration, PMs have an increased risk of venlafaxine adverse effects. It has been suggested that high concentration of parent drug together with slight differences in reuptake inhibition profiles between venlafaxine and ODV might explain increased risk of venlafaxine adverse effects in PMs (Lessard et al. 1999, Shams et al. 2006). The CYP2D6 inhibitor, quinidine, has been shown to decrease venlafaxine oral clearance from 100 l/h to 17 l/h in EMs of CYP2D6 (Lessard et al. 1999) and CYP3A4 inhibitor, ketoconazole, has been reported to increase the AUC of venlafaxine by 36% and AUC of ODV by 26%, in healthy volunteers (Lindh et al. 2003).

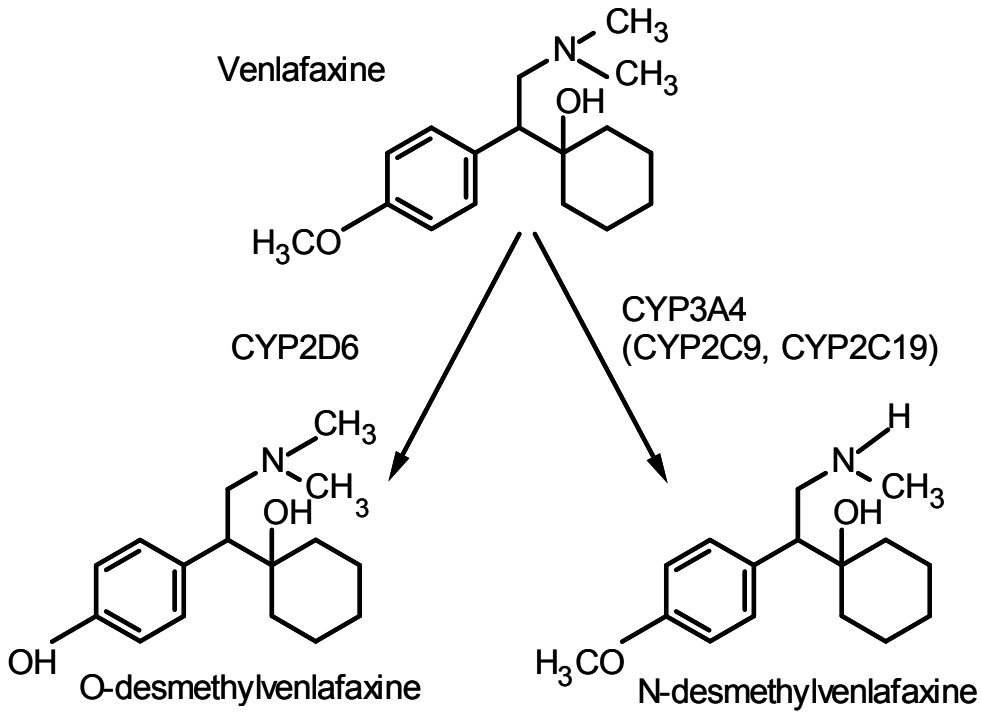


Figure 2. The metabolism of venlafaxine

2.9 Employed CYP inhibitors

The azoles are a group of synthetic antifungal agents that include two classes; imidazoles and triazoles. The antifungal activity of azoles is based on the inhibition of the fungal 14-α-demetylase, a cytochrome P450-dependent enzyme system responsible for converting lanosterol to ergosterol, the main sterol in the fungal cell membrane (Lamb et al. 1999, Jeu et al. 2003). Although the main effect focuses on fungi membrane, all azoles also inhibit human CYP enzymes. The degree of inhibition varies with each azole and is different for various CYP isoforms on the basis of azole's physiochemical characteristics and pharmacokinetics. The CYP inhibition by azoles has been mostly reported to be competitive in nature (Venkatakrishnan et al. 2000).

2.9.1 Voriconazole

Voriconazole is a novel antifungal agent first introduced into the market in USA in 2001. It is structurally derived from fluconazole with an extended spectrum of activity against a wide variety of fungi. Voriconazole has become a first line drug in the treatment of systemic aspergillosis, but it is also used in the treatment of e.g. fluconazole-resistant Candida infections as well as in the treatment of infections caused by Scedosporium and Fusarium species (Herbrecht et al. 2002, Herbrecht 2004, Kullberg et al. 2005).

After an oral dose, the C_{max} of voriconazole is achieved within 1-2 h, and the oral bioavailability of voriconazole is over 90% (Purkins et al. 2003a, Roffey et al. 2003). The C_{max} and AUC values of voriconazole have been shown to increase disproportionately with ingreasing doses of voriconazole, indicating nonlinear pharmacokinetics of voriconazole, most likely due to the saturation of its metabolism (Purkins et al. 2003a, b). About 58% of voriconazole binds to plasma proteins (Purkins et al. 2003b). The mean elimination half-life of voriconazole is 6 h, but due to nonlinear pharmacokinetics, the $t_{½}$ of voriconazole depends on the dose administered (Purkins et al. 2002, Purkins et al. 2003a). The steady state concentration of voriconazole is reached after 5-7 days of multiple oral dosing of 200 mg twice daily, but can be achieved within 24 h by using the oral loading dose of 400 mg twice a day for one day (Purkins et al. 2002). Variability between an individual's plasma voriconazole concentrations is high and can be at least partly explained by CYP2C19 polymorphism (Purkins et al. 2003a). Data published so far indicate approximately 3- to 4-fold higher voriconazole AUC or C_{max} values in CYP2C19 PMs than in homozygous EMs for CYP2C19 (Mikus et al. 2006, Ikeda et al. 2004, Rengelshausen et al. 2005).

The most frequently reported adverse effects of voriconazole are visual disturbances. Approximately 30% of patients experience altered or enhanced visual perception, blurred vision, colour vision change, or photofobia, which typically occur 30 minutes after intake of voriconazole during the first week of therapy and are spontaneusly resolved within 1 hour. Other commonly reported adverse effects include liver function test abnormalities and various kinds of skin reactions (Jeu et al. 2003).

In vitro studies have indicated that voriconazole inhibits the CYP2C9 catalyzed tolbutamide hydroxylation, CYP2C19 catalyzed S-mephenytoin 4`-hydroxylation, and CYP3A4 catalyzed nifedipine oxidation, whereas it is not found to inhibit CYP1A2, CYP2E1, or CYP2D6 catalyzed reactions. The inhibition of CYPs by voriconazole was not stimulated by preincubation, suggesting that voriconazole is not a mechanism-based inhibitor (Niwa et al. 2005a, Niwa et al. 2005b). *In vivo*, voriconazole has been shown to increase the concentrations of the CYP3A4 substrate midazolam (Saari et al. 2005), the CYP2C19 substrate omepaprazole (http://www.emea.eu.int./humandocs/Humans/EPAR/vfend/vfend.htm.), and to potentiate the CYP2C9 substrate warfarin induced prothrombin time prolongation (Purkins et al. 2003c).

2.9.2 Fluconazole

The first triazole antifungal agent, fluconazole, was released in 1990. It has retained its position as a leading drug of antifungal prophylaxis and therapy of invasive candidas (Charlier et al. 2006). Fluconazole is almost completely absorbed from GI tract, its oral bioavailability being over 90%. Peak plasma levels are reached normally 1-2 h after ingestion. Only 11% of fluconazole is bound to plasma proteins. The elimination $t_{½}$ is from 27 to 37 h, with a minimum of 6 days needed to reach steady-state levels. However, using the double dose during the first day, steady state concentrations can be achieved within 2 days (Debruyne & Ryckelynck 1993, Tett et al. 1995).

Fluconazole has been shown to be an inhibitor of CYP2C9 (Kunze et al.1996, Niwa et al. 2005a) and CYP2C19 (Wienkers et al. 1996, Niwa et al. 2005a) *in vitro*. These findings have been confirmed by *in vivo* studies, where fluconazole has been shown to inhibit CYP2C9 catalyzed 6- and 7-hydroxylation of S-warfarin by approximately 70% (Black et al. 1996) and to inhibit the CYP2C19 mediated metabolism of omeprazole, leading to about 6-fold increase in the AUC of omeprazole (Kang et al. 2002). Today, fluconazole is recommended as a first choice CYP2C9 inhibitor to be used for *in vivo* pharmacokinetic studies investigating the effect of CYP2C9 inhibition on the metabolism of an investigational drug (Huang et al. 2007). *In vitro,* fluconazole seems to be a weaker inhibitor of CYP3A4 than ketokonazole and itraconazole (von Moltke et al. 1996), but also fluconazole inhibits CYP3A4 mediated metabolism of midazolam *in vivo*, although the magnitude of interaction is smaller than that with itraconazole (Olkkola et al. 1996). The CYP inhibition produced by fluconazole seems to be mainly competitive in nature (Kunze et al.1996, von Moltke et al. 1996, Niwa et al. 2005a).

2.9.3 Miconazole

Miconazole is an imidazole antifungal agent that has been available since the 1970s. It was originally developed for systemic use, but due to its low oral bioavailability and high incidence of adverse effects in systemic use, it is nowadays used almost solely as a topical preparation. Miconazole has a broad-spectrum antifungal activity against most frequent Candidas observed in mouth (Kuriyama et al. 2005) and is thereby widely used as an oral gel in the treatment of oral candidiasis. Instructions for miconazole oral gel use include that the oral gel is kept in the mouth for as long as possible before swallowing. Administration of a typical 60 mg dose of miconazole oral

gel results in peak plasma concentrations of 31-49 ng/ml within two hours postdose. Absorbed miconazole is bound to plasma proteins (88.2%) and its $t_{½}$ is 20 hours (Daneshmend & Warnock 1983).

In vitro studies have revealed that miconazole is a nonselective inhibitor of several CYPs, namely CYP3A4, CYP2C9, CYP2C19, CYP1A2, CYP2A6 and CYP2B6 (Zhang et al. 2002, Niwa et al. 2005a, Niwa et al. 2005b). *In vivo*, the interaction between systemically administered miconazole and CYP2C9 substrate, warfarin, is well established (O`Reilly et al. 1992) and in addition, several case reports have reported that miconazole oral gel can affect warfarin induced anticoagulation (Ariyaratnam et al. 1997, Silingardi et al. 2000, Pemberton et al. 2004). However, there seem to be no controlled clinical trials investigating the effect of miconazole oral gel on the pharmacokinetics of different CYP substrates.

2.9.4 Itraconazole

Itraconazole is a triazole antifungal agent first brought into the market in 1990. It is used for prophylaxis and treatment of many systemic fungal infections (*Candida*, *Blastomyces*, *Histoplasma* infections) as well as for superficial fungal infections such as onychomycosis (Chapman et al. 2000, Wheat et al. 2000, Pappas et al. 2004, Finch & Warshaw 2007). The bioavailability of itraconazole 100 mg capsules is reported to be approximately 55%, and the C_{max} is reached in 3-4 h. Itraconazole is highly bound (>99%) to plasma albumin. The major metabolite, hydroxy-itraconazole, has a similar antifungal activity than the parent drug. The elimination half-life of itraconazole is 30 h and that of hydroxy-itraconazole about 14 h. However, the elimination kinetics of itraconazole depends on the dose used, and the overall clearance is reduced after high doses (Hardin et al. 1988, Heykants et al. 1989).

Itraconazole has been shown to be a potent competitive inhibitor of CYP3A4, without an inhibitory effect on CYP2C9, CYP2C19, CYP2D6, CYP1A2, and CYP2E1 catalyzed reactions *in vitro* (von Moltke et al. 1996, Niwa et al. 2005a, Niwa et al. 2005b). *In vivo*, itraconazole increases the AUC of oral midazolam, CYP3A4 substrate, over 10-fold (Olkkola et al. 1996), but does not inhibit CYP2C9 catalyzed metabolism of losartan (Kaukonen et al. 1998). In addition, also itraconazole metabolites; hydroxy-itraconazole, keto-itraconazole, and N-desalkyl-itraconazole have been found to be potent CYP3A4 inhibitors (Isoherranen et al. 2004). Nowadays, itraconazole is recommended as a standard CYP3A4 inhibitor to be used in drug-drug interaction studies (Huang et al. 2007). Itraconazole also has the ability to inhibit the function of P-glycoprotein (Partanen et al. 1996, Wang et al. 2002).

2.9.5 Terbinafine

Terbinafine belongs to the allylamine group of antifungal agents, first marketed in Europe in 1991. It blocks the biosynthesis of ergosterol in fungi by inhibiting squalene epoxidase enzyme (Ryder 1985). Terbinafine is effective against many dermatophytes, yeasts, and moulds and is therefore widely used to treat skin infections, especially onychomycosis (Darkes et al. 2003). Following the oral administration of a single dose

of terbinafine, its oral bioavailability is 47%, and C_{max} is achieved within 1.3-2 h. Terbinafine is 94% bound to plasma proteins. After a single dose, the initial $t_{½}$ of terbinafine is 16-26 h, whereas the terminal $t_{½}$ can be as long as 90 h (Balfour & Faulds 1992).

Compared with azoles, terbinafine has a limited ability to inhibit different CYP enzymes. *In vitro*, terbinafine competitively inhibits CYP2D6 without any significant effect on other CYP enzymes (Back et al. 1989). These findings are consistent with significant interaction of terbinafine in clinical studies with CYP2D6 substrate, dextromethorphan (Abdel-Rahman et al. 1999), and with minimal or nonexistent interactions of terbinafine with the CYP1A2 substrate, theophylline, the CYP2C9 substrate, warfarin, and the CYP3A4 substrate midazolam (Ahonen et al. 1995, Guerret et al. 1997, Trepanier et al. 1998).

3 AIMS OF THE STUDY

The overall goal of the present studies was to investigate the effect of the CYP inhibition, using antifungal agents as typical CYP inhibitors, on the pharmacokinetics of NSAIDs and venlafaxine, drugs used in the treatment of pain. The specific aims of the studies were:

1. To investigate the effect of voriconazole on the pharmacokinetics of *S*-(+)- and *R*-(-)-ibuprofen and to compare its effect with that of fluconazole (Study I)

2. To investigate the effect of voriconazole on the pharmacokinetics of diclofenac (Study II)

3. To investigate the effect of miconazole oral gel on the pharmacokinetics and COX-1 inhibition of etoricoxib and to compare its effect with that of voriconazole (Study III)

4. To investigate the effect of voriconazole on the pharmacokinetics and COX-1 inhibition of meloxicam and to compare its effect with that of itraconazole (Study IV)

5. To investigate the effects of terbinafine and voriconazole on the pharmacokinetics of venlafaxine (Study V)

4 MATERIALS AND METHODS

4.1 Subjects

Altogether 45 healthy male volunteers participated in the studies, 7 of whom participated in 2 studies and 3 of whom participated in 3 studies. The number of subjects and their demographics are shown in Table 3. Before entering the study, each subject was ascertained to be healthy by medical history, clinical examination, and routine laboratory tests including complete blood count, plasma creatinine, alkaline phosphatase, alanine aminotransferase, and urinalysis. In addition, 12-lead electrocardiogram was obtained in study V. Exclusion criteria were identical in all studies (Table 4). The volunteers were not allowed to drink grapefruit juice or take any drugs known to cause CYP enzyme inhibition or induction for four weeks before the study.

Table 3. The mean (range) demographics of the subjects in studies I-V

Study	No of subjects	Age, mean (range)	BMI (kg/m^2)
I	12	21 (19-23)	23 (20-26)
II	10	22 (20-31)	24 (21-27)
III	12	24 (20-28)	23 (20-25)
IV	12	26 (20-39)	23 (21-26)
V	12	23 (20-29)	22 (21-26)

4.2 Study design

All studies were carried out in an open-label, randomized, controlled, crossover design. Studies I and III-V had three phases and study II had two phases. The drug free washout period between the phases was two weeks in studies I-III and four weeks in studies IV and V. In all studies, volunteers were given in a randomized order either no pretreatment (control phase) or oral voriconazole pretreatment (voriconazole phase) for 2 days or depending on the study, another oral antifungal pretreatment (Table 5). Pretreatment drugs were self-administered by subjects according to a dosing schedule, except for the last doses, which were administered by the study personnel. Compliance to pretreatment was verified by use of mobile phone short message service and by tablet counting.

On study days, after overnight fasting, the subjects arrived in the clinical laboratory of the Department of Pharmacology, Drug Development and Therapeutics, where the study drug was administered precisely one hour after the last dose of pretreatment drug, at 9 AM with 150 ml of water. Venous blood samples were collected for the determination of plasma concentrations of study drugs as well as concentrations of

pretreatment drugs at least for period of 3 elimination half-lives of the study drug. Plasma was separated within 30 minutes and stored at -70 °C until analysis of drug concentrations. In study II, urine was collected for 24 hours for the determination of diclofenac. The subjects were offered standardized meals 4 and 8 hours after study drug ingestion. On study days, subjects stayed at the clinical laboratory from 7 AM till 9 PM.

Table 4. Exclusion criteria in all studies

History of intolerance to the study drugs	Notable psychological or emotional problems
Concomitant drug therapy	History of alcoholism or drug abuse
Age under 18 or over 40	Existing significant disease
Existing significant disease	Smoking
Participation in other studies involving investigational or marketed drug products concomitantly	Donation of blood for 4 weeks prior to the study

4.3 Determination of plasma drug concentrations

4.3.1 Ibuprofen

Plasma concentrations of *R*-(-)- and *S*-(+)-ibuprofen were determined by high-performance liquid chromatography (HPLC) with UV detection, as previously described (Menzel-Soglowek et al. 1990, Pettersson et al. 1991). In addition, because voriconazole metabolite interfered with the HPLC analysis of *R*-(-)-ibuprofen, the concentrations of *R*-(-)-ibuprofen during the voriconazole phase were quantified by a liquid chromatography-tandem mass spectrometry system (MDS SCIEX, Applied BioSystems, Q Trap LC/MS/MS System, Foster City, CA) The interday coefficient of variation (CV) was less than 12% for both enantiomers at the concentrations 500 ng/ml, 5000 ng/ml, and 25000 ng/ml. The limit of quantification for *R*-(-)- and *S*-(+)-ibuprofen was 250 ng/ml.

4.3.2 Diclofenac

The concentrations of diclofenac in plasma and urine were determined by HPLC (Lansdorp et al. 1990, Zecca et al. 1991). Flufenamic acid was used as the internal standard. The interday CV was 3.4%, 3.1% and 5.9% at 100 ng/ml, 1500 ng/ml, and 6000 ng/ml, respectively. The limit of quantification for diclofenac was 15 ng/ml.

4.3.3 Etoricoxib

Etoricoxib plasma concentrations were determined by HPLC using UV detection and using rofecoxib as an internal standard (Chavez-Eng et al. 2000). The interday CV was 7.7%, 2.2%, and 2.8% at 29.3 ng/ml, 290 ng/ml, and 1078 ng/ml, respectively. The limit of quantification was 6 ng/ml.

Table 5. Study drugs and CYP inhibitors used in studies I to V

Study	Pretreatment			Study drug		Wash-out
	Drug	*Dose*	*Duration*	*Drug*	*Dose*	
I	Voriconazole (Vfend®) Voriconazole (Vfend®) Fluconazole (Fluconazol ratiopharm®) Fluconazole (Fluconazol ratiopharm®)	400 mg x 2 po 200 mg x 2 po 400 mg x 1 po 200 mg x 1 po	1. day 2. day 1. day 2. day	Ibuprofen (Burana®)	400 mg po	2 weeks
II	Voriconazole (Vfend®) Voriconazole (Vfend®)	400 mg x 2 po 200 mg x 2 po	1. day 2. day	Diclofenac (Diclomex Rapid®)	50 mg po	2 weeks
III	Voriconazole (Vfend®) Voriconazole (Vfend®) Miconazole oral gel (Daktarin 2 % oral gel)	400 mg x 2 po 200 mg x 2 po 85 mg x 3	1. day 2. day 3 days	Etoricoxib (Arcoxia®)	60 mg po	2 weeks
IV	Voriconazole (Vfend®) Voriconazole (Vfend®) Itraconazole (Sporanox®)	400 mg x 2 po 200 mg x 2 po 200 mg x 1	1. day 2. day 4 days	Meloxicam (Mobic®)	15 mg po	4 weeks
V	Voriconazole (Vfend®) Voriconazole (Vfend®) Terbinafine (Lamisil®)	400 mg x 2 po 200 mg x 2 po 250 mg x 1	1. day 2. day 4 days	Venlafaxine (Efexor®)	75 mg po	4 weeks

4.3.4 Meloxicam

Plasma concentrations of meloxicam were measured, as described earlier (Ji et al. 2005), using piroxicam as an internal standard and using a Q Trap liquid chromatography-tandem mass spectrometry system (Sciex Division of MDS, Toronto, Ontario, Canada). The interday CV for meloxicam was 7.0%, 5.6%, 6.1% at 100 ng/ml, 500 ng/ml, and 1000 ng/ml, respectively. The limit of quantification was 10 ng/ml.

4.3.5 Venlafaxine

Plasma concentrations of venlafaxine and ODV were quantified by use of a Dionex Ultimate 3000 liquid chromatography system and a Dionex RF 2000 fluorescence detector (Dionex Softron GmbH, Germering, Germany). Plasma (1.0 ml) and the internal standard citalopram (20 µg in 10 ml methanol/water, 1:1, v/v), were vortexed and applied to an Oasis MCX solid-phase extraction cartridge (1 ml, 30 mg; Waters Corp, Milford, USA) with prior conditioning with 1 ml methanol and 1 ml water. Cartridges were washed with 1 ml 0.1 M HCl and 3 ml methanol, and then they were eluted with 1 ml 2% (v/v) ammonium hydroxide in methanol. Samples were evaporated to dryness under a nitrogen stream, reconstituted with 100 µl of 50 mM ammoniumdihydrogenphosphate/acetonitrile/methanol, and transferred to autosampler vials. Chromatography was performed on a Hypersil BDS-C18 analytic column (3 µm, 4.0 x 100 mm) with a Hypersil BDS-C18 guard column (5 µm, 4.0 x 4.0 mm, Agilent Technologies, Santa Clara, USA) by use of gradient elution in pH 4.4. The interday CV for venlafaxine was 4.3%, 2.2%, 2.0% at 5.0 ng/ml, 50 ng/ml, and 150 ng/ml, respectively, and for ODV 4.3%, 2.2%, and 1.6% at 5.0 ng/ml, 50 ng/ml, and 150 ng/ml, respectively. The limit of quantification for both venlafaxine and ODV was 1 ng/ml.

4.3.6 Voriconazole

After a solid phase extraction of plasma voriconazole, its concentration was determined by HPLC, using a fluconazole analog as the internal standard as described earlier (Gage & Stopher 1998, Pennick et al. 2003). The limit of voriconazole quantification was 50 ng/ml in studies I, III, and IV and 20 ng/ml in studies II and V. The interday CV was less than 4% at the relevant concentrations (50 ng/ml, 1000 ng/ml, 10000 ng/ml) in all studies.

4.3.7 Fluconazole

The concentrations of plasma fluconazole were determined, after a solid phase extraction, by HPLC, using UK 54373 as the internal standard (Inagaki et al. 1992). The limit of fluconazole quantification was 0.2 mg/l. The interday CV was less than 2% at concentrations 3 mg/l and 18 mg/l (n=7).

4.3.8 Miconazole

Plasma concentrations of miconazole were determined by use of an API 2000 liquid chromatography-tandem mass spectrometry system (MDS Sciex, Toronto, Ontario,

Canada) (Compas et al. 1996, Roberts & Bersuder 2006). The limit of quantification for miconazole was 1.0 ng/ml, and the interday CV was 15.4%, 7.9%, and 7.4% at 4.3 ng/ml, 37.0 ng/ml, and 143 ng/ml, respectively.

4.3.9 Itraconazole

Itraconazole plasma concentrations were quantified by HPLC as described earlier (Gubbins et al. 1998). The interaday CV for itraconazole was 6.1%, 2.8%, 2.9% at 19 ng/ml, 192 ng/ml, and 1200 ng/ml, respectively. The limit of quantification for itraconazole was 10 ng/ml.

4.3.10 Terbinafine

Plasma concentrations of terbinafine were determined by HPLC (Kovarik et al. 1992). The limit of quantification was 20 ng/ml for terbinafine. The CV was 2.5% and 3.7% at 25 ng/ml and 100 ng/ml, respectively.

4.4 Genotyping

In studies I, II, and IV, the subjects were genotyped for *CYP2C9*2 and CYP2C9*3* using a TaqMan assay, as previously described (Yasar et al. 2001). Alleles containing no **2 or *3* were named *CYP2C9*1*. In addition, in study I, subjects were genotyped for *CYP2C8*3* (Yasar et al. 2002).

In study V, the genotyping for *CYP2D6*3* and *CYP2D6*4* alleles were determined by the TaqMan allele discrimination method (Heim & Meyer 1990). Detection of the CYP2D6 gene duplication was performed by long polymerase chain reaction (Lundqvist et al. 1999).

4.5 Pharmacokinetic calculations

The peak plasma concentration (C_{max}) and time needed to reach C_{max} (t_{max}) for each subject were derived directly from the plasma concentration data. All other pharmacokinetic variables were calculated using standard non-compartmental methods. Elimination rate constant (k_{el}) was determined by a linear regression analysis of the terminal linear part of the logarithmic plasma concentration versus time curve using at least 3 time points above the quantification limit. The $t_{½}$ was calculated by the following equation: $t_{½} = \ln 2/k_{el}$. The area under plasma concentration-time curve (AUC) was calculated from zero to either last measured time point, or was extrapolated to infinity, by using the linear trapezoidal rule for the rising phase of the plasma concentration-time curves and the logarithmic trapezoidal rule for the descending phase. The extrapolation of AUC to infinity was calculated by dividing the concentration measured at the last time point (above the quantification limit) by k_{el}. In study II, the renal clearance (CL_R) of diclofenac was calculated by dividing the amount of diclofenac excreted into urine within 24 hours by the plasma $AUC_{(0\text{-}24)}$ of diclofenac. All pharmacokinetic calculations were performed with WinNonlin pharmacokinetic program (version 4.1; Pharsight, Mountain View, California).

4.6 Pharmacodynamics

In studies III and IV, pharmacodynamics of etoricoxib and meloxicam were assessed by measuring thromboxane B_2 (TxB_2), a stable metabolite of TxA_2, formation by platelets in spontaneously clotting whole blood. The decrease in TxB_2 generation is shown to reflect the degree of COX-1 inhibition and is widely used to examine the inhibitory effect of NSAIDs on COX-1. (Brideau et al. 1996, Patragnani et al. 1997). On study days, blood samples for TxB_2 assay were drawn before, and at 1.5, 4, 8, 24, and 48 h after the etoricoxib administration in study III and before, at 5, 8, 12, 24, and 48 h after meloxicam administration in study IV. Blood samples were collected into glass tubes containing no anticoagulant and were immediately incubated for 1 hour at 37°C to stimulate the TxB_2 production in platelets during coagulation. Thereafter, serum was collected, centrifuged, and stored at -70°C until assayed for TxB_2 by enzyme immunoassay kit (Amersham Thromboxane B_2 Enzymeimmunoassay Biotrak™ System, GE Healthcare, UK). The limit of detection was 10 ng/ml, and the interassay CV was 17% and 10% in study III and IV, respectively. The decrease in TxB_2 generation was calculated by comparing TxB_2 concentration at different time points with the individual baseline value, which was the average of TxB_2 concentrations, measured before the study drug administration at the beginning of every phase of the study.

In study V, subjective effects of venlafaxine (no effects of the drug to very strong effects of the drug, very good performance to very poor performance) were assessed by using 100 mm visual analogue scales (VAS), and subjects were asked about typical serotonergic adverse effects with a structured questionnaire. In addition, the systolic and diastolic blood pressure and heart rate were measured by an automatic oscillometric blood pressure monitor. The sitting measurement was taken twice in the forearm after 5 minutes of rest, and the mean value was used in the calculations. Each pharmacodynamic variable was assessed before venlafaxine administration and 2, 4, 6, 8, 12 and 24 hours after the administration. The area under the response-time curve was determined by use of the trapezoidal rule for 24 hours for each pharmacodynamic variable.

4.7 Statistical analysis

The number of subjects in each study was based on pre-study sample size analysis with the power of 80% and a significance level of 0.05 in every study. The sample size needed was calculated to detect mean percentage change of 30% in the AUC of the study drug. Standard deviations of the study drug AUC were derived from previous studies. In the three-phase-studies, the pharmacokinetic and pharmacodynamic variables were compared by use of analysis of variance (ANOVA) for repeated measures, and a posteriori testing was performed by use of the Tukey test. T_{max} was analyzed with Friedman`s test, and Wilcoxon signed rank test was used for pairwise comparisons. In study III, an additional statistical analysis was performed for the change in TxB_2 values from baseline, which were analyzed by using the fixed subject effects model, including subject, treatment, period, time, period × time, and treatment

× time effects. Time was used as a repeated effect assuming the unstructured covariance structure. Study II was the only two-phase-study, and then the Student two-tailed t test for paired samples was used for the statistical testing of the pharmacokinetic results, and in the case of t_{max}, the Wilcoxon signed-rank test was used.

As recommended for bioequivalence testing, 90% confidence intervals about the geometric mean ratio of the pharmacokinetic variables were calculated in studies I and III. Bioequivalence (i.e., lack of interaction) was concluded if the 90% CI of the geometric mean ratios for both C_{max} and AUC were within the acceptance limit of 0.8 to 1.25. Correlations between the ratio of AUC of the study drug after antifungal treatment to the AUC during the control phase and the AUC or trough concentration of the antifungal were assessed by using Pearson correlation test when the data were normally distributed, and Spearman rank test was used for non-normally distributed data. In study IV, also the correlation between meloxicam C_{max} or AUC and decrease in TxB_2 formation was tested using Pearson correlation. Statistical analysis was carried out using the statistical program SYSTAT for Windows (version 10.2; Systat Software, Richmond, California). The chosen statistical significance level was $P < 0.05$.

4.8 Ethical considerations

All study protocols were conducted according to the Declaration of Helsinki and approved by the Ethics Committee of the Hospital District of Southwest Finland as well as by the National Agency for Medicines, Finland. The subjects received both verbal and written information on the study and they were told that they could withdraw from the study at any time they wanted. After this, volunteers gave their written informed consent before entering the studies. In all studies, the doses of drugs investigated were selected to be small enough so that they could be safely administered to the healthy volunteers. In addition, all studies were done in facilities where the treatment of any study drug related toxic effects could be done appropriately.

5 RESULTS

The mean pharmacokinetic changes and 95% confidence intervals of the NSAIDs studied are shown in figures 3 and 4.

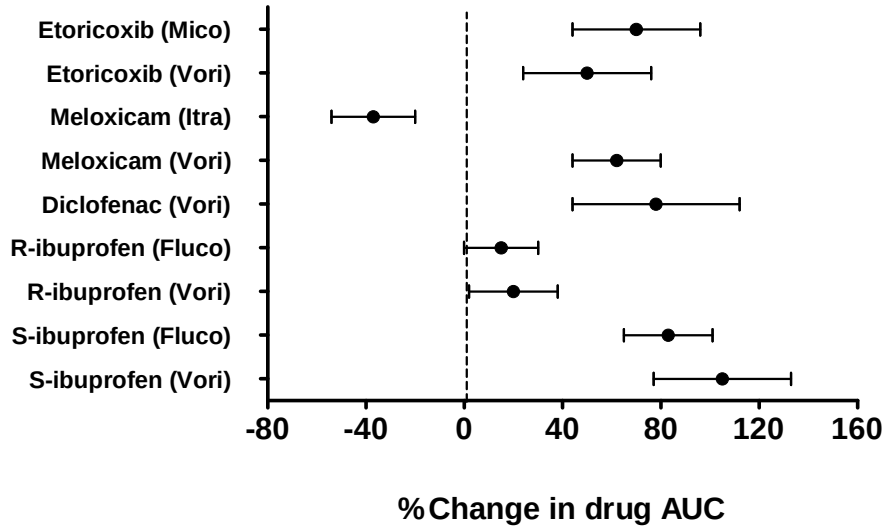
5.1 Effects of azole antifungals on NSAIDs metabolized by CYP2C9 (I, II, IV)

Voriconazole increased the mean $AUC_{(0-\infty)}$ of *S*-(+)-ibuprofen by 105% ($P < 0.001$) and prolonged the mean $t_{½}$ of *S*-(+)-ibuprofen by 43%, from 2.4 to 3.2 h ($P < 0.01$). In addition, the mean C_{max} of *S*-(+)-ibuprofen was 22% ($P < 0.01$) higher, whereas the median t_{max} of *S*-(+)-ibuprofen remained unchanged after voriconazole treatment. After fluconazole pretreatment, the mean $AUC_{(0-\infty)}$ of *S*-(+)-ibuprofen was increased by 83% ($P < 0.001$) and the mean C_{max} was increased by 16% ($P < 0.05$), compared with the control values. The mean $t_{½}$ of *S*-(+)-ibuprofen was prolonged by 34%, from 2.4 to 3.1 h ($P < 0.05$) and was also achieved later (3 h vs. 1 h; $P < 0.05$). The increase in the $AUC_{(0-\infty)}$ of *S*-(+)-ibuprofen was evident in all subjects after both voriconazole and fluconazole. The pharmacokinetic variables of *S*-(+)-ibuprofen after fluconazole pretreatment did not differ from those observed after voriconazole pretreatment.

The mean AUC of *R*-(-)-ibuprofen was increased by 20% ($P < 0.05$), whereas the $t_{½}$ was slightly shortened by 7% ($P < 0.01$) by voriconazole, compared with the control values. The mean C_{max} and median t_{max} of the *R*-(-)-ibuprofen remained unaffected. Fluconazole had no significant effects on the pharmacokinetics of *R*-(-)-ibuprofen

Compared with control phase values, the mean $AUC_{(0-\infty)}$ of diclofenac was increased by 78% ($P < 0.001$) and the mean C_{max} of diclofenac by 114% ($P < 0.05$) after voriconazole treatment. Again, the increase in the $AUC_{(0-\infty)}$ of diclofenac was observed in all subjects. The mean $t_{½}$ of diclofenac was found to be 22% ($P > 0.05$) shorter after voriconazole pretreatment, whereas the median t_{max} of diclofenac remained unaffected.

The AUC_{0-72} of meloxicam was increased in every subject after voriconazole treatment, the mean increase being 47% ($P < 0.001$). Compared with the control phase, the mean $t_{½}$ of meloxicam was prolonged by 51%, from 17.4 to 26.7 h ($P < 0.01$), but the mean C_{max} and median t_{max} of meloxicam were unaffected by voriconazole. By contrast, itraconazole decreased the mean $AUC_{(0-72)}$ meloxicam by 37% ($P < 0.001$) and its mean C_{max} by 64% ($P < 0.001$) compared with control values. The decrease of $AUC_{(0-72)}$ and C_{max} was seen in all subjects. The median t_{max} of meloxicam was reached later (24 h vs. 4 h; $P < 0.01$) and the mean $t_{½}$ was prolonged by 54%, from 17.4 to 27 h ($P < 0.01$) by itraconazole. The plasma protein binding of meloxicam was 99.83%, 99.83%, and 99.82% during the control, voriconazole, and itraconazole phase, respectively, measured from the plasma samples taken 5 h after meloxicam ingestion.



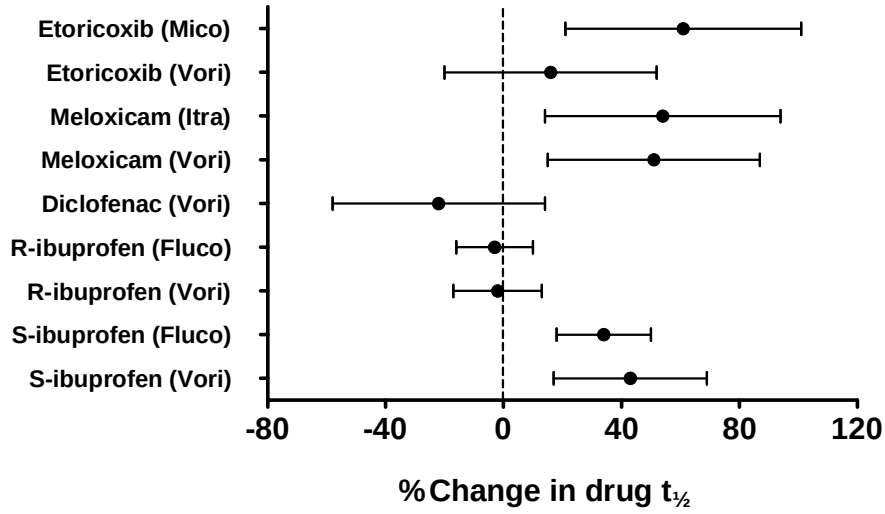


Figure 3. Percent changes in the area under the plasma concentration-time curve (AUC) and elimination half-life ($t_{½}$) of NSAIDs, with 95% confidence intervals, after pretreatment with voriconazole (Vori), fluconazole (Fluco), itraconazole (Itra), and miconazole (Mico).

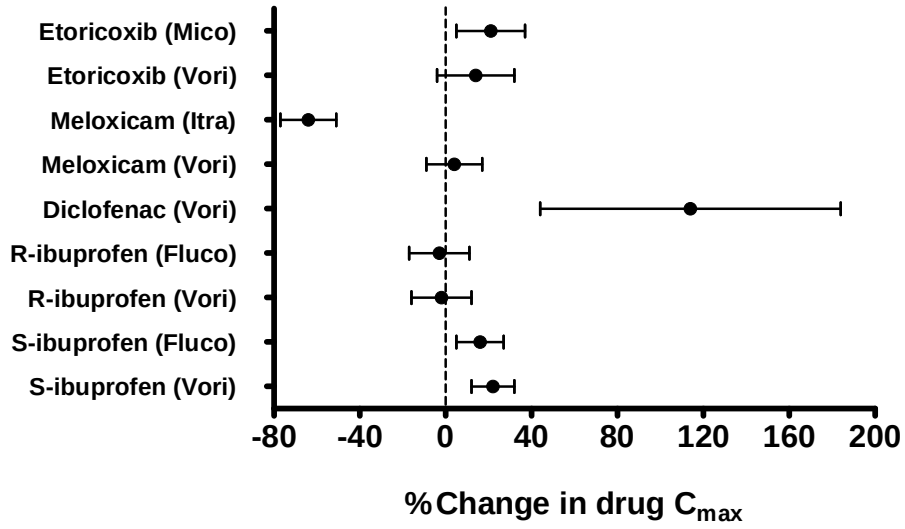


Figure 4. Percent changes in the peak plasma concentration (C_{max}) of NSAIDs, with 95% confidence intervals, after pretreatment with voriconazole (Vori), fluconazole (Fluco), itraconazole (Itra), and miconazole (Mico).

5.2 Effects of azole antifungals on etoricoxib (III)

Compared with the control values, voriconazole increased the mean $AUC_{(0-\infty)}$ and C_{max} of etoricoxib by 50% ($P < 0.01$) and by 21% ($P < 0.05$), respectively. The increase of etoricoxib $AUC_{(0-\infty)}$ was observed in all 12 subjects. The statistically nonsignificant prolongation of $t_{½}$ of etoricoxib (mean 16%) was seen in 10 out of 12 subjects. Voriconazole did not affect the median t_{max} of etoricoxib.

Miconazole oral gel increased the $AUC_{(0-\infty)}$ of etoricoxib in all subjects, the mean increase being 75% ($P < 0.001$), compared with the control values. In addition, the mean $t_{½}$ of etoricoxib was prolonged by 61%, from 18.9 to 31.3 hours ($P < 0.01$), whereas the mean C_{max} or median t_{max} of etoricoxib remained unaffected. Compared with the $t_{½}$ of etoricoxib in the voriconazole phase, the mean $t_{½}$ of etoricoxib was 40% longer ($P < 0.01$) after miconazole oral gel pretreatment.

5.3 Effects of terbinafine and voriconazole on venlafaxine (V)

The mean $AUC_{(0-\infty)}$ and C_{max} of venlafaxine was increased by 390% ($P < 0.001$) and by 167% ($P < 0.001$) after terbinafine pretreatment, respectively. The mean $t_{½}$ of venlafaxine was prolonged by 78%, from 5.1 to 8.6 h ($P < 0.001$), but the median t_{max} did not change after terbinafine pretreatment. The mean $AUC_{(0-\infty)}$ and C_{max} of ODV were 57% ($P < 0.001$) and 33% ($P < 0.001$) respectively, of the control values. Also, the mean $t_{½}$ of ODV was prolonged by 80%, from 10.3 to 18.6 hours ($P < 0.001$) and its median t_{max} from 5 to 10 hours ($P < 0.05$). The ratio of ODV $AUC_{(0-\infty)}$ to venlafaxine $AUC_{(0-\infty)}$ was 18% of the respective ratio in the control phase. The $AUC_{(0-}$

$_{\infty)}$ of the venlafaxine active moiety (the sum of $AUC_{(0-\infty)}$ of venlafaxine plus $AUC_{(0-\infty)}$ of ODV) was increased by 22% ($P < 0.05$) by terbinafine (Figure 5).

Voriconazole pretreatment increased the $AUC_{(0-\infty)}$ of venlafaxine in 11 out of the 12 subjects and the $AUC_{(0-\infty)}$ of ODV in 10 out of 12 subjects, but the mean increases were not statistically significant. However, the $AUC_{(0-\infty)}$ of the venlafaxine active moiety was increased by 31% ($P < 0.001$). Otherwise, the pharmacokinetic parameters of venlafaxine or ODV were not affected by voriconazole.

The AUC from 0 to 24 hours for overall drug effect (VAS) was increased by 100% ($P < 0.05$), during the voriconazole phase compared with the control phase. The other pharmacodynamic variables remained unchanged.

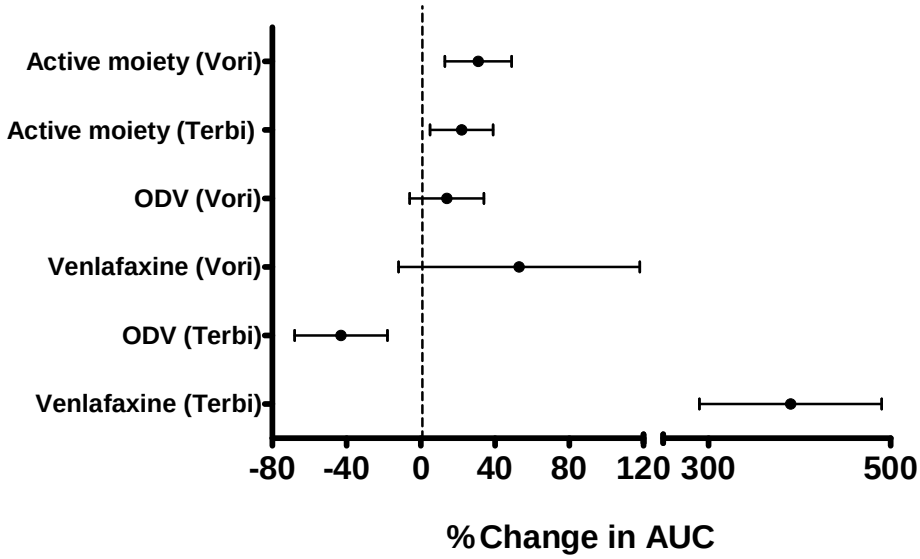


Figure 5. Percent changes in the area under the plasma concentration-time curve (AUC) of venlafaxine, ODV, and active moiety of venlafaxine, with 95% confidence intervals, after pretreatment with terbinafine (Terbi) or voriconazole (Vori). ODV = O-desmethylvenlafaxine, active moiety = AUC of venlafaxine plus AUC of ODV.

5.4 Effects of CYP genotypes

Genotyping in studies I, II, and IV revealed that altogether 4 subjects had the *CYP2C9*1/*3* genotype (2 subjects in study I, 1 subject in study II and study IV), 3 subjects had *CYP2C9*1/*2* genotype (1 subject in study II, 2 subjects in study IV), and 1 subject had *CYP2C9*2/*2* genotype (study IV). Other 24 were homozygous for the wild-type *CYP2C9*1* allele. In study I, where subjects were genotyped also for *CYP2C8,* one subject had the *CYP2C8*1/*3* genotype, whereas the other 11 were homozygous for the wild-type *CYP2C8*1* allele.

Two subjects with *CYP2C9*1/*3* and *CYP2C8*1/*1* genotype had the longest $t_{½}$ of *S*-(+)-ibuprofen in the control phase, and one subject with *CYP2C9*1/*3* genotype had the longest $t_{½}$ of meloxicam in the control phase. The pharmacokinetic parameters of diclofenac of one subject with *CYP2C9*1/*3* genotype were comparable with the mean values of the other subjects. The prolongation of $t_{½}$ of *S*-(+)-ibuprofen by voriconazole and fluconazole was smallest with *CYP2C9*1/*3* and *CYP2C8*1/*1* genotypes, whereas the greatest prolongation of meloxicam $t_{½}$ by voriconazole and itraconazole was observed with *CYP2C9*1/*3* genotype. The subject with the *CYP2C9*1/*1* and *CYP2C8*1/*3* genotype had the greatest $AUC_{(0-\infty)}$ of *S*-(+)-ibuprofen in the control phase and seemed to have the strongest inhibitory effect on *S*-(+)-ibuprofen $AUC_{(0-\infty)}$ by both voriconazole and fluconazole. *CYP2C9*1/*2* and *CYP2C9*2/*2* genotypes seemed to have no effect on the pharmacokinetics of NSAIDs.

Genotyping in study V for CYP2D6 showed that 8 subjects were EMs (6 *CYP2D6*1/*1*, and 2 *CYP2D6*1/*4*), one subject was PM (*CYP2D6 *3/*4*), and 3 were UMs (*CYP2D6*1/*1x2*). The $AUC_{(0-\infty)}$ of venlafaxine of the PM subject was 7-, 2.7-, and 9-fold in the control, terbinafine, and voriconazole phase, respectively, compared with the mean $AUC_{(0-\infty)}$ of venlafaxine of the other 11 subjects in the corresponding phases. In contrast, his $AUC_{(0-\infty)}$ of ODV was the lowest in the control and terbinafine phases, and no ODV was measurable during the voriconazole phase. The $AUC_{(0-\infty)}$ of venlafaxine active moiety of this subject was 1.8-fold greater compared with the other 11 subjects after voriconazole pretreatment. The mean $AUC_{(0-\infty)}$ of venlafaxine in the three UMs in the control phase was about half of the mean $AUC_{(0-\infty)}$ of venlafaxine of EMs (333 vs. 642 ng/ml h). After pretreatment with voriconazole or terbinafine, the pharmacokinetic parameters in the UMs were comparable with those of the EMs.

5.5 Inhibition of TxB_2 synthesis (III, IV)

No statistically significant inhibition of TxB_2 synthesis was observed at any time point after ingestion of etoricoxib alone or after etoricoxib with voriconazole or miconazole. Meloxicam alone and meloxicam after voriconazole pretreatment decreased the $AUC_{(0-48)}$ of TxB_2 by 38% ($P < 0.001$) and 37% ($P < 0.001$), respectively, compared with baseline. In the itraconazole phase, the $AUC_{(0-48)}$ of TxB_2 was decreased by 7% ($P > 0.05$). The inhibition of TxB_2 synthesis at different time points is shown in Table 6. The inhibition of TxB_2 synthesis was significantly greater, from 5 to 12 h after meloxicam ingestion in the control and in the voriconazole phase compared with the itraconazole phase, whereas no difference was observed in the inhibition of TxB_2 synthesis between the control and the voriconazole phase.

5.6 Concentrations of antifungals

The pharmacokinetic variables of antifungals are shown in Table 7. There was a large interindividual variation in the concentrations of voriconazole. The mean trough plasma voriconazole concentration (C_{trough}) of all studies was 1.2 µg/ml. The mean

Table 6. Effect of a single oral dose of 15 mg meloxicam on thromboxane B_2 (TxB_2) generation, when given alone or after pretreatment with voriconazole or after pretreatment with itraconazole

TxB_2 (ng/ml)	Meloxicam	Meloxicam with voriconazole	Meloxicam with itraconazole
Baseline	145 ± 61	144 ± 47	124 ± 48
5 hours postdose	92.6 ± 35.3	80.2 ± 41.8	146 ± 64.0
% Change from baseline	- 30%*	-37%**	10%
8 hours postdose	82.7 ± 36.9	70.2 ± 34.2	128 ± 49.2
% Change from baseline	-37%*	-46%***	-0.3%
12 hours postdose	58.1 ± 26.8	66.8 ± 37.1	110 ± 39.5
% Change from baseline	-56%***	-49%***	-11%
24 hours postdose	88.1 ± 30.3	84.8 ± 37.0	118 ± 37.2
% Change from baseline	-28%*	-31%*	-5%
48 hours postdose	103 ± 23.0	104 ± 47.9	89 ± 29.1
% Change from baseline	-25%*	-14%	-29%*

The results are mean ± standard deviation. Percent change from baseline was calculated individually for each subject. Individual baseline value was the average of predose TxB_2 concentrations, which were measured before meloxicam ingestion in every phase of the study.
*Significantly ($P < 0.05$) different from baseline
**Significantly ($P < 0.01$) different from baseline
***Significantly ($P < 0.001$) different from baseline

C_{max}, $AUC_{(0-\infty)}$, and $t_{½}$ of voriconazole were 2.3 μg/ml, 30850 μg h/ml, and 10 h measured in studies III and IV. The mean trough concentration of fluconazole was 4.2 μg/ml (Study I). Miconazole plasma concentrations were quantifiable (4 ng/mL or more) up to 24 hours. The mean C_{trough}, C_{max}, $AUC_{(0-\infty)}$, and $t_{½}$ of miconazole were 0.019 μg/ml, 0.083 μg/ml, 0.78 μg h/ml and 22.3 h, respectively (Study III). The respective values for itraconazole were 0.11 μg/ml 0.39 μg/ml, 11 μg h/ml, and 31 h (Study IV). The mean C_{trough} of terbinafine was 0.34 μg/ml (Study V).

There was a significant correlation between voriconazole (Spearman $r = 0.82$; $P < 0.01$) and fluconazole (Pearson $r = 0.76$, $P < 0.01$) trough plasma concentration and the increase in *S*-(+)-ibuprofen $AUC_{(0-\infty)}$. In the other studies, the concentration of the antifungal did not correlate with the extent of interaction between antifungal and NSAID studied or venlafaxine.

5.7 Adverse effects

The use of NSAIDs did not cause any adverse effects in our studies. All 12 subjects experienced mild to moderate nausea after venlafaxine administration. Four of the subjects (two EMs, one UM, and one PM) vomited after the administration of

voriconazole plus venlafaxine. In addition, two out of these four subjects (one EM and one PM) also vomited after terbinafine plus venlafaxine administration. Vomiting was not associated with higher plasma concentrations of venlafaxine, ODV, or venlafaxine active moiety around the time of emesis compared with the subjects who did not vomit.

Voriconazole pretreatment caused visual disturbances, including photophobia and altered colour vision changes, in 34% of subjects (20 cases of total 58 voriconazole exposures). Visual disturbances were typically experienced shortly after voriconazole intake and were resolved within 1 h without any medical intervention. No other clinically relevant adverse effects of antifungals were recorded during the studies

Table 7. The pharmacokinetic variables of antifungals used in studies I-V

Antifungal	C_{trough} (μg/ml)	AUC (μg h/ml)	C_{max} (μg/ml)	$t_{½}$ (h)
Voriconazole	1.2 (0.2-3.9)	31 (5.9-120)	2.3 (0.3-4.4)	10 (6.0-26)
Fluconazole	4.2 (3.0-5.9)	NA	NA	NA
Miconazole	0.019 (0.0059-0.063)	0.78 (0.41-2.1)	0.083 (0.045-0.24)	22 (11-44)
Itraconazole	0.11 (0.048-0.17)	11 (6.8-16)	0.39 (0.25-0.62)	31 (15-49)
Terbinafine	0.34 (0.15-0.73)	NA	NA	NA

The results are mean (with range). C_{trough} = trough concentration measured just before the last dose of antifungals, AUC = area under plasma concentration-time curve extrapolated to infinity, C_{max} = maximum plasma concentration, $t_{½}$ = elimination half-life, NA = not available (only C_{trough} was measured).

6 DISCUSSION

6.1 Methodological aspects

All studies were carried out in an open, randomized, balanced, crossover study design. In crossover studies, where each subject serves as his or her own control, the changes in the pharmacokinetic or pharmacodynamic variables are calculated within subject, which minimizes the effect of interindividual variability. Thus, the number of healthy volunteers needed in each study could be kept as low as possible. Since the main aim of the studies was to investigate pharmacokinetics, and because also pharmacodynamic measurements in studies III and IV were determined from blood, a double blind, placebo controlled design was not considered necessary. However, the use of a double blind design would have allowed a more reliable assessment of pharmacodynamic effects of venlafaxine in study V. As the studies were conducted by using single doses of NSAIDs and venlafaxine in healthy volunteers, the results can not be directly extrapolated to elderly people or to long-term concomitant use of the drugs investigated.

Wash-out periods from 2 to 4 weeks between study phases were used to eliminate the possible carry-over effects. The length of wash-out periods proved to be sufficient in studies I-IV, where no carry-over antifungals or NSAIDs were measurable after the wash-out period, at the beginning of the next phase. In study V, trace amounts of terbinafine were detected in plasma samples in 8 subjects before the intake of venlafaxine, at the beginning of the following control phase. However, the pharmacokinetics of venlafaxine in the control phase seemed not to differ in those subjects with trace amounts of terbinafine detected in plasma compared with others. In addition, in the previous study it was shown that CYP2D6 activity returns to baseline in most subjects within 4 weeks after discontinuation of terbinafine medication (Abdel-Rahman et al. 1999).

The aim of pretreatment with voriconazole, fluconazole, and miconazole oral gel was to attain a steady state concentration before the intake of study drug. Voriconazole dosing was based on a previous study indicating that the steady state concentration of voriconazole can be achieved in 2 days, using a loading dose of 400 mg twice daily on the first day followed by 200 mg twice daily on the second day (Purkins et al. 2003b). The dose of fluconazole was 400 mg once daily on the first day followed by 200 mg once daily on the second day, which is the highest recommended dose of fluconazole in clinical use and leads to steady state fluconazole levels on the second day (Debruyne & Ryckelynck 1993, Tett et al. 1995). The pharmacokinetics of miconazole oral gel is poorly described. Based on $t_{½}$ of miconazole (20 h) (Daneshmend & Warnock 1983), it was estimated that a dosing schedule of 3.5 ml (approximately 85 mg) every 8 hours for 3 days increases the plasma miconazole concentration into the steady state level. The dosing schedule was selected to be close to the therapeutic regimen (2.5 ml every 6 h) and to allow the subjects not to take miconazole at night time.

Four-day pretreatment with itraconazole and terbinafine was too short to achieve steady state concentrations of itraconazole and terbibafine, which are reached only 10-14 days after the beginning of treatment (Hardin et al. 1988, Jensen 1989). Four-day pretreatment was selected, because it is not desirable to expose healthy volunteers to a 2 week pretreatment of itraconazole and terbinafine and because also 4-day pretreatment with similar doses of itraconazole as used in our study has been shown to strongly inhibit CYP3A4 mediated metabolism of midazolam (Backman et al. 1998). In addition, 4-day pretreatment with 250 mg daily dose of terbinafine has been shown to produce therapeutic plasma concentrations of terbinafine (Ahonen et al. 1995).

A weakness in our study design was that administration of voriconazole was not continued after etoricoxib and meloxicam ingestion in studies III and IV. The half-life of voriconazole was short, 10 h in studies III and IV, compared with that of etoricoxib and meloxicam, 19 h and 17 h in studies III and IV, respectively. In addition, in clinical use, voriconazole is used twice daily. Accordingly, in clinical use voriconazole concentrations are higher than those observed in our studies from 12 hours on. Therefore the extent of the interaction during the elimination phase of etoricoxib and meloxicam may have been somewhat underestimated, as these NSAIDs are eliminated more slowly than voriconazole.

Blood sampling times were sufficient for reliable calculation of all pharmacokinetic parameters in studies I-III and V. In study IV, the sampling period of 72 h turned out to be inadequate for the determination of k_{el} of meloxicam in 4 subjects in the itraconazole phase. These subjects had t_{max} of meloxicam as late as 48 hours after its ingestion, and there were no measurements between 48 h and 72 h time points. Accordingly, because AUC values of meloxicam could not be extrapolated reliably to infinity in all 12 subjects in the itraconazole phase, $AUC_{0\text{-}72}$ was used for comparison of AUC values between the phases in study IV.

In these studies, subjects were not selected into the studies according to their genotypes, and genotyping was done rather to control the expected variability in the pharmacokinetic variables between subjects. Therefore, bearing in mind the low number of individuals carrying variant genotypes in our studies, our data cannot be used to evaluate the genotype effect precisely.

Synthesis of TxB_2, a stable metabolite of TxA_2, by platelets in spontaneously clotting whole blood was used for assessment of COX-1 activity of etoricoxib and meloxicam in studies III and IV. Platelet aggregation depends on their ability to generate TxA_2 from prostaglandin H_2, which is synthesized from arachidonic acid by COX enzymes. Since platelets lack COX-2 enzyme, the whole synthesis of TxA_2 and also the synthesis of TxB_2 is indirectly mediated by COX-1. Traditional NSAIDs have been shown to dose-dependently inhibit the synthesis of TxB_2 during blood clotting, and this method is widely used for assessing the ability of NSAIDs to inhibit COX-1 at therapeutic plasma concentrations (Patrignani et al. 1997, Panara et al. 1999, Blain et al. 2002).

6.2 Effects of CYP inhibitors on NSAIDs metabolized by CYP2C9

No studies have previously investigated the effect of CYP inhibitors on the pharmacokinetics of ibuprofen, diclofenac, and meloxicam. In the present studies, voriconazole increased the exposure to *S*-(+)-ibuprofen, diclofenac and meloxicam 2.1-, 1.8-, and 1.5- fold, respectively, and fluconazole increased the exposure to *S*-(+)-ibuprofen 1.8-fold, as judged by AUC. As the $t_{½}$ of *S*-(+)-ibuprofen and meloxicam were prolonged and C_{max} remained roughly unchanged, it seems that the interactions between voriconazole or fluconazole and *S*-(+)-ibuprofen and between voriconazole and meloxicam are due to the inhibition of metabolism of *S*-(+)-ibuprofen and meloxicam during the elimination phase. By contrast, the C_{max} of diclofenac was substantially increased, whereas its $t_{½}$ was unaffected by voriconazole, which indicates that this interaction occurred to a great extent during the first pass metabolism. This is plausible, because the oral bioavailability of diclofenac is 50-60% (John 1979) and that of ibuprofen and meloxicam is 100% and 89%, respectively (Davies 1997, Turck et al.1997).

The effects of fluconazole and voriconazole on the AUC of *S*-(+)-ibuprofen were similar in magnitude, which supports the findings of *in vitro* studies that voriconazole is at least as strong an inhibitor of CYP2C9 as fluconazole (Niwa et al. 2005a). However, although the interactions described here are most likely due to the inhibition of CYP2C9 mediated metabolism of *S*-(+)-ibuprofen, diclofenac, and meloxicam, the inhibition of minor metabolic pathways may also be involved in the interactions, since these NSAIDs are also metabolized to some extent by other CYPs and since both voriconazole and fluconazole also have an inhibitory effect on CYP2C19 and CYP3A4 (Niwa et al. 2005a).

Itraconazole was used in study IV to investigate the effect of CYP3A4 inhibition on the pharmacokinetics of meloxicam. It was supposed that the inhibition of CYP3A4 mediated metabolism of meloxicam would increase the concentrations of meloxicam. Unexpectedly, we found that itraconazole caused a notable decrease in the plasma concentrations of meloxicam. The plasma concentrations of meloxicam were clearly lower during the first 24 h following the ingestion of meloxicam in the itraconazole phase compared with the control phase. The $AUC_{0\text{-}72}$ and C_{max}, were considerably decreased and the t_{max} was greatly prolonged by itraconazole. The plasma protein binding of meloxicam was very high (99.8%), but there was no difference in its percentage binding between different phases of the study. Thus, a displacement of meloxicam from plasma protein by itraconazole does not explain the observed interaction. The findings suggest that itraconazole decreased the exposure to meloxicam by impairing its gastrointestinal absorption. The mechanism behind impaired absorption remains unclear, but itraconazole could have inhibited some transport system in the gut wall, which is needed for the absorption of meloxicam. However, this postulate is not further supported in the literature, since the involvement of a certain transport system in the absorption of meloxicam is not known, and similar effects of itraconazole on the absorption of other drugs have not been reported earlier.

In vitro studies have indicated that *R*-(-)-ibuprofen is mainly metabolized by CYP2C8 and to a minor extent by CYP2C9 (Leemann et al. 1993, Hamman et al. 1997). Our results are in accordance with this, because CYP2C9 inhibitors, voriconazole and fluconazole, had very little effect on the pharmacokinetics of *R*-(-)-ibuprofen. In addition, it can be concluded that neither voriconazole nor fluconazole notably inhibit CYP2C8 enzyme as suggested previously with regard to fluconazole (Walsky et al. 2005). However, the precise effect of CYP2C8 or CYP2C9 inhibition on the pharmacokinetics of *R*-(-)-ibuprofen is difficult to assess, since about 60% of *R*-(-)-ibuprofen is converted to *S*-(+)-ibuprofen (Lee et al. 1985), and therefore, the inhibition of the metabolism of *R*-(-)-ibuprofen most likely increases the concentration of both ibuprofen enantiomers.

6.3 Effects of CYP inhibitors on etoricoxib

Unlike the other NSAIDs studied, etoricoxib is mainly metabolized by CYP3A subfamily (60%), the rest being catalyzed equally between CYP2C9, CYP2C19, CYP2D6, and CYP1A2 (Kassahun et al. 2001). Miconazole oral gel and voriconazole increased the exposure to etoricoxib 1.8- and 1.5-fold, respectively, as judged by $AUC_{(0-\infty)}$. Miconazole oral gel also prolonged the $t_{½}$ of etoricoxib, but had no effect on the C_{max} of etoricoxib, whereas voriconazole slightly increased both C_{max} and $t_{½}$. In addition, as the oral biovailability of etoricoxib is almost 100% (Agrawal et al. 2003), it is likely that the inhibitory effect of miconazole and voriconazole mainly focused on the metabolism of etoricoxib during the elimination phase.

The effect of voriconazole on the pharmacokinetics of etoricoxib was substantially weaker than what was observed in a previous study between voriconazole and the CYP3A substrate, midazolam, in which voriconazole caused a 9-fold increase in the AUC of oral midazolam (Saari et al. 2006). This was not surprising, since with drugs like etoricoxib, which have many metabolic pathways, the inhibition of the main CYP mediated pathway can usually be compensated by alternative pathways. Therefore, the increase in the concentration of etoricoxib remains small compared with increase in the concentration of drugs like midazolam, which are almost solely metabolized by CYP3A. Furthermore, contrary to midazolam, etoricoxib has a high oral bioavailability, which reduces its susceptibility to the inhibitory effect of voriconazole as well.

In vitro studies have indicated that miconazole is a nonselective inhibitor of several CYPs (Zhang et al. 2002, Niwa et al. 2005a, Niwa et al. 2005b). However, as the ability of miconazole to be absorbed from oral gel preparation is low, miconazole oral gel was commonly considered a safe treatment regarding possible drug interactions, until several case reports described hazardous interaction between miconazole oral gel and warfarin (Ariyaratnam et al. 1997, Silingard et al. 2000, Pemberton et al. 2004). In our study, a 3-day use of miconazole oral gel with doses used in clinical practice resulted in quantifiable plasma concentrations of miconazole for up to 24 hours after the last dose and caused a similar increase in the concentrations of etoricoxib as systemic oral voriconazole. These findings strongly support the conclusion derived

from case reports that miconazole, also administered as an oral gel, has a potential to be absorbed in sufficiently large amounts to inhibit CYP-mediated metabolism of drugs.

6.4 Effects of antifungals on venlafaxine

Terbinafine strongly inhibited the CYP2D6 catalyzed O-demethylation of venlafaxine, detected as 82% decrease in the $AUC_{(0-\infty)}$ ratio of ODV over venlafaxine. As both C_{max} and $t_{½}$ of venlafaxine were increased, it is likely that the inhibition occurred during both first pass metabolism and elimination. In the previous study, 3-week terbinafine pretreatment caused a similar (5-fold) increase in the AUC of the sensitive CYP2D6 substrate desipramine (Madani et al. 2002). In addition, the 80% decrease in apparent oral clearance of venlafaxine in the present study was similar to that produced by quinidine, a strong CYP2D6 inhibitor, which has been shown to decrease venlafaxine oral clearance from 100 l/h to 17 l/h (Lessard et al. 1999). Accordingly, it can be concluded that a 4-day terbinafine treatment, as used in our study, is a sufficient time period to investigate CYP2D6 inhibition also in future.

Voriconazole had no significant effect on the $AUC_{(0-\infty)}$ ratio of ODV over venlafaxine, which supports the previous *in vitro* finding that voriconazole does not inhibit CYP2D6 (Niwa et al. 2005b). In addition, voriconazole caused only slightly increased plasma levels of venlafaxine and ODV, suggesting that inhibition of CYP3A4- (and to a lesser extent CYP2C9-, and CYP2C19-) mediated N-demethylation of venlafaxine causes only small changes in the pharmacokinetics of venlafaxine, due to a minor role of CYP3A4 in the overall metabolism of venlafaxine. This finding is comparable to the effect of another CYP3A4 inhibitor, ketoconazole, which has been reported to increase the AUC of venlafaxine by 36% and AUC of ODV by 26% in healthy volunteers (Lindh et al. 2003).

6.5 Effects of genotypes

Previous studies have indicated that the apparent oral clearance of *S*-(+)-ibuprofen is decreased in individuals who are hetero- or homozygous for *CYP2C9*3* or for *CYP2C8*3* (Kirchheiner et al. 2002, Martinez et al. 2005), whereas different variant *CYP2C9* genotypes have no effect on the pharmacokinetics of diclofenac (Yasar et al. 2001, Kirchheiner et al. 2003, Brenner et al. 2003). Our findings were somewhat similar, because the two subjects with the *CYP2C9*1/*3* and *CYP2C8*1/*1* genotype had the longest $t_{½}$ of *S*-(+)-ibuprofen in the control phase, and one subject with *CYP2C8*1/*3* and *CYP2C9*1/*1* genotype had the highest AUC of *S*-(+)-ibuprofen in the control phase. In addition, voriconazole and fluconazole produced almost no effect on *S*-(+)-ibuprofen $t_{½}$ with the *CYP2C9*1/*3* and *CYP2C8*1/*1* genotype subjects, suggesting that these individuals were less prone to the inhibitory effect of CYP2C9 by voriconazole or fluconazole, probably due to low baseline CYP2C9 activity. Furthermore, the *CYP2C9*1/*2* or *CYP2C9*1/*3* genotypes did not seem to affect the pharmacokinetics of diclofenac. In the case of meloxicam, the subject with the *CYP2C9*1/*3* genotype had the longest $t_{½}$ of meloxicam in the control phase, which

might mean that this genotype impairs the metabolism of meloxicam, but this has not been studied earlier.

In study V, subjects were genotyped for defective alleles **3* and **4* for CYP2D6, which covers over 75% of PMs (Dahl et al. 1992). Based on the venlafaxine metabolic ratio of subjects, the existence of additional PMs with undetected defective alleles in our study was unlikely. The prevalence of 25% UMs was much more than the expected 1% prevalence in Northern Europe (Dahl et al. 1995). As expected, the three UMs had lower venlafaxine concentrations, whereas the PM subject had remarkably higher venlafaxine concentration during the control phase compared with the EMs (Veefkind et al. 2000, Shams et al. 2006). In addition, the greatest increase in the AUC of venlafaxine active moiety by voriconazole was observed in the PM indicating a bigger role of the minor CYP3A4, CYP2C9, or CYP2C19 mediated N-demethylation pathway in PM possessing impaired CYP2D6 activity.

6.6 COX-1 inhibition

Traditional NSAIDs dose-dependently inhibit the activity of both COX-1 and COX-2, whereas coxibs are highly COX-2 selective with clinically used doses (Cryer & Feldman 1998, Leese et al. 2000, Dallob et al. 2003)**.** Our results are in accordance with this, because we found that etoricoxib did not cause any significant COX-1 inhibition among the study phases, as indicated by non-significant changes in the platelet TxB_2 generation. By contrast, meloxicam dose-dependently inhibited the COX-1 mediated synthesis of TxB_2 as shown also in previous studies (Panara et al. 1999, de Meijer et al. 1999). The maximum decline of the synthesis of TxB_2 was 56% and 49% in the control and in the voriconazole phase, respectively, which is comparable with other studies, in which TxB_2 synthesis was inhibited from 35% to 66% by 15 mg meloxicam (Panara et al. 1999, de Meijer et al. 1999). After itraconazole, low meloxicam concentrations were associated with clearly reduced COX-1 inhibition. The maximum inhibition of TxB_2 synthesis in the itraconazole phase occurred 48 h after meloxicam ingestion and was 29%, which corresponds to the degree of inhibition reported previously 5 h after ingestion of 7.5 mg of meloxicam, without itraconazole (Blain et al. 2002).

6.7 Plasma voriconazole and adverse effects

The variability in plasma voriconazole trough concentrations was nearly 20-fold between subjects. This might be caused by nonlinear pharmacokinetics of voriconazole, which is likely due to saturation of its metabolism (Purkins et al. 2002, Purkins et al. 2003a). In addition, CYP2C19 exhibits genetic polymorphism resulting in an approximately 4-fold higher voriconazole exposure in poor metabolizers than in extensive metabolizers (Mikus et al. 2006, Ikeda et al. 2004, Rengelshausen et al. 2005). One possible reason is also noncompliance, because only the last dose of voriconazole was administered by the study personnel and the first three doses were self-administered by subjects at home. In our studies the incidence of visual disturbances (34%) during voriconazole treatment was similar to that previously reported (Jeu et al. 2003).

6.8 Clinical aspects

NSAIDs are generally well tolerated drugs with a wide therapeutic index. In the present series of studies, azole antifungals, with the exception of itraconazole, typically caused a 1.5- to 2-fold increase in the exposure to NSAIDs, but did not cause any obvious NSAID related adverse effects in healthy young adults after a single dose. In addition, in the case of etoricoxib or meloxicam, the higher concentrations were not associated with greater COX-1 inhibition. Accordingly, dosing adjustments of the NSAIDs studied are most likely not necessary when single doses of these NSAIDs are coadministered with azole antifungals or other CYP inhibitors. On the other hand, as the analgesic effects of NSAIDs are concentration dependent (Laska et al. 1986, Collins et al. 1998, Malmstrom et al. 2004), it appears logical that lower doses of ibuprofen, diclofenac, etoricoxib, or meloxicam are adequate for patients receiving CYP inhibitors to gain pain relief. In addition, the risk for gastrointestinal, cardiovascular, and renal adverse effects of NSAIDs seems to increase when they are used in high doses for prolonged periods of time (Whelton & Hamilton, 1991, Brater 2001, Schwartz et al. 2002, Henry et al. 1996, Solomon et al. 2006, Cannon et al. 2006, Helin-Salmivaara et al. 2006), and therefore, long-term concomitant use of CYP inhibitors with NSAIDs might predispose patients for these adverse effects.

In contrast to other azole antifungals, itraconazole substantially decreased meloxicam concentrations, and this was associated with clearly reduced pharmacodynamic effect. Thus, the clinical efficacy of meloxicam is most likely reduced, at least in the short term, when given during itraconazole treatment. In long term use, the situation might be different, since as discussed earlier, the AUC of etoricoxib was not extrapolated to infinity, but was calculated using values up to 72 h postdose. Therefore, the actual extent of absorption of meloxicam remained unclear, and it is not known whether the absorption of meloxicam is truly decreased or only delayed. If the absorption of meloxicam is only delayed, the steady state concentrations of meloxicam will most likely be close to normal levels, despite the concomitant use of itraconazole. The mechanism of interaction between itraconazole and meloxicam could not be resolved in our study. Thus, it is even difficult to speculate whether itraconazole has similar effects on the absorption of other drugs as well, but it is certainly a matter for further studies.

Study III was the first controlled clinical trial showing the CYP3A4 inhibition potential of miconazole oral gel. As the interaction between miconazole oral gel and etoricoxib was observed in the elimination phase, it is likely that mostly hepatic and not intestinal CYP3A4 was involved in the interaction. Therefore, drugs that have extensive first pass metabolism in the intestinal wall might be more prone to interact with miconazole oral gel, assuming that most of the miconazole dose is not absorbed but retained in the intestine. In addition, as etoricoxib is not very sensitive CYP3A4 substrate, it is likely that the inhibitory effect of miconazole oral gel is much greater on drugs that are more sensitive CYP3A4 substrates such as midazolam, triazolam, simvastatin, and felodipine.

Parent venlafaxine and ODV are considered to be equal in their antidepressive efficacy. However, increased exposure to parent venlafaxine, due to low CYP2D6 activity, has been shown to increase the risk for cardiovascular toxicity of venlafaxine as well as for other adverse effects of venlafaxine, such as nausea, vomiting, diarrhoea, and hyponatremia (Lessard et al. 1999, Shams et al. 2006). It has been speculated that slight differences in the reuptake inhibition profiles between venlafaxine and ODV might be responsible for these findings. Accordingly, although not seen in our study, the inhibition of CYP2D6 catalyzed O-demetylation of venlafaxine by terbinafine, leading to a considerably high venlafaxine concentration, might predispose patients to venlafaxine related adverse effects. On the other hand, although the inhibition of CYP3A4, CYP2C9, and CYP2C19 mediated N-demetylation of venlafaxine by voriconazole only slightly increased concentrations of venlafaxine active moiety, it was accompanied with a stronger subjective feeling of drug effect. Therefore, careful clinical monitoring of patients regarding the adverse effects of venlafaxine is needed when using either CYP2D6 or CYP3A4 inhibitors with venlafaxine. This is especially important with poor metabolizers for CYP2D6, who might be more prone to experience adverse effects of venlafaxine if coadministered with CYP3A4 inhibitors, since then both of the metabolic pathways of venlafaxine are blocked and the concentration of parent venlafaxine can be very high.

7 CONCLUSIONS

1. Azole antifungals, with the exception of itraconazole, increase the exposure to *S*-(+)-ibuprofen, diclofenac, etoricoxib, and meloxicam 1.5- to 2.1-fold.

2. Unexpectedly, itraconazole significantly decreases the concentrations and pharmacodynamic effect of meloxicam, most likely by impairing the absorption of meloxicam.

3. Miconazole, from oral gel preparation, is absorbed in sufficiently large amounts to inhibit CYP3A4 mediated metabolism of drugs.

4. Terbinafine increases the exposure to parent venlafaxine 5-fold by inhibiting the CYP2D6 mediated O-demetylation of venlafaxine, but causes only a minor increase in the exposure to venlafaxine active moiety. The inhibition of CYP2C9, CYP2C19, and CYP3A4 catalyzed metabolism of venlafaxine by voriconazole, results in 30% increase in the exposure to venlafaxine active moiety.

5. Voriconazole has no substantial inhibitory effect on CYP2D6 or CYP2C8 activity.

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