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THE ROLE OF CYTOCHROME P450 3A INDUCERS AND INHIBITORS IN THE METABOLISM AND THE EFFECTS OF OXYCODONE

by

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To my family

Nieminen, Tuija:

The Role of Cytochrome P450 3A Inducers and Inhibitors in the Metabolism and the Effects of Oxycodone

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ABSTRACT

Oxycodone is an opioid used in the treatment of moderate or severe pain. It is principally metabolized in the liver by cytochrome P450 3A (CYP3A) enzymes whereas approximately 10% is metabolized by CYP2D6. Little is known about the interactions between oxycodone and other drugs, herbals and nutritional substances. In this work the effects of CYP3A inducers rifampicin and St. John's wort and CYP3A inhibitors voriconazole, grapefruit juice, ritonavir and lopinavir/ritonavir were investigated on the pharmacokinetics and pharmacodynamics of oxycodone.

All studies were randomized, balanced, placebo-controlled crossover clinical studies in healthy volunteers. The plasma concentrations of oxycodone and its metabolites were determined for 48 hours and pharmacodynamic parameters were recorded for 12 hours in each study. Pharmacokinetic parameters were calculated by noncompartmental methods.

Rifampicin decreased the plasma concentrations, analgesic effects, and oral bioavailability of oral oxycodone. St. John's wort reduced the concentrations of oxycodone and diminished the self-reported drug effect. Voriconazole increased the exposure to oral oxycodone by 3.6-fold whereas grapefruit juice, which inhibits predominantly the intestinal CYP3A, elevated the mean concentrations of oxycodone by 1.7-fold. Ritonavir and lopinavir/ritonavir increased the mean AUC of oxycodone by 3.0- and 2.6-fold, respectively, and prolonged its elimination half-life. In spite of increased oxycodone plasma concentrations during concomitant administration of CYP3A inhibitors, the analgesic effects were not increased.

These studies show that the induction or inhibition of CYP3A alters the pharmacokinetics and pharmacologic effects of oxycodone. The exposure to oxycodone decreased after induction and increased after inhibition of CYP3A. As a conclusion, the clinicians should avoid concomitant administration of CYP3A inducers or inhibitors and oral oxycodone. If this is not possible, they should be prepared to interactions leading to impaired analgesia after CYP3A inducers or increased adverse effects after CYP3A inhibitors and oral oxycodone.

Keywords: oxycodone, interaction, CYP3A, pharmacokinetics, pharmacodynamics

Nieminen, Tuija:

Sytokromi P450 3A-entsyymien toimintaa kiihdyttävien ja estävien aineiden vaikutus oksikodonin aineenvaihduntaan ja lääkevasteeseen

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TIIVISTELMÄ

Oksikodoni on opioidi, jota käytetään keskivaikean tai vaikean kivun hoidossa. Se metaboloituu pääosin maksassa sytokromi P450 3A (CYP3A)-entsyymien avulla, kun taas noin 10 % metaboloituu CYP2D6-entsyymin välityksellä. Oksikodonin yhteisvaikutuksista muiden lääkkeiden, rohdosvalmisteiden tai ravintoaineiden kanssa tiedetään vain vähän. Tässä työssä tutkittiin CYP3A-entsyymin toimintaa kiihdyttävien rifampisiinin ja mäkikuisman sekä CYP3A-entsyymin toimintaa estävien vorikonatsolin, greippimehun, ritonaviirin ja lopinaviiri/ritonaviirin vaikutusta oksikodonin farmakokinetiikkaan ja farmakodynamiikkaan.

Kaikki tutkimukset olivat satunnaistettuja, balansoituja, plasebo-kontrolloituja, vaihtovuoroisia kliinisiä tutkimuksia terveillä vapaaehtoisilla koehenkilöillä. Oksikodonin ja sen aineenvaihduntatuotteiden plasmapitoisuuksia määritettiin 48 tunnin ajan ja lääkevasteita rekisteröitiin 12 tunnin ajan kussakin tutkimuksessa. Farmakokineettiset määritykset tehtiin tilamallista riippumattomalla menetelmällä.

Rifampisiini pienensi suun kautta annostellun oksikodonin plasmapitoisuuksia ja analgeettisia vaikutuksia sekä heikensi oraalista hyötyosuutta. Mäkikuisma laski oraalisen oksikodonin pitoisuuksia ja pienensi itsearvioitua lääkevaikutusta. Vorikonatsoli lisäsi suun kautta annostellun oksikodonin altistusta 3.6-kertaiseksi, kun taas pääasiallisesti suolen seinämän CYP3A-entsyymien toimintaa estävä greippimehu lisäsi keskimääräisen oksikodonin pitoisuuksia 1.7-kertaiseksi. Ritonaviirin ja lopinaviiri/ritonaviirin vaikutuksesta oksikodonin keskimääräinen AUC kasvoi 3.0- ja 2.6-kertaiseksi ja eliminaation puoliintumisaika pitkittyi. Huolimatta samanaikaisesti annosteltujen CYP3A-entsyymien estäjien aiheuttamasta oksikodonin plasmapitoisuuksien kasvusta, sen analgeettiset vaikutukset eivät lisääntyneet.

Nämä tutkimukset osoittavat, että CYP3A-entsyymien toiminnan kiihtyminen tai estyminen muuttaa oksikodonin farmakokinetiikkaa ja lääkevaikutuksia. CYP3A-entsyymien kiihtyminen vähensi oksikodonialtistusta, kun taas estyminen lisäsi sitä. Yhteenvetona voidaan todeta, että CYP3A-entsyymien toimintaa kiihdyttävien ja hidastavien aineiden samanaikaista käyttöä suun kautta annosteltavan oksikodonin kanssa tulisi välttää. Mikäli tämä ei ole mahdollista, tulisi varautua analgeettisen tehon heikkenemiseen CYP3A-entsyymejä kiihdyttävien aineiden ja suun kautta annostellun oksikodonin yhteisvaikutuksen seurauksena, kun taas CYP3A-entsyymiä hidastavien lääkkeiden samanaikainen käyttö oksikodonin kanssa saattaa lisätä sivuvaikutuksia.

Avainsanat: oksikodoni, lääkeyhteisvaikutus, CYP3A, farmakokinetiikka, farmakodynamiikka

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ABBREVIATIONS

ADR	adverse drug reaction
AUC_{0-t}	area under plasma concentration–time curve from zero to t hours
$AUEC_{0-12}$	area under effect–time curve from zero to 12 hours
AUC_m/AUC_p	metabolite-to-parent drug area under plasma concentration–time curve ratio
CI	confidence interval
CL	plasma clearance
CL/F	apparent oral clearance
C_{max}	peak plasma concentration
CPT	cold pain threshold
CYP	cytochrome P450
CV	coefficient of variation
DDI	drug-drug interaction
DSST	digit symbol substitution test
EDTA	ethylenediaminetetraacetic acid
EC_{50}	median effective concentration
EM	extensive metabolizer
F	oral bioavailability of drug
FDA	Food and Drug Administration
HPLC	high performance liquid chromatography
IM	intermediate metabolizer
K_i	inhibition constant
k_e	elimination rate constant
LC–MS	liquid chromatography–mass spectrometric method
LC–MS/MS	liquid chromatography–tandem mass spectrometric method
LLQ	lower limit of quantification
ln	natural logarithm
MWT	Maddox Wing Test
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	reduced form of NADP
P-gp	P-glycoprotein
PM	poor metabolizer
SD	standard deviation
SEM	standard error of mean
$t_{½}$	elimination half-life
t_{max}	time to peak concentration
UM	ultrarapid metabolizer
VAS	visual analogue scale

LIST OF ORIGINAL PUBLICATIONS

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

I Nieminen TH, Hagelberg NM, Saari TI, Pertovaara A, Neuvonen M, Laine K, Neuvonen PJ, Olkkola KT. Rifampin greatly reduces the plasma concentrations of intravenous and oral oxycodone. Anesthesiology 2009;110:1371–1378

II Hagelberg NM, Nieminen TH, Saari TI, Neuvonen M, Neuvonen PJ, Laine K, Olkkola KT. Voriconazole drastically increases exposure to oral oxycodone. Eur J Clin Pharmacol 2009; 65:263–271

III Nieminen TH, Hagelberg NM, Saari TI, Neuvonen M, Laine K, Neuvonen PJ, Olkkola KT. St. John's wort greatly reduces the concentrations of oral oxycodone. Eur J Pain 2010; 14:854–859

IV Nieminen TH, Hagelberg NM, Saari TI, Neuvonen M, Laine K, Neuvonen PJ, Olkkola KT. Grapefruit juice enhances the exposure to oral oxycodone. Basic Clin Pharmacol Toxicol 2010; 107:782–788

V Nieminen TH, Hagelberg NM, Saari TI, Neuvonen M, Laine K, Neuvonen PJ, Olkkola KT. Oxycodone concentrations are greatly increased by the concomitant use of ritonavir or lopinavir/ritonavir. Eur J Clin Pharmacol 2010; 66:977–985

1. INTRODUCTION

Drug-drug interactions (DDIs), drug-herb interactions or drug-food interactions can be a remarkable source of increased adverse drug reactions (ADRs), and can alter the efficacy of medication. It has been estimated that the prevalence of ADRs as a cause of admission to the hospital can be up to 6.5% (Pirmohamed et al. 2004). Of the patients admitted to the hospital due to ADRs, the estimated incidence of fatal ADRs has been reported to be 0.05–0.13% representing approximately 5% of all deaths (Lazarou et al. 1998, Juntti-Patinen and Neuvonen 2002). Besides ADRs, DDIs can lead to treatment failure without causing any clinically visible harm to the patient. According to a large study using data from a university hospital medication database, every fifth patient receiving prodrugs losartan, codeine or tramadol, had concomitant medication with the potential to inhibit the activation of these drugs (Tirkkonen and Laine 2004).

Pain is a common reason for medical interventions. Pain patients are often treated with a combination of several drugs (Vadivelu et al. 2010), and can have other chronic diseases requiring drug therapy. As the risk of DDIs increases exponentially together with the number of drugs used concomitantly, pain patients are prone to DDIs. Oxycodone is a commonly prescribed opioid, which has been in clinical use for almost one hundred years (Falk 1917). In Finland it has been the most frequently used strong opioid in the treatment of severe pain conditions (Pöyhiä 1994a, Hamunen et al. 2009). After the introduction of controlled release oral oxycodone, its market share in the US grew from 10% in 1996 to 53% in 2000 (Coluzzi and Mattia 2005). Oxycodone is effective in acute and cancer pain (Kalso et al. 1991, Silvasti et al. 1998, Heiskanen et al. 1997), but can be used as a second line treatment also in neuropathic and other chronic pain states (Dworkin et al. 2010). Despite of the long history of oxycodone in the treatment of pain, little was known about its interactions with other drugs, herbals or food before these studies.

Cytochrome P450 (CYP) enzymes are major enzymes involved in drug metabolism (Guengerich 2008). CYP3A mediates the metabolism of approximately half of the marketed drugs and thus plays a critical role in the drug metabolism (Guengerich 2008). The concomitant use of CYP3A substrates and CYP3A inducers or inhibitors can lead to a diminished drug effect or exaggerated effect with increased ADRs or toxicity (Villikka et al. 1997, Friedman et al. 1999). Rifampicin is known as a potent CYP3A inducer, which has previously diminished the concentrations of several CYP3A substrates (Backman et al. 1996, Villikka et al. 1997). St. John's wort has been previously associated with serious drug-herbal interactions through the reduction of immunosuppressive drug concentrations (Ruschitzka et al. 2000). Voriconazole is a potent inhibitor of CYP3A (Saari et al. 2006b), whereas grapefruit juice is a part of normal nutrition, but can elevate the concentrations of many orally administered drugs (Bailey et al. 1991, Lilja et al. 2004). Ritonavir and lopinavir/ritonavir are used in the treatment of human immunodeficiency

virus infection (HIV) and have been reported to effectively inhibit CYP3A (Olkkola et al. 1999, Yeh et al. 2006).

Oxycodone is mainly metabolized by CYP3A, although it has also minor metabolic routes such as CYP2D6-mediated O-demethylation and the reductive metabolism (Lalovic et al. 2004). Concomitant use of oxycodone and CYP3A inducers rifampicin and St. John's wort or CYP3A inhibitors voriconazole, grapefruit juice, ritonavir and lopinavir/ritonavir may alter the concentrations and effects of oxycodone. Although oxycodone is a commonly used drug, its interactions with other drugs have not been thoroughly investigated. Thus, it was considered important to study interactions between oxycodone and other selected products.

2. REVIEW OF THE LITERATURE

2.1 Oxycodone

2.1.1 Basic pharmacology

Oxycodone (6-deoxy-7,8-dihydro-14-hydroxy-3-O-methyl-6-oxomorphine) is a semisynthetic opioid, which was developed from the opioid alkaloid thebaine. The first clinical use of oxycodone was documented in Germany in 1917, and it was approved for the US market in 1939 (Coluzzi and Mattia 2005). The worldwide use of oxycodone increased after the approval of orally administered controlled-release formulas in 1995. The pharmacological characteristics of oxycodone and morphine are summarized in Table 1.

Table 1. Pharmacological characteristics of oxycodone and morphine.

Parameter	***Oxycodone***	***Morphine***	***Reference***
MW (g/mol)	315	285	(1)
pK_a	8.53	8.08	(2–3)
Oral bioavailability (%)	55–87	19–30	(4–7)
Lipid solubility P_{app}	0.7	0.5	(8)
Lipid solubility ClogD	1.26	–0.49	(9)
Protein binding (%)	38–45	31–35	(8, 10)
[3H]-Diprenorphine displacement (K_i) (nmol/L)			
μ-receptor	16	3.2	(11)
κ-receptor	> 1000	–	(11)
δ-receptor	> 1000	–	(11)

MW = Molecular weight; pK_a = Dissociation constant; P_{app} = partition coefficient of opioids in n-octanol and tris-buffer at pH 7.4 and 37 ˚C; ClogD = Log Octanol/Water partition coefficient of opioid at 25 ˚C and pH 7.4; K_i = inhibition constant

1) (accessed http://pubchem. ncbi.nlm.nih.gov /summary)
2) Tien 1991
3) Roy and Flynn 1989
4) Pöyhiä et al. 1992
5) Leow et al. 1995
6) Saari et al. 2010
7) Osborne et al 1990
8) Pöyhiä and Seppälä 1994b
9) Lemberg et al. 2008
10) Leow et al. 1993
11) Lalovic et al. 2006

Oxycodone is a μ-receptor agonist, but morphine binds to the μ-receptor with 4-fold stronger affinity than oxycodone (Chen et al. 1991, Lalovic et al. 2006, Narita et al. 2008). Opioid receptors belong to the large superfamily of seven transmembrane-spanning $G_{i/o}$-protein–coupled receptors. Each receptor is a component of the molecular complex consisting of an extracellular N-terminus, 7 transmembrane proteins, 3 extracellular and

3 intracellular loops and intracellular C-terminus. Classical opioid receptors are μ-, δ- and κ-receptors, which are encoded by three genes showing 50–70% structural homology (Evans et al. 1992, Meng et al. 1993, Wang et al. 1993). Furthermore, an opioid receptor-like protein (ORL) with a 65% structural homology to the classical receptors has been cloned (Meunier et al. 1995). Several subtypes of each opioid receptor have been identified. The structural differences of the subtypes can lead to alterations in signaling and ligand-binding properties (Pan et al. 1999, Pasternak 2010).

When an endogenous or exogenous opioid agonist, such as oxycodone, binds at the opioid receptor, $G_{i/o}$-protein is activated leading to the inhibition of adenylate cyclase enzyme and thereby a decrease of intracellular cyclic 3',5'-adenosine monophosphate (cAMP) production and/or to the regulation of ion-channels. Transient inhibition of Ca^{2+} influx, hyperpolarization of the cell membrane by K^+-ion efflux and decreased release of neurotransmitters such as glutamate, substance P and/or calcitonin gene related peptide, inhibit the neuronal excitability resulting in analgesia (Zöllner and Stein 2007). Opioid receptors are located mainly in the cell membranes of presynaptic nerve endings in the brain (cortex, thalamus, hypothalamus, amygdala), in the brainstem (periaqueductal gray and the rostral medulla), and the spinal cord (dorsal horn). They are found also in postsynaptic nerve endings, peripheral tissues such as peripheral nerve terminals of primary afferent neurons (Hassan et al. 1993, Zöllner and Stein 2007), and neuroendocrine, immune and ectodermal tissues (Slominski et al. 2000). Opioid receptors are synthesized in the dorsal horn and transported along intra-axonal microtubules to the peripheral endings of the sensory neurons (Besse et al. 1990). During inflammation, the synthesis of peripheral opioid receptors is accelerated (Hassan et al. 1993, Kalso et al. 1997).

2.1.2 Pharmacokinetics

Absorption

Oral bioavailability of oxycodone in humans is 55–87% (Table 1) due to moderate first-pass metabolism. After intranasal or rectal administration its bioavailability is lower (46–62%) (Leow et al. 1995, Takala et al. 1997), and after sublingual administration, the bioavailability of oxycodone has been less than 20% in adults at normal pH (Weinberg et al. 1988).

Fatty foods were reported to modestly (in the range of 20%) increase the maximal plasma concentrations of immediate-release oxycodone, but did not alter the absorption of controlled-release oxycodone (Benziger et al. 1996). Controlled-release oxycodone is absorbed in a bi-exponential fashion with a rapid phase accounting for 38% of the dose and a slow phase with an absorption half-life of 6.2 h, which accounts for the residual 62% of the dose (Mandema et al. 1996). Steady-state plasma concentrations of both immediate-release and controlled-release oxycodone are achieved in approximately 24 h (Reder et al. 1996). The time to mean maximal plasma concentration (t_{max}) is 1–1.5 h

after the immediate-release oxycodone and 2.6–3.2 h after controlled-release oxycodone (Pöyhiä et al. 1992, Mandema et al. 1996, Reder et al. 1996, Lalovic et al. 2006).

Distribution

The protein binding of oxycodone is similar to morphine (Table 1) and oxycodone is mainly bound to albumin (Leow et al. 1993). The lipid solubility of oxycodone is equal to morphine (Table 1) (Pöyhiä and Seppälä 1994b). The volume of distribution has been 2.0–3.3 L/kg after single doses in healthy volunteers (Pöyhiä et al. 1991, Takala et al. 1997, Saari et al. 2010). The distribution kinetics in the brain tissue was studied in conscious sheep (Villesen et al. 2006). Oxycodone had 7-fold higher permeability across the blood-brain barrier (BBB) than morphine and approximately 2.5-fold higher cerebral volume of distribution. Equilibration half-time of the deep compartment for oxycodone was short (7 min), whereas the corresponding values for morphine and fentanyl were 10 min and for methadone 17 min.

In animal studies, the unbound concentrations of oxycodone in the brain tissue have been 2–6-fold higher than concentrations in the plasma suggesting high BBB permeability (Boström et al. 2006, Lalovic et al. 2006, Villesen et al. 2006). The plasma and brain tissue concentrations of the metabolites noroxycodone, oxymorphone, noroxymorphone and β-oxycodol have been studied in rats (Lalovic et al. 2006). The concentrations of noroxycodone in the plasma were highest, being 8-fold higher compared to those of oxycodone, but the brain/plasma ratio was only 5% compared to oxycodone suggesting low BBB permeability. In the same study, the concentrations of active metabolite oxymorphone in the brain were less than 10% compared to oxycodone and the brain/plasma ratio was low, only approximately 10% of those of oxycodone. Although noroxymorphone was present in high concentrations in rat plasma (7-fold higher than oxycodone), the brain tissue concentrations were only 1% of those in plasma, indicating extremely low BBB penetration (Lalovic et al. 2006). β-oxycodol concentrations were low both in plasma and the brain tissue.

Metabolism and the role of metabolites

Oxycodone is extensively metabolized, mainly via oxidative pathways in the liver, and to a lesser extent in the intestinal microsomes (Lalovic et al. 2004). The predominant oxidative pathway of oxycodone is the N-demethylation to noroxycodone (Figure 1) in a reaction catalyzed by CYP3A (Pöyhiä et al. 1991, Pöyhiä et al. 1992, Kaiko et al. 1996, Heiskanen et al. 1998, Lalovic et al. 2004, Lalovic et al. 2006, Grönlund et al. 2010a,b, Saari et al. 2010). Concerning CYP3A, CYP3A4 and CYP3A5 have been involved in the metabolism of oxycodone and displayed the highest activity for the predominant route from oxycodone to noroxycodone (Lalovic et al. 2004). The CYP3A4 content in human liver microsomes varied 13-fold and CYP3A5 even 100-fold (Lalovic et al. 2004).

About 10% of oxycodone is O-demethylated to oxymorphone by CYP2D6 (Lalovic et al. 2006). Noroxymorphone is the secondary metabolite of oxycodone and it is O-demethylated mainly from noroxycodone by CYP2D6. Only negligible

N-demethylation activity of oxymorphone to noroxymorphone catalyzed by CYP3A and CYP2D6 has been observed, and it was measurable only at the highest oxycodone concentrations, and at 20-fold slower rate than the noroxycodone O-demethylation (Lalovic et al. 2004, Lalovic et al. 2006). Reduced metabolites of oxycodone are α- and β-oxycodol, α- and β-noroxycodol and α- and β-oxymorphol (Figure 1).

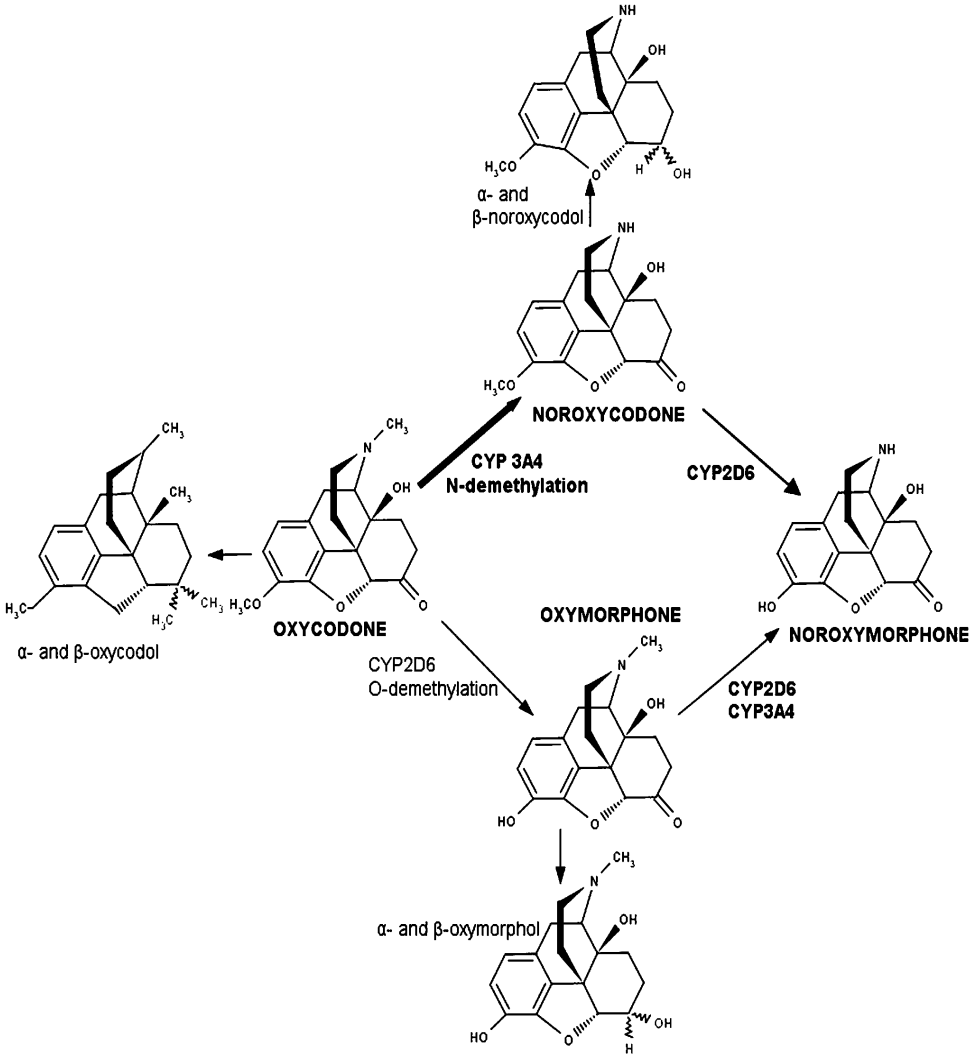

Figure 1. The metabolism of oxycodone. Modified from Lalovic et al. 2006.

Previously it was thought, that oxycodone was a prodrug like codeine, and CYP2D6 dependent metabolite oxymorphone was suggested to be responsible for its effects (Otton et al. 1993). The μ-opioid receptor affinity of oxymorphone is over 40-fold compared to that of oxycodone, and it activates the G-protein/opioid receptor complex in GTP[^{35}S]

γ assay at 8-fold lower concentrations compared with oxycodone (Lalovic et al. 2006). However, in pharmacokinetic studies, the concentrations of oxymorphone have been low or absent (Pöyhiä et al. 1991, Pöyhiä et al. 1992, Kaiko et al. 1996, Lalovic et al. 2006). The formation of oxymorphone has been significantly dependent on CYP2D6 function (Zwisler et al. 2009 and 2010a).

High concentrations of noroxycodone are present in plasma after the administration of oxycodone, but its binding affinity to the μ-receptor is only 28% compared with oxycodone (Lalovic et al. 2006). Noroxycodone activates the G-protein/opioid complex at only over 5-fold higher concentrations compared with oxycodone, and has very low BBB permeability (Lalovic et al. 2006). The effects of noroxycodone have not been studied in humans, but in animal studies its antinociceptive effects have been low during subcutaneous, intrathecal or intracerebroventral administration (Leow and Smith 1994, Lemberg et al. 2006). Noroxymorphone has over 2-fold higher affinity to the μ-opioid receptor and it binds to the G-protein/opioid receptor complex at two times higher concentrations compared with oxycodone (Lalovic et al. 2006). Noroxymorphone has low lipid solubility and its BBB permeability is extremely low or absent (Lalovic et al. 2006, Lemberg et al. 2008).

The role of transporters

In addition to the CYP system, there may be transporter systems affecting the pharmacokinetics of oxycodone. P-glycoprotein (P-gp) is a transmembrane efflux transporter, which has been identified, for example, in the luminal surface of brain capillary endothelium, the intestine, liver and kidney (Hebert 1997). P-gp limits the absorption of its substrates in the intestine and across BBB. Furthermore, it can diminish the reabsorption of xenobiotics from the renal proximal tubule. Many opioids such as loperamide, morphine and methadone are P-gp substrates (Wandel et al. 2002, Kharasch et al. 2003, Kharasch et al. 2004). P-gp and CYP enzymes share many inhibitors and inducers (Zhou 2008).

The role of P-gp in the pharmacokinetics and pharmacodynamics of oxycodone is under debate. Animal studies have yielded controversial findings about its role in the transport of oxycodone via BBB (Boström et al. 2005, Hassan et al. 2007). Boström et al. found negligible the role of P-gp in the permeability of oxycodone, but this was argued by the findings of Hassan et al. They considered that a too low dose of oxycodone in the previous study had been insufficient to show the role of P-gp and instead of that, oxycodone was proposed to be a P-gp substrate. The high brain to plasma ratios of oxycodone have been suggested to be due to the existence of an active influx transporter (Boström et al. 2006, Boström et al. 2008). Recently it has been postulated, that the pyrilamine transporter, an organic cation transporter, might act as an influx protein of oxycodone in rats (Okura et al. 2008). The role of the pyrilamine transporter in humans has not been studied.

Elimination

The elimination half-life of oxycodone is 3–5 h regardless of the route of administration (Pöyhiä et al. 1991, Leow et al. 1995, Kaiko et al. 1996, Lalovic et al. 2006, Grönlund

et al. 2010a,b, Saari et al. 2010). Its total plasma clearance in healthy volunteers is 0.78–0.82 L/min (Pöyhiä et al. 1991, Takala et al. 1997), and renal clearance 0.07 L/min (Pöyhiä et al. 1992). Less than 10% of the oxycodone dose is excreted unchanged in urine (Pöyhiä et al. 1992, Kirvelä et al. 1996, Lalovic et al. 2006).

Noroxycodone is the main metabolite in urine excreted primarily as free forms, whereas oxymorphone is principally excreted as conjugates. Noroxymorphone was the second common metabolite in urine excreted both as conjugated and unconjugated forms (Pöyhiä et al. 1992, Lalovic et al. 2006). The reductive metabolites have accounted for about 18% of the oxycodone dose in urine compared with 47% for oxidative metabolites (Lalovic et al. 2006). Only 72% of the dose was recovered in urine. It is not known, whether the remaining 28% of the dose represents either an unidentified metabolic or excretory pathway or incomplete gastrointestinal absorption.

Oxycodone has been found in breast milk. In a study with 50 breast-feeding mothers and their 41 neonates after a Caesarean section, oxycodone median milk:plasma ratio during a 24 h period was 3.2:1. Although oxycodone levels determined in breast milk ascended up to 168 ng/mL, only one neonate had detectable concentrations of oxycodone in their plasma during the following 72 h (Seaton et al. 2007).

Effect of age, gender, hepatic and renal failure

The clearance of oxycodone is decreased and elimination half-life prolonged in patients aged 70–90 years resulting in two-fold higher plasma concentrations of oxycodone, when compared to young adults (Liukas et al. 2008). No gender-associated differences were observed in any of the pharmacokinetic parameters. Kaiko et al. investigated young and old female and male volunteers after a single dose of controlled-release oxycodone (Kaiko et al. 1996). Group sizes were small, only seven subjects in each. The weight-adjusted clearance of oxycodone was reported to be 25% slower in healthy women when compared to men.

Hepatic failure increases the exposure of oxycodone. In end-stage liver cirrhosis, the elimination half-life decreased from the value of 13.9 h before liver transplant to 3.4 h after it (Tallgren et al. 1997). The corresponding oxycodone clearance increased from 0.26 L/min to 1.13 L/min. The pharmacokinetics and excretion of oxycodone was studied in 10 uremic patients (mean serum creatinine concentration 644 ± 131 μmol/l) undergoing renal transplantation, and in healthy patients (mean serum creatinine concentration 72 ± 11 μmol/l) undergoing general surgery (Kirvelä et al. 1996). After a single intravenous oxycodone dose, the median elimination half-life of oxycodone was 2.3 h in general surgical patients and 3.9 h in renal transplantation patients. The difference was explained with the increased volume of distribution and decreased clearance. Great interindividual differences were detected. In a case report, oxycodone and its metabolites were shown to be removable by haemodialysis (Lee et al. 2005). This supports the possibility to use oxycodone in patients undergoing haemodialysis treatment, but individual dose adjustments are needed.

2.1.3 Pharmacodynamics

The mechanism of action of oxycodone is typical for μ-opioid receptor agonists. In addition to analgesia, oxycodone produces several adverse effects such as respiratory depression, sedation, miosis, constipation, euphoria and increased tolerance (Silvasti et al. 1998, Cicero et al. 2005). Parent oxycodone seems to be responsible for the main clinical effects of the drug, although oxymorphone may contribute to the effects (Kaiko et al. 1996, Lalovic et al. 2006). Noroxymorphone has low or absent BBB permeability, but after intrathecal administration it was shown to produce long-lasting analgesia in rats (Lemberg et al. 2008). Noroxycodone has not been studied in humans, but studies in rats and mice have shown, that noroxycodone has poor antinociceptive effects regardless of the administration route (Leow and Smith 1994, Lemberg et al. 2006).

Oxycodone has been more effective than morphine in treating postoperative abdominal pain (Kalso et al. 1991, Lenz et al. 2009). Persistent advocates for the contribution of other opioid receptors such as κ- receptor have been claimed to be involved in the efficacy of oxycodone (Ross and Smith 1997, Nielsen et al. 2007). However, recent animal studies have failed to reduce the antinociceptive effect of oxycodone with selective κ- and δ-receptor antagonists (Lemberg et al. 2006, Narita et al. 2008). It may be due to the fact that oxycodone binds to κ-and δ-receptors with a more than 50-fold lower affinity compared to μ-receptor (Lalovic et al. 2006).

Analgesic effect in experimental pain

Opioid effects have been investigated in healthy volunteers with several experimental pain models such as using cold, warm, electrical or pressure stimulation applied onto the skin, muscle, oesophagus, or aiming at evoking central integration of pain and hyperalgesia (Arendt-Nielsen et al. 1997, Enggaard et al. 2001, Luginbuhl et al. 2001, Staahl et al. 2006b). When five experimental pain models were compared, the cold pain pressure test, electrical and pressure thresholds were clinically relevant in the investigation of alfentanil concentration-response curves, whereas in the heat pain test with a rapid temperature increase 2 ˚C/s, no alfentanil effects were detected (Luginbuhl et al. 2001). The cold pressor test has been sensitive to analgesic effects of oxycodone in healthy subjects (Kolzenburg et al. 2006). After administration of epidural morphine in healthy volunteers, the pain detection thresholds for the heat pain, pressure pain and electrical pain stimulus increased significantly (Brennum et al. 1993), and the changes in heat pain detection were reduced by naloxone. Weak and strong opioids such as codeine, tramadol, morphine, fentanyl and alfentanil have decreased cold pressure pain (Jones et al. 1988, Poulsen et al. 1996, Enggaard et al. 2001, Luginbuhl et al. 2001).

Oxycodone has been investigated in well designed and validated pain models (Staahl et al. 2006a, Staahl et al. 2008). Each test battery consisted of mechanical, thermal computer driven heat stimulation) and electrical stimulations onto the skin and viscera. Furthermore, mechanical and electrical stimulations in muscles were conducted. Morphine and oxycodone relieved pain from skin and muscles similarly, but oxycodone

was superior compared to morphine relieving pain arising from the mechanical and thermal stimulation of the oesophagus (Staahl et al. 2006a). For oxycodone, the analgesia has been related to the plasma concentrations with no delay for most visceral measures and 17 minutes delay for somatic pain measures whereas the median delay for morphine has been 34 minutes (Staahl et al. 2008). Grach et al. used the cold pain pressure test to compare the analgesic synergy of oral oxycodone and morphine in humans (Grach et al. 2004). Unfortunately they did not take into consideration the different bioavailabilities of these two drugs and the findings were difficult to compare. Recently, the cold pain pressure test has been also used in studies investigating the effect of CYP3A and CYP2D6 blockade to the analgesic effect of oxycodone (Grönlund et al. 2009, Samer et al. 2010b, Saari et al. 2010, Zwisler 2010a), but the results have been conflicting.

Clinical pain

In Finland, oxycodone has been the most frequently used opioid during the last fifty years in the treatment of moderate or severe pain (Pöyhiä 1994a, Hamunen et al. 2009). Oxycodone is used also in the treatment of severe chronic non-malignant pain when all other medical therapies have failed. Clinical trials have demonstrated the effect of oxycodone in nociceptive and neuropathic non-cancer pain. The effectiveness of oxycodone in acute pain has been shown in many clinical studies (Kalso et al. 1991, Silvasti et al. 1998, Lenz et al. 2009). Silvasti et al. studied 50 patients after plastic reconstruction of the breast or a major operation of the vertebrae, such as spinal fusion, being treated with doses of oxycodone (30 μg/kg) or morphine (45 μg/kg) with patient-controlled analgesia for postoperative pain (Silvasti et al. 1998). More doses were needed after oxycodone thus delaying the onset of pain relief, but the analgesic efficacy was similar for both opioids at equal doses. In osteoarthritis pain, oxycodone has been superior to the placebo with a mean daily dose 40 mg (Caldwell et al. 1999, Roth et al. 2000). Oxycodone can be effective in neuropathic pain, although it is not recommended as a first-line drug. In a RCT in diabetic neuropathy patients, controlled-release oxycodone was more effective than the placebo and improved the quality of life significantly (Watson et al. 2003). The number needed to treat to attain at least 50% pain relief for one patient, was 2.6. It has also been effective in the treatment of postherpetic pain (Watson and Babul 1998, Raja et al. 2002).

Oxycodone is specifically suitable for oral administration because of its high oral bioavailability. Immediate-release and controlled-release oxycodone have provided comparable analgesia and adverse effects in many RCTs (Bruera et al. 1998, Kaplan et al. 1998, Salzman et al. 1999, Stambaugh et al. 2001). Compared to controlled-release morphine, controlled-release oxycodone provides comparable analgesia with probably fewer adverse effects (Heiskanen and Kalso 1997, Mucci-LoRusso et al. 1998, Heiskanen et al. 2000). At least hallucinations have been more rarely connected to the use of oxycodone compared to morphine (Hagen and Babul 1997, Mucci-LoRusso et al. 1998). After epidural administration, the equianalgesic dose ratio between morphine and oxycodone was approximately 1:10 (Backlund et al. 1997).

The equianalgesic doses of oxycodone and morphine have varied depending on the study population and settings. In abdominal surgery patients, an equianalgesic dose ratio of intravenous oxycodone and morphine was 2:3 as calculated on the basis of total consumption of opioid needed during the whole 2 h study period (Kalso et al. 1991). The first state of pain relief was achieved faster with oxycodone and it lasted longer with less sedation compared to morphine. A similar equianalgesic dose ratio was reported after a gynaecological operation and PCA (Lenz et al. 2009). On the other hand, in a study with patients undergoing breast reconstruction or a major operation of the vertebrae, the doses and effects of intravenous oxycodone or morphine with PCA were similar (Silvasti et al. 1998).

In studies with cancer patients, 30% more intravenous/intramuscular oxycodone was required to produce the same analgesia as with intravenous/intramuscular morphine (Kalso and Vainio 1990). In other studies, comparable analgesia, but fewer adverse effects were recorded with the use of controlled-release oxycodone in cancer pain when compared to controlled-release morphine (Heiskanen and Kalso 1997, Mucci-LoRusso et al. 1998, Heiskanen et al. 2000). Equianalgesic oral doses of oxycodone in cancer patients have been 2:3–3:4 (Heiskanen and Kalso 1997).

Other effects

The adverse effects of oxycodone are similar to morphine (Silvasti et al. 1998). The most common adverse effects reported are sedation, nausea and constipation. After high doses, sedation and respiratory depression occur and may even have fatal consequences. Oxycodone is not releasing histamines such as morphine (Pöyhiä et al. 2004). It can alter the hypothalamic-pituitary-adrenal or hypothalamic-pituitary-gonad response resulting in increased plasma prolactine or decreased cortisol concentrations (Saarialho-Kere et al. 1989, Sithisarn et al. 2008).

Pharmacological tolerance develops after repeated exposure to opioids. Although it can have multiple causes, mechanisms involving receptor trafficking, such as receptor phosphorylation/desensitization, internalization, recycling or degradation are often included (Rijn et al. 2010). This phenomenon can lead to reduced analgesia if the doses are not increased. Tolerance does not develop to opioid-induced constipation or miosis. After continuous exposure to opioids physical dependence develops and discontinuation of the drug abruptly leads to withdrawal symptoms such as restlessness, mydriasis, runny nose, diarrhoea, and shaking chills.

2.1.4 Pharmacogenetics

The genetic polymorphism of drug metabolizing enzymes, receptors and transporters can alter the pharmacokinetics and pharmacodynamics of opioids. Approximately 7% of Caucasians are poor CYP2D6 metabolizers with no enzyme activity (Dahl et al. 1995, Sachse et al. 1997). This polymorphism has been shown to diminish the CYP2D6-mediated O-demethylation of oxycodone to oxymorphone resulting in significantly lower

oxymorphone concentrations (Zwisler et al. 2009, Zwisler et al. 2010a). The influence of pharmacogenetics on the analgesic effect of oxycodone is controversial. In an experimental pain model with 16 healthy extensive metabolizers (EMs) and 17 poor metabolizers (PMs), PMs had diminished analgesic effect of oxycodone in 3 out of 5 tests, including pain detection and tolerance for electrical stimulation and cold pain $AUC_{0-\infty}$ (Zwisler et al. 2009). This was not supported in another study with 270 postoperative patients (246 EMs and 24 PMs), where the analgesic differences between PMs and EMs were not found in spite of the decreased formation of oxymorphone (Zwisler et al. 2010a). It has also been suggested, based on a sample of two individuals, that CYP2D6 ultrarapid metabolizers (UMs) experienced a 1.5- and 6-fold increase in the analgesic effects of oxycodone (Samer et al 2010a). Further studies with adequate sample sizes are needed to make conclusions about the effect of the *CYP2D6* genotype on the analgesic effect of oxycodone.

Although all opioid receptors can mediate opioid effects, the μ-opioid receptor has a primary role in analgesia. The most prevalent single nucleotide polymorphism (SNP) of the gene for the μ-opioid receptor, *OPRM1*, is A118>G. Patients carrying one (heterozygote A118G) or two (homozygote G118G) variant G alleles, may require higher doses of opioids (Chou et al. 2006, Campa et al. 2008). Polymorphism in the P-gp encoding gene *ABCB1/MDR1* affects the function of the efflux pump, P-gp, which is most effective with the homozygous C/C genotype. It has been shown, that subjects with the *3435TT* genotype (inefficient P-gp) had higher digoxin concentrations than those with the *3435CC* genotype (Johne et al. 2002).

The antinociceptive effect and adverse reactions of oxycodone were studied in relation to the genetic variations of *OPRM1* and *ABCB1/MDR1* in 33 healthy subjects (Zwisler et al. 2010b). In subjects with the *A118G* genotype, the antinociceptive effect of oxycodone was reduced in an experimental pain model of sural nerve stimulation, but not in a cold pain test. Variant alleles of *C3436T* and *G2677T/A* (lower expression of P-gp) were associated with fewer adverse drug reactions, but not with a diminished antinociceptive effect of oxycodone (Zwisler et al. 2010b). As the role of P-gp in the absorption of oxycodone is controversial, these findings need further elucidation.

2.2 Cytochrome P 450

2.2.1 Principles of drug metabolism

The xenobiotics and drugs are eliminated from the body during the metabolism. This is achieved by converting lipid-soluble compounds into water-soluble or hydrophilic forms to enable their excretion by the kidneys. Without this biotransformation, lipid-soluble xenobiotics would be reabsorbed from renal tubules leading to accumulation. Drug metabolism is essential for the activation of pro-drugs such as codeine, which is inactive itself, but is metabolized into active metabolite by CYP2D6 (Desmeules et al.

1991). Drug metabolism can also lead to intoxication if a drug is converted into toxic metabolites such as paracetamol (Albano et al. 1985).

The most important organ for drug metabolism is the liver, because it has the highest amount of drug metabolizing enzymes (Shimada et al. 1994, Pelkonen et al. 2008). The gut is also an important organ for drug metabolism. Xenobiotics are metabolized also in many other organs such as the brain, placenta or lungs (Krishna and Klotz 1994). In addition to metabolism, the drug transport across biological membranes can be restricted by efflux transporters such as P-gp or multidrug resistance-associated proteins (Oswald et al. 2007). CYP3A and P-gp have many common substrates (Zhou 2008).

Drug metabolism is divided into two phases. In a phase I reaction, a functional group is added to the xenobiotic, whereas during phase II reactions, a water-soluble molecule is added to the functional group and a more hydrophilic complex is formed. Phase I reactions are performed mainly by CYP enzymes involved in oxidation, reduction and hydrolysis, and phase II reactions involve glucuronidation, sulfation, acetylation and methylation (Yan and Caldwell 2001). Most drugs undergo both reactions sequentially, but the involvement of only one of the phases is possible.

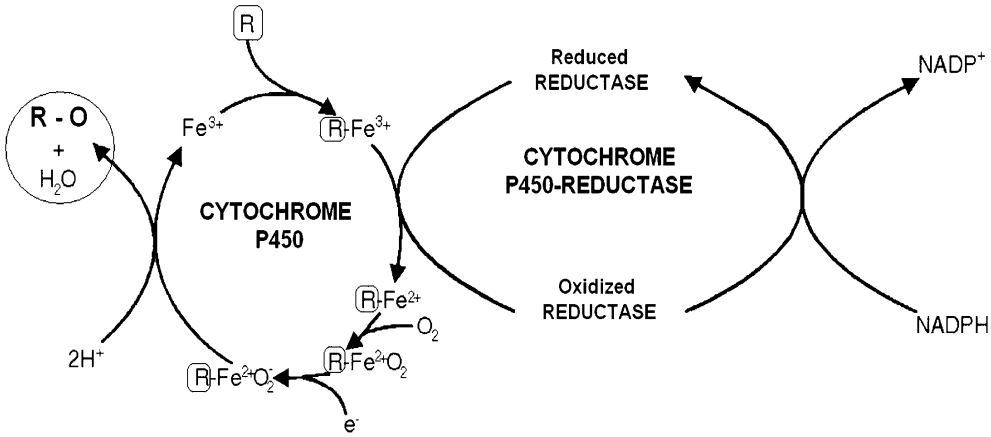

Figure 2. Simplified CYP catalytic oxidation cycle. Modified from Koulu and Tuomisto, 2001.
R = CYP substrate; NADP = Nicotinamide adenine dinucleotide phosphate; NADPH = reduced for of NADP; R–O = oxidized drug.

Oxidation, catalyzed by the haem containing CYP enzymes, is the most common phase I reaction (Figure 2). In the beginning of the oxidation cycle, the drug (R) binds reversibly to the active domain of the CYP enzyme. The haem group is reduced to the ferrous state by an electron generated from NADPH by the CYP reductase. During the next step, an oxygen molecule binds to the ferrous CYP-substrate complex. NADPH provides a second electron resulting in the cleavage of O-O complex. In the end of the oxidation cycle, the substrate is oxidized and released from the CYP enzyme (Lin and Lu 1998,

Yan and Caldwell 2001). CYP inhibitors can interrupt the oxidation cycle resulting in a deceleration of the catalytic cycle and an increase of the parent substrate concentrations and action.

2.2.2 Cytochrome P450 (CYP) enzymes

CYP enzymes have been identified in most organisms from plants to humans. The members of the CYP superfamily are haem-containing mono-oxygenases, which are involved in over 50% of all drug metabolisms (Nebert and Russell 2002, Guengerich 2008). The haem acts as an oxidation reaction center and the apoprotein determines the substrate specifity and binding affinity of individual isozymes. CYP enzymes catalyze oxidation reactions of both xenobiotics and endogenous chemicals such as steroids, fatty acids, vitamins and eicosanoids (Nebert and Russell 2002, Guengerich 2008). The expression of CYPs is affected by physiological, pathological, genetic and environmental factors such as hormones, infections, polymorphism, diet and environmental pollutants (Yang et al. 2008).

In the human genome, 57 CYP genes have been identified (www.cypalleles.ki.se) comprising 18 families and 42 subfamilies. The nomenclature of CYP enzymes is based on the amino acid similarities (Figure 3), and is designated by a family number, a subfamily letter, a number for an individual enzyme within the subfamily, and an asterisk followed by a number and a letter for each genetic (allelic) variant. In the same family, enzymes have at least 40% amino acid sequence homology and in the same subfamily, the similarity increases over 55% (Nebert and Russell 2002, Pelkonen et al. 2008). CYP enzymes are found mainly in the liver and intestine, but also in many other tissues such as the adrenal gland, brain, kidneys, lungs, testis and skin (Krishna and Klotz 1994, Pelkonen et al. 2008). CYP1–3 families are the main contributors in the metabolism of drugs.

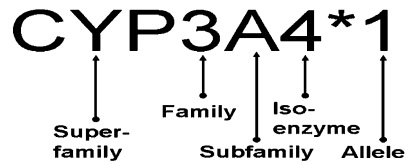

Figure 3. An example of the nomenclature of a CYP enzyme.
Modified from Koulu and Tuomisto, 2001.

CYP1 and 2 families

The CYP1 family consists of three members: CYP1A1, CYP1A2 and CYP1B1, of which the CYP1A2 is the only one present in the liver, accounting for 13% of liver CYP proteins (Pelkonen et al. 2008). CYP1A1 and CYP1B1 are present in extrahepatic tissues. CYP1A2 catalyzes the metabolism of theophylline, caffeine, oral melatonin

and ropivacaine (Shimada et al. 1994, Jokinen et al. 2000, Pelkonen et al. 2008). It is induced by cigarette smoking or charcoal-grilled food and inhibited by fluvoxamine, oral hormone replacement therapy and oral contraceptives (Laine et al. 1999, Nebert and Russell 2002, Pelkonen et al. 2008).

CYP2 is the largest P450 family comprising 16 genes, 5 families and 13 subfamilies (Nebert and Russell 2002). CYP2C and CYP2E1 account for more than 15% of liver CYP enzymes, whereas the corresponding value for CYP2A6 is approximately 10% and for CYP2D6, CYP2C8, CYP2C19 and less than 5% (Shimada et al. 1994, Pelkonen et al. 2008). CYP2B6, CYP2C9, CYP2C19 and CYP2D6 present the most important CYP polymorphisms clinically with ethnic differences in their distributions. Subjects with one or two functional *CYP2D6* alleles are classified as normal, EM genotype.

Approximately 7% of Caucasians have two nonfunctional CYP2D6 alleles and are classified thereby as PMs (Sachse et al. 1997). The PM genotype results in a diminished metabolism and effects of prodrugs that are CYP2D6 substrates, such as codeine or tramadol (Desmeules et al. 1991, Dayer et al. 1997, Bradford 2002). 1–2% of Caucasians and more than 25% of Ethiopians are UM with over two functional copies of the *CYP2D6* gene exhibiting extremely high enzyme activity resulting in the accelerated metabolism of CYP2D6 substrates (Dahl et al. 1995, Gasche et al. 2004). This can result in an increased risk of life-threatening central nervous system depression of breastfed infants of mothers, who are receiving codeine treatment (Madadi et al. 2009). The clinical impact of intermediate CYP2D6 metabolizers (IMs) with one non-functional and one allele of decreased activity or two alleles with decreased activity is not clear.

Approximately 30% of all drugs are CYP2D6 substrates such as codeine and tramadol (Desmeules et al. 1991, Dayer et al. 1997). CYP2D6 can be inhibited by for example quinidine, fluoxetine or paroxetine (Otton et al. 1993, Heiskanen et al. 1998, Lemberg et al. 2010), but CYP2D6 seems to not be inducible by other drugs.

The CYP3 family

The CYP3A subfamily is the most important CYP subfamily consisting of four isoenzymes CYP3A4, CYP3A5, CYP3A7 and CYP3A43, which are encoded by genes located in chromosome 7 (Finta and Zaphiropoulos 2000).

CYP3A4 exhibits a dominant role among the CYP3A proteins. Although several variant alleles of the *CYP3A4* gene have been defined, most of them seem to be too rare to contribute significantly to the CYP3A4 variability (Eiselt et al. 2001). CYP3A4 is undetectable in fetal liver, but rapidly rises after the birth, and becomes the predominant CYP in the adult liver (Schuetz et al. 1994). CYP3A4 accounts for approximately 30% of total CYP proteins (Shimada et al. 1994). As in the liver, CYP3A4 is the most abundant CYP also in the intestine, representing approximately 50–70% of its total intestinal CYP content (Kolars et al. 1994, Paine et al. 1997, Thummel and Wilkinson 1998, Paine et al. 2006). CYP3A4 expression represents high interindividual heterogeneity and its

function has been estimated to vary over 11-fold in both liver microsomes and duodenal biopsies (Lown et al. 1994, Shimada et al. 1994, Paine et al. 1997). The expression of CYP3A4 diminishes along the intestinal tract, thus the greatest CYP3A content is determined in the duodenum and lowest in the ileum (Paine et al. 1997).

CYP3A4 and CYP3A5 share an 84% amino sequence similarity and their substrate specifity is almost equal, although some differences in catalytic activities have been found. CYP3A5 genotype has not significantly influenced on the pharmacokinetics or pharmacodynamics of CYP3A substrates alfentanil or midazolam (Kharasch et al. 2007), but it is involved in the metabolism of oxycodone (Lalovic et al. 2004). CYP3A5 is polymorphically expressed and is found in 2–30% of the livers of Caucasians and up to 60% of African-Americans (Hustert et al. 2001, Kuehl et al. 2001, Westlind-Johnsson et al. 2003). In individuals expressing the CYP3A5 enzyme, it can represent over 50% of the total CYP3A activity in the liver, thus having an important role in the interindividual variability in drug metabolism (Kuehl et al. 2001). Individuals having at least one CYP3A5*1 allele, have functional CYP3A5, whereas a single nucleotide polymorphism in CYP3A5*3 or CYP3A5*6 allele reduced translation and caused the absence of functional protein (Kuehl et al. 2001). In the Finnish population, the frequency of the homozygous CYP3A5*1 gene expression (high activity of enzyme) has been higher than in other Caucasian populations (Hilli et al. 2007). CYP3A5 is expressed specifically in the extrahepatic tissues, such as the lung, oesophagus and colon (Westlind-Johnsson et al. 2003). In renal samples, CYP3A5 is the most frequently expressed CYP, whereas CYP3A4 is present in only 30% of the samples (Haehner et al. 1996). Still, the role of CYP3A5 in drug elimination remains conflicting.

CYP3A7 is the major fetal liver CYP3A, but its amount declines after birth and it gets replaced by CYP3A4 (Schuetz et al. 1994, Lacroix et al. 1997). Ten per cent of adult livers express significant levels of CYP3A7 (Sim et al. 2005), whereas the clinical implication of recently identified CYP3A43 has not been clarified and it has been suggested to be a result of a pseudogene (Pelkonen et al. 2008). At least the low level of expression and low activity suggests that its biological significance might be negligible (Domanski et al. 2001).

CYP3A is involved in the metabolism of approximately 50% of the all marketed drugs. The inducible or inhibitable character of CYP3A4 makes it prone to the drug-drug interactions. The structure of CYP3A4 is shown in Figure 4. The active site of CYP3A is flexible, allowing multiple small molecules to be present simultaneously (Williams et al. 2004, Ekroos and Sjögren 2006). When a ligand binds to the active center of CYP3A4, it undergoes dramatic conformational changes with an increase of its volume by over 80% (Ekroos and Sjögren 2006). CYP3A4 can also be activated (heterotropic cooperativity) by several other compounds, which may lead to increased metabolism of the substrate (Niwa et al. 2008).

Typical CYP3A4 substrates (Table 2) are calcium channel blockers verapamil, nifedipine, felodipine, benzodiazepines alprazolam, midazolam, triazolam, and atorvastatin, ketoconazole, voriconazole, itraconazole or antiretroviral ritonavir (Zhou 2008). The induction and inhibition have resulted in 400- fold differences in the exposure to oral midazolam (Backman et al. 1998).

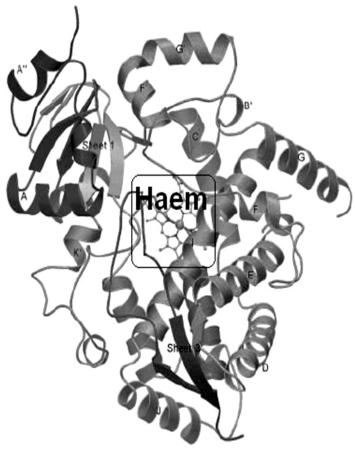

Figure 4. The structure of CYP3A4. Modified from Williams et al. 2004.

Table 2. Some of the clinically relevant CYP3A subtrates, inhibitors and inducers. (Accessed http://medicine.iupui.edu/clinpharm/DDIs/ClinicalTable.asp)

CYP3A substrates			
alfentanil	hydrocortisone	atorvastatin	nifedipine
fentanyl	dexamethasone	lovastatin	verapamil
methadone	haloperidol	simvastatin	cyclosporine
dextromethorphan	lidocaine	clarithromycin	tacrolimus
alprazolam	ondansetron	erythromycin	propranolol
buspirone	estradiol	telithromycin	risperidone
midazolam	progesterone	ritonavir	tamoxifen
triazolam	testosterone	diltiazem	taxol
zolpidem	terfenadine	felodipine	terfenadine
CYP3A inhibitors			
chlaritromycin	ketoconazole	ritonavir	diltiazem
erythromycin	itraconazole	nelfinavir	verapamil
telithromycin	voriconazole	indinavir	grapefruit juice
CYP3A inducers			
			pioglitazone
barbiturates	phenobarbital	rifabutin	troglitazone
carbamazepine	phenytoin	rifampicin	St. John's wort

2.2.3 CYP3A induction

Mechanism of CYP3A induction

During an exposure to multiple exogenous drugs or chemicals, CYP induction may serve as a detoxifying process, but it can also decrease the efficacy of drug therapy or lead to the increased formation of toxic metabolites (Albano et al. 1985, Bauer et al. 2003). CYPs are induced in a similar mechanism involving de novo RNA and protein synthesis. The inducer binds to the nuclear receptors of a cell, such as pregnane X receptor (PXR), constitutive androstane receptor or aryl hydrocarbon receptor, leading to enhanced cytochrome transcription (Lehmann et al. 1998). Alternative mechanisms of induction are the stabilization of enzymes and the inhibition of protein degradation (Yang et al. 2008).

Rifampicin, carbamazepine, phenobarbital and St. John's wort (Table 2) are CYP3A inducers (Thummel and Wilkinson 1998, Zhou 2008). When CYP3A is induced, an inducing agent binds to the nuclear PXR forming a heterodimer with the retinoid X receptor (Lehmann et al. 1998, Lin and Lu 1998). The heterodimer then binds to the regulatory region of the *CYP3A4* gene. This results in an up-regulated transcription of DNA and increased synthesis RNA, which causes an enhanced CYP3A4 amount, and a reduction in the plasma concentrations of CYP3A4 substrates. As an induction requires changes in protein transcription and translation it is a slow process and it takes 1–14 days to reach a new steady-state level between biosynthesis and degradation (Kolars et al. 1992, Yang et al. 2008). After the withdrawal of the inducer, the CYP activity returns to the initial level during 3 days to 3 weeks, depending on the substance.

Rifampicin

Rifampicin (Figure 5) was introduced into clinical use in 1968. Rifampicin is a semisynthetic derivative of rifamycin B. It is mainly a bacteriocidic antibiotic, and inhibits the bacterial DNA-dependent RNA polymerase activity leading to suppression of RNA synthesis in susceptible bacteria (Campbell et al. 2001). Rifampicin has been used against microorganisms of the genus *Mycobacteris*, specifically *M. tuberculosis*. It is a useful oral alternative in the treatment of both methicillin-sensitive and methicillin-resistant staphylococcus infection (Turnidge and Grayson 1993). Despite of its wide spectrum, it is used principally in combination with other antibacterial agents, because of the rapid development of resistant strains.

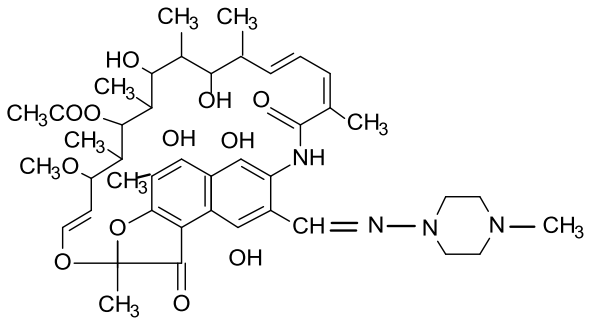

Figure 5. Chemical structure of rifampicin.

Oral rifampicin is almost completely absorbed when administrated into an empty stomach (Acocella 1978). After a single dose of 600 mg, the peak plasma concentration is achieved in 2 hours. Approximately 80% of rifampicin found in the serum is bound to plasma protein, mainly to albumin. Rifampicin is well distributed into cerebrospinal fluid and other tissues, and high concentrations have been determined in sputum and the cavitary fluid in tuberculosis. Its minimal inhibitory concentration (MIC) against mycobacterials is 0.1–2 μg/ml. The elimination half-life is dose dependent and after a 600 mg oral dose, it is 3.3 h (Acocella 1978). During rifampicin, CYP3A induction has been observed as soon as 1 day after the administration and it lasts for 3 days after the withdrawal *in vitro* (Kolars et al. 1992).

The main metabolite of rifampicin is desacetylrifampicin, which is active, but a weaker antimicrobial agent than the parent drug (Acocella 1978). Rifampicin is excreted mainly into the bile by the liver and also into urine by the kidneys. Transient elevation of bilirubin has been observed. Rifampicin has a remarkable autoinductive effect, which can shorten its elimination half-life for 2–3 h (Loos et al. 1985). Adverse events during continuous treatment are rare. Urine, feces, saliva, sputum, sweat and tears may be colored reddish orange by rifampicin and its metabolites, but this disappears after the discontinuation of the medication.

Rifampicin is one of the most potent inducers of orally administered CYP3A and/or P-gp substrates. *In vitro* the hepatic CYP3A4 mRNA content has been reported to increase by 2.4–4.7-fold after rifampicin administration and intestinal CYP3A4 mRNA by 5- to 8-fold when compared to the controls (Kolars et al. 1992). This can lead to a total loss of efficacy of triazolam or midazolam with 95% decreases in their AUCs (Backman et al. 1996, Villikka et al. 1997). Induction of the efflux protein P-gp has been demonstrated with P-gp substrate diqoxin. The oral bioavailability of digoxin was reduced from 63% to 44% after rifampicin in healthy volunteers (Greiner et al. 1999). Furthermore, rifampicin induces several other enzymes such as CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP3A5 (Niemi et al. 2003).

St. John's wort

The medicinal plant, St. John's wort (*Hypericum Perforatum*), has been known for over 2000 years. Since the time of the Swiss physician Paracelsus in the Middle Ages it has been used to treat psychiatric diseases (Bilia et al. 2002). The modern history of St. John's wort extracts began in 1984, when the German Federal Health Agency recommended it for the treatment of a depressed mood, nervousness and anxiety (Wurglics and Schubert-Zsilavecz 2006). St. John's wort is a wild plant found all over the world, including Europe, North America, Asia and Northern Africa. The height of the plant growing wild is 60 cm and the flowers are bright yellow. The drug consists of dried flowers or aerial parts of the plant and its harvesting time is just before or during the blooming (Bilia et al. 2002).

St. John's wort consists of several components such as hyperforin (Figure 6), hypericin, pseudohypericin and quercetin (Obach 2000). Hyperforin has been identified as the possible active constituent in the treatment of depression and the antidepressive effect has been correlated to the hyperforin content (Laakmann et al. 1998, Mueller et al. 1998). Animal studies suggest that St. John's wort inhibits the uptake of serotonin, norepinephrine, dopamine, L-glutamate and GABA (Singer et al. 1999, Muller et al. 2000, Wonnemann et al. 2000). St. John's wort has been more effective than a placebo and well tolerated in the treatment of mild to moderate depression (Linde et al. 1996).

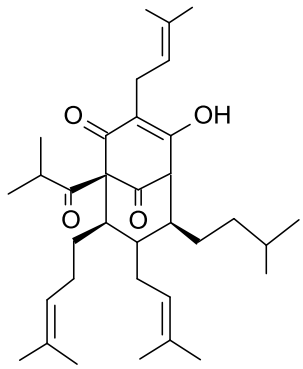

Figure 6. Structure of hyperforin, the suggested active constituent of St. John's wort.

In a study with healthy volunteers, hyperforin could be determined in the plasma up to 24 h in healthy volunteers after the administration of a single dose of 300 mg of hypericum extract containing 14.8 mg hyperforin (Biber et al. 1998). The maximum plasma levels were approximately 150 ng/ml and the time to the peak concentration 3.5 h. The elimination half-life of hyperforin was approximately 9 h and the pharmacokinetics was linear up to the dose of 600 mg of the extract. Hyperforin has been determined in the brains of rodents after oral administration unlike most of the other ingredients (Keller et al. 2003). The contents of St. John's wort preparations have been variable. When eight different preparations were compared, hyperforin content varied over 35-fold

and hypericin content over 2-fold (Wurglics et al. 2001). Furthermore, the differences between the batches varied depending on the product. Despite the high tolerability of St. John's wort products, complaints of skin reactions and gastrointestinal symptoms have been most commonly present (Brattström 2009). Photosensitization has been an extremely rare adverse event (1 per 300 000 treated cases) with the recommended dosage (Schulz 2001).

Interactions of St. John's wort with other drugs are common. The extent of interactions depends on the hyperforin content (Mueller et al. 2006, 2009). When 42 volunteers were treated with 6 different St. John's wort preparations (daily hyperforin varied between 0.1 mg and 41 mg) for two weeks, midazolam $AUC_{0-\infty}$ decreased by 21%–79% (Mueller et al. 2006). The extent of midazolam AUC decrease correlated significantly with the increasing hyperforin dose, but not with the hypericin dose. Furthermore, the duration of the exposure is important. Administration of St. John's wort increased the exposure of voriconazole by 22% during the first 10 hours, but reduced the $AUC_{0-\infty}$ after the treatment for two weeks (Rengelshausen et al. 2005).

The main mechanism behind the interaction of St. John's wort in humans is the induction of intestinal and hepatic CYP3A, as well as intestinal P-gp (Dürr et al. 2000). Long-term administration of St. John's wort reduces the AUCs of many CYP3A substrates such as midazolam (Rengelshausen et al. 2005), cyclosporin (Bauer et al. 2003), alprazolam (Markowitz et al. 2003), omeprazole (Wang et al. 2004) and simvastatin (Sugimoto et al. 2001). It reduces the concentratios of P-gp substrates digoxin and fexofenadine (Dürr et al. 2000, Dresser et al. 2003). St. John's wort has also been shown to induce CYP2C19 by reducing the concentrations of voriconazole (Rengelshausen et al. 2005) and CYP2C19-dependent hydrozylation of omeprazole (Wang et al. 2004).

2.2.4 CYP3A inhibition

CYP inhibition is the most common cause of clinically significant DDIs (Lin and Liu 2001). It can increase the bioavailability of a parent drug with extensive first-pass metabolism, or lead to a decreased elimination of compounds with high systemic clearance resulting in increased concentrations or toxicity of the substrate. With prodrugs, the inhibition can result in diminished metabolism and decreased concentrations of active metabolite responsible for the clinical effects. The mechanism of CYP inhibition can be categorized as reversible inhibition, quasi-irreversible inhibition and irreversible inhibition (Lin and Lu 1998, Pelkonen et al. 2008). Of these, reversible inhibition is probably the most common mechanism responsible for the interaction. It can be further classified as competitive, non-competitive, uncompetive or mixed-type inhibition.

Reversible inhibition involves probably only the first step of the CYP catalytic cycle, where the substrate reversibly binds to the active site of the CYP enzyme (Figure 2). In competitive inhibition, substrate and inhibitor compete on the same binding site on the CYP enzyme, whereas in non-competitive inhibition the binding sites of substrate

and inhibitor are different. This binding occurs rapidly with weak bonds, which get broken easily without destroying the enzyme. In uncompetitive inhibition, the inhibitor binds to the enzyme-substrate complex without affecting the free enzyme entity. Mixed-type inhibition is frequently seen in practice, consisting of both competitive and non-competitive inhibition of CYP. The potency of inhibitors can be characterized by the inhibition constant K_i, which expresses the affinity of a compound to an enzyme and is determined *in vitro*. To achieve the inhibition of enzyme activity, the concentration of the inhibitor has to be higher than K_i. The most potent reversible inhibitors such as ketoconazole, itraconazole or ritonavir (Table 2), have K_i values below 1 μM (Lin and Lu 1998, Thummel and Wilkinson 1998, Pelkonen et al. 2008).

Quasi-irreversible inhibition involves the formation of noncovalent, but a tight bond between the metabolic intermediate and the prothetic haem of the CYP enzyme. This metabolic intermediate complex is slowly reversible *in vitro,* but stable *in vivo*, therefore named as quasi-irreversible. Compounds acting via the formation of a metabolic intermediate complex with CYP3A are erythromycin, clarithromycin or diltiazem (Lin and Lu 1998, Thummel and Wilkinson 1998, Pelkonen et al. 2008).

In irreversible mechanism-based or "suicide inhibition," metabolic activation is required for enzyme inactivation. This inactivation is time-, concentration- and NADPH-dependent. The CYP enzyme is inactivated by the formation of a tight binding between inhibitory metabolites and the heme and/or protein of CYP. These complexes are so stable, that biosynthesis of new enzymes is the only way to restore the function of CYP, which makes this type of inhibition long-lasting. Examples of mechanism-based CYP3A inhibitors are ethinylestradiol, gestodene and ritonavir (Lin and Lu 1998, Thummel and Wilkinson 1998, Pelkonen et al. 2008).

Voriconazole

Voriconazole has been in clinical use since 2002. It is the first second-generation wide-spectrum triazole antifungal agent, which has a chemical structure derived from fluconazole (Figure 7). The mechanism of action is the inhibition of the fungal CYP-dependent enzyme, lanosterol C-14 α-demethylase, which is involved in the conversion of lanosterol to ergosterol. Ergosterol is a crucial component of the fungal membrane and the loss of ergosterol may be responsible for the antifungal activity of voriconazole. Voriconazole is a drug of choice for the treatment of invasive aspergillosis (Herbrecht et al. 2002). It is also approved for the treatment of invasive candidemias in non-neutropenic patients, esophageal candidiasis and for less frequent fungal infections such as fusariosis and scedosporiosis (Ally et al. 2001, Kullberg et al. 2005).

The molecular weight of voriconazole is 349.3 and plasma protein binding is moderate at 58%. The oral bioavailability of voriconazole is estimated to be high, over 90%, and peak plasma concentrations are achieved in 1-2 hours after dosing (Purkins et al. 2003a). Voriconazole pharmacokinetics is nonlinear, which results in a dose-dependent AUCs, maximum concentrations and terminal elimination half-lives. The administration of

200 mg of voriconazole b.i.d. with food has been shown to decrease the bioavailability by approximately 22%, requiring dosing into an empty stomach (Purkins et al. 2003b). High, even 100-fold interindividual variation in the plasma concencentrations of voriconazole have been reported, necessitating therapeutic drug monitoring (Denning et al. 2002). An association between survival and voriconazole trough concentrations over 1000 ng/mL have been reported ensuing 88% response rates in adults with severe invasive mycosis (Pascual et al. 2008).

Voriconazole steady-state in adults is achieved on day 6, but after a recommended intravenous or oral loading dose plasma concentrations close to a steady-state are achieved within the first 24 hours after dosing. The volume of distribution at a steady state for voriconazole is estimated to be 4.6 l/kg, suggesting extensive distribution into tissues. Voriconazole has been detected in a wide range of tissues, such as the central nervous system and the lungs (Lutsar et al. 2003, Capitano et al. 2006).

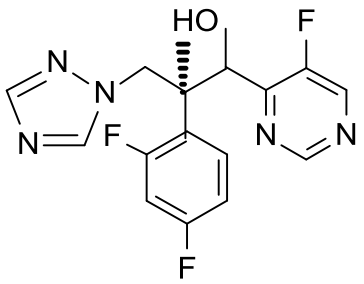

Figure 7. Structure of voriconazole.

Voriconazole is extensively metabolized in the liver by human hepatic CYP enzymes CYP2C19, and to a lesser extent by CYP2C9 and CYP3A, with only less than 2% of the dose excreted unchanged in urine (Denning et al. 2002, Hyland et al. 2003). The main route of metabolism is the N-oxidation to UK-121,265 by CYP2C19, which exhibits genetic polymorphism (Scholz et al. 2009).Voriconazole is a competitive inhibitor of CYP2B6 (K_i < 0.5 μM), CYP2C9 (K_i < 2.79 μM), CYP2C19 (K_i < 5.1 μM) and noncompetitive inhibitor of CYP3A (K_i < 2.97 μM) *in vitro*, but CYP2D6 is not affected (Niwa et al. 2005a, Niwa et al. 2005b, Jeong et al. 2009). *In vivo,* this inhibition has been detected in several drug-drug interaction studies conducted recently. The inhibition of CYP3A has increased the AUC of intravenous and oral midazolam by 3.8- and 10.3-fold, respectively (Saari et al. 2006b). Furthermore, the AUC of intravenous alfentanil and fentanyl have been shown to increase after oral voriconazole by 6- and 1.4-fold (Saari et al. 2006a, Saari et al. 2008). CYP2C19 has been involved in the interaction between voriconazole and, e.g. diazepam (Saari et al. 2007), and CYP2C9 between voriconazole and, e.g. varfarin, diclofenac, meloxicam and ibuprofen (Purkins et al. 2003c, Hynninen et al. 2006, Hynninen et al. 2007, Hynninen et al. 2009). Interaction between voriconazole and methadone is suggested to be due to CYP2B6, and propably CYP3A inhibition by voriconazole (Liu et al. 2007b).

The main adverse effects of voriconazole are visual disturbances, elevations in liver function tests and skin reactions. Approximately 30% of subjects receiving voriconazole have been reported to experience visual disturbances such as blurred vision, altered visual perception, altered color perception or photophopia (Ally et al. 2001, Herbrecht et al. 2002, Johnson and Kauffman 2003). Skin reactions are the second most common adverse effects and include mild erythema or photosensitivity, whereas few severe reactions such as Stevens-Johnson syndrome or toxic epidermal necrolysis have been reported (Johnson and Kauffman 2003). Increases in aspartate transaminase, alanine transaminase and alkaline phosphatase have been observed in approximately 10–20% of patients (Ally et al. 2001, Perfect et al. 2003), therefore the manufacturer recommends controlling these tests during the treatment.

Grapefruit juice

Grapefruit juice (GFJ, "Forbidden fruit", *Citrus Paradisi*) is believed to originate as far back as from 18th century India. It is a stabilized hybrid between pummelo (*Citrus Maxima*) and sweet orange (*Citrus Sinesis*), scientifically described first in Jamaica by the botanist James Macfadyen in 1837 (Kumamoto et al. 1987). The first suspicions about grapefruit juice–drug interactions were raised accidentally by Bailey et al. 1989, when they used grapefruit juice to mask ethanol taste in an ethanol-felodipine interaction study (Bailey et al. 1989). An increase in felodipine concentrations was detected when grapefruit juice-ethanol was ingested instead of a placebo concomitantly with felodipine. Thereafter, about 40 drugs have been reported to interact with grapefruit juice.

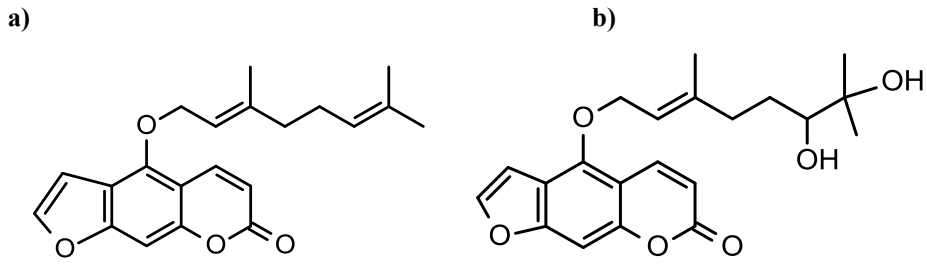

Figure 8. The chemical structure of the major active constituents of grapefruit juice: a) bergamottin and b) 6',7'-dihydrobergamottin.

Grapefruit juice is a mixture of several hundred ingredients with a wide variability between the products, and a lot depending on their genetic background, growing circumstances and fruit processing (Bailey et al. 2000, Ho et al. 2000, De Castro et al. 2006). It has been suggested to have health benefits for overweight, hyperlipidemia and cardiovascular diseases (Gorinstein et al. 2006, Dallas et al. 2008). Furanocoumarins (bergamottin, 6′,7'-dihydroxybergamottin), flavonoids (naringin, naringenin) and quercetin have been suggested to contribute to the interactions of grapefruit juice-drug *in vitro* (Ho et al. 2001, Paine et al. 2004). *In vivo*, furanocoumarins bergamottin and

6',7'-dihydroxybergamottin (Figure 8) might be involved in the interactions (Goosen et al. 2004, Kakar et al. 2004, Paine et al. 2008). However, the active components responsible for the *in vivo* interaction activity of grapefruit juice need further investigation.

The mechanism underlying the grapefruit juice interactions when consumed in usual volumes, is the mechanism-based and reversible inhibition of the intestinal, but not liver CYP3A4 enzyme (Ducharme et al. 1995, Lown et al. 1997, Lundahl et al. 1997). The concentrations of CYP3A4 in the small bowel epithelia have been shown to decrease significantly after grapefruit juice ingestion without a corresponding change in the CYP3A4 mRNA levels (Lown et al. 1997). Even one glass of grapefruit juice has been reported to achieve maximal inhibition of CYP3A4, which remains for over 24 h (Lundahl et al. 1995, Lundahl et al. 1998). However, there are findings suggesting that some ingredients of grapefruit juice might have an effect on liver metabolism and could increase the elimination half-life when the intake is repeated (Lown et al. 1997, Lilja et al. 2000a, Lilja et al. 2003, Lilja et al. 2005).

Grapefruit juice has been reported to interact with many CYP3A4 substrates. These interactions have been greatly variable between the substances ranging from 16-fold enhancement of simvastatin AUC to an 87% decrease in celiprolol AUC (Lilja et al. 1998b, Lilja et al. 2003). The reduction has been explained with the inhibition of the intestinal uptake transporter OATP. The most notable effects are found among CYP3A4 substrates with low oral bioavailability (Kantola et al. 1998, Lilja et al. 1998b, Kivistö et al. 1999). Interestingly, some CYP3A4 substrates with moderate bioavailability such as quinidine or clarithromycin (Min et al. 1996, Cheng et al. 1998) are lacking interactions with grapefruit juice. P-gp substrate digoxin with a rather high oral bioavailability of 60% to 70%, had no interactions with grapefruit juice (Becquemont et al. 2001).

Protease inhibitors

The introduction of protease inhibitors (PIs) and highly active antiretroviral therapy (HAART) have diminished the morbidity and mortality associated with acquired immunodeficiency syndrome (AIDS) (Palella et al. 1998). HIV belongs to the retroviruses and its genome codes for several precursor proteins such as gag and gag-pol, which require a posttranslational proteolytic cleavage mediated by HIV protease enzyme to yield mature, infectious virus proteins. HIV protease inhibitors prevent the function of this aspartic protease enzyme leading to the production of immature, non-functional virions (Kohl et al. 1988, Bugelski et al. 1994). Thus, the main antiviral action of PIs is to prevent the subsequent infection of susceptible cells, as it has no effect on cells in which viral DNA is already integrated.

Ritonavir

Ritonavir, formerly known as ABT-538, was approved by the US Food and Drug Administration (FDA) in 1996, and its structure is shown in Figure 9. It is a competitive HIV protease inhibitor with over 500-fold more selectivity in inhibition of HIV-1 and HIV-2 aspartic protease enzyme compared to human aspartic protease (Kempf et al.

1995, Hsu et al. 1997). It has a molecular weight of 720.9 and 98–99% of the drug is bound to plasma albumin and alfa1-acid glycoprotein. The elimination half-life of ritonavir is 3–5 h and the apparent clearance at a steady-state is low, approximately 7–11 L/h indicating a minimal hepatic first-pass metabolism and high oral bioavailability of the drug. Its oral bioavailability has been estimated to be high, although bioavailability studies with ritonavir have not been conducted in humans.

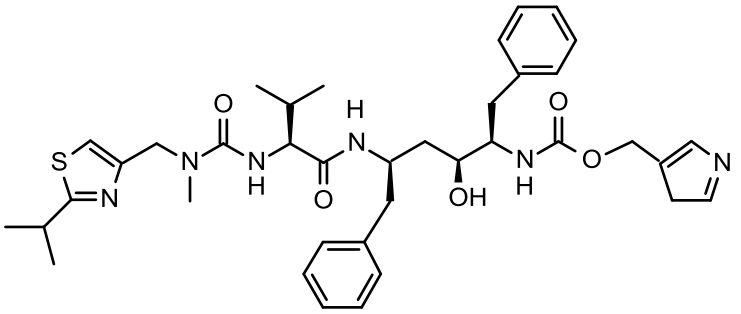

Figure 9. Chemical structure of ritonavir.

The recommended dose of ritonavir in adults is 600 mg twice daily with or without food. To avoid adverse effects, the doses need to be titrated during two weeks. After multiple-dose administration of ritonavir to HIV infected humans, through concentrations have decreased by 30–70% after 2 weeks administration, suggesting the probability of autoinduction. However, the AUCs remained unchanged (Hsu et al. 1997). *In vitro* studies have identified the CYP3A group as the major contributor to the *in vitro* biotransformation of ritonavir with a lesser contribution from CYP2D6 (Kumar et al. 1996). The elimination occurs primarily via hepatobiliary routes. After one 600 mg oral dose; over 80% of the dose is excreted in the feces during the next 6 days, approximately one third of the dose as the unchanged parent drug. Only approximately 11% of the dose is excreted into the urine (Denissen et al. 1997).

Ritonavir is a potent mechanism-based CYP3A inhibitor *in vitro,* with K_i values of less than 0.10 μM quantified using testosterone 6β–hydroxylation as a marker of CYP3A activity (Eagling et al. 1997, Granfors et al. 2006). *In vivo* a paradoxical time- and dose-dependent inhibitory/induction effect of CYP3A and P-gp has been observed. Short-term ritonavir has inhibitory effects on the metabolism of many CYP3A substrates, such as fentanyl (Olkkola et al. 1999), alprazolam (Greenblatt et al. 2000), clarithromycin (Ouellet et al. 1998a) and prednisolone (Penzak et al. 2005). After longer exposure, the extent of inhibition decreases, but net inhibition still remains (Kharasch et al. 2008a). Because ritonavir has a high affinity to the CYP3A, its metabolism is only minimally affected by other CYP inhibitors such as fluconazole, voriconazole and clarithromycin (Cato et al. 1998, Ouellet et al. 1998a, Liu et al. 2007a).

In addition, P-gp seems to be inhibited during ritonavir administration. This has been shown as an increase in digoxin and fexofenadine AUC (Ding et al. 2004, Kharasch et al. 2008a), although at a steady-state mild induction of P-gp was seen, diminishing the net inhibitory effect. Even a small dose of ritonavir has decreased the CYP2D6 activity moderately (Aarnoutse et al. 2005). Furthermore, induction of CYP2B6, CYP2C9, CYP2C19 and CYP2E1 have been observed producing decreased concentrations of methadone, voriconazole and ethinyl estradiol (Ouellet et al. 1998b, Liu et al. 2007a, Kharasch et al. 2008b)

Over 90% of patients treated with ritonavir have been reported with some adverse events (Markowitz et al. 1995). The most frequently complained adverse events among adults receiving ritonavir were diarrhoea (over 60%), nausea and headache (approximately 30%), altered taste sensations, vomiting, circumoral parestesia and asthenia (over 15%) or abdominal pain (10%). Asymptomatic elevations of triglycerides and hepatic transaminases were observed as well. Difficult dosing regimens, the development of resistance and adverse effects have diminished the use of ritonavir in therapeutical doses, but low doses are combined with to other PIs to enhance the oral bioavailability.

Lopinavir/ritonavir

Lopinavir/ritonavir is a coformulation of lopinavir and low-dose ritonavir and was approved by the FDA in 2000. It has been proved to be an effective component in the treatment of HIV among treatment-naive or -experienced patients (Cohen et al. 2005, Murphy et al. 2008). Lopinavir (Figure 10) is a ritonavir analogue where the valine residue at position 82, which is sensitive to mutations, is replaced with the cyclic urea producing a high genetic barrier against resistance (Kempf et al. 1995).

The absolute bioavailability of lopinavir coformulated with ritonavir in humans has not been established. In animal studies, the oral bioavailability of lopinavir has been reported to be as low as 25% (Sham et al. 1998). The coformulation of lopinavir and ritonavir is needed to enhance the oral bioavailability of lopinavir by exploiting the favourable CYP3A inhibition. In healthy volunteers, the combination of a single dose of 400 mg lopinavir and 50 mg ritonavir has been reported to increase the AUC_{0-24} of lopinavir by 77-fold. This results in elevated mean lopinavir concentrations exceeding the EC_{50} for over 24 h (Sham et al. 1998). Ritonavir peak concentrations have remained less than 7% below the levels observed after the administration of therapeutic doses in single-PI regimen indicating lopinavir to be responsible for the clinical efficacy of lopinavir/ritonavir (Sham et al. 1998, Cvetkovic and Goa 2003). Fatty food has been reported to increase the oral bioavailability of lopinavir/ritonavir solution and capsules, but has an insignificant effect on the absorption of tablet formulas (Klein et al. 2007).

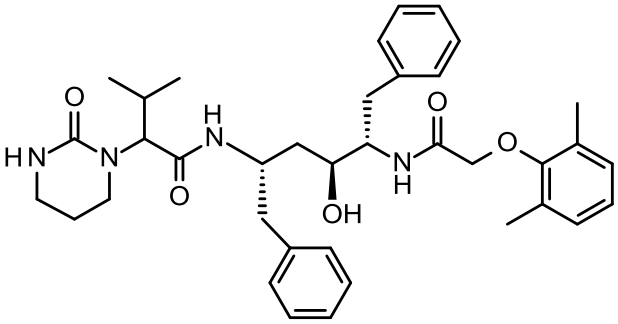

Figure 10. The chemical structure of lopinavir.

The molecular weight of lopinavir is 628.80 and at a steady-state, 98.2–99.2% of lopinavir is bound to plasma proteins (Hurst and Faulds 2000, Cvetkovic and Goa 2003). After the coadministration of 400 mg of lopinavir with 100 mg of ritonavir twice daily, the C_{max} of lopinavir has been 9.8 mg/L, the t_{max} 4.1–5.8 h and the trough plasma concentration 5.5–7.1 mg/L. The EC_{50} of lopinavir is approximately 10-fold lower than that of ritonavir. Lopinavir undergoes primarily oxidative metabolism by hepatic CYP3A, and at least 13 oxidative metabolites have been identified in humans. The reported mean apparent elimination half-life at steady state in naive and treatment-experienced patients has been 4.1–5.8 h, and apparent clearance 6.0–7.1 L/h. 80% of lopinavir/ritonavir is excreted via fecal routes and over 10% into urine (Hurst and Faulds 2000, Cvetkovic and Goa 2003).

The clinically most important drug-drug interactions associated with the use of lopinavir/ritonavir originate from their inhibitory effect on CYP3A. This inhibition results in the decreased elimination and elevated concentrations of CYP3A substrates such as midazolam, cyclosporine A or irinotecan (Vogel et al. 2004, Yeh et al. 2006, Corona et al. 2008). Lopinavir/ritonavir has also been suggested to inhibit P-gp and CYP2D6 (Aarnoutse et al. 2005, van Heeswijk et al. 2006, Wyen et al. 2008). These changes are mainly attributed to the low-dose ritonavir only, but *in vitro* studies suggest that also lopinavir is a reversible inhibitor of CYP3A with K_i 1.0 μM and might be a more potent inhibitor of P-gp than ritonavir (Weemhoff et al. 2003, Storch et al. 2007, Zhou et al. 2008). Furthermore, *in vitro* studies showed a weak or negligible inhibition of human CYP1A2, 2B6, 2C9, 2C19 and 2D6 (Weemhoff et al. 2003). However, in human studies, lopinavir/ritonavir has been reported to induce its own metabolism and biotransformation of some drugs metabolized by CYP2B6, CYP2C9, CYP2C19 and CYP1A2, or by UDP-glucuronosyltransferase enzymes resulting in decreased substrate concentrations (van der Lee et al. 2006, Yeh et al. 2006, Hogeland et al. 2007).

The most frequent adverse reactions reported by over 2% of adult antiretroviral-naive patients after lopinavir/ritonavir 400/100 mg b.i.d. are diarrhoea, nausea and vomiting according to the Summary of Product Characteristics. Furthermore, increased incidence of hypertriglyceridaemia and hypercholesterolemia has been associated with the antiretroviral treatment with lopinavir/ritonavir (Cvetkovic and Goa 2003, Calza et al. 2004).

2.3 Interactions of oxycodone

Although oxycodone has been in clinical use for over 90 years, some features of its metabolism have not been described until recently. Because of CYP-mediated metabolism, oxycodone is prone to drug-drug interactions (Table 3).

In a case report, a patient with chronic pain using fluoxetine and acetaminophen/oxycodone medication had insufficient analgesia in spite of increasing opioid doses (Otton et al. 1993). The authors suggested, that the inhibition of CYP2D6-mediated metabolic route of oxycodone to its active metabolite oxymorphone could explain this lack of efficacy. In an RCT in healthy volunteers, the inhibition of CYP2D6 by quinidine almost totally blocked the formation of oxymorphone, but did not affect the psychomotor or subjective drug effects (Heiskanen et al. 1998). In chronic pain patients using controlled-release oxycodone, the administration of paroxetine 20 mg/day for one week increased oxycodone concentrations and decreased oxymorphone exposure, but did not alter the analgesic effect of oxycodone (Lemberg et al. 2010). Grönlund et al. investigated healthy volunteers receiving pre-treatment with a placebo, paroxetine alone or paroxetine in combination with itraconazole (Grönlund et al. 2010b). Although paroxetine decreased the exposure to oxymorphone by 44%, no significant changes in the AUC of oxycodone was observed, suggesting the negligible clinical importance of CYP2D6 inhibition. After the inhibition of both CYP3A and CYP2D6 routes by itraconaxole and paroxetine, the exposure to oxycodone increased by 3.0-fold. In another recent study with quinidine and ketoconazole, the high interindividual variability in the activity of CYP2D6 and CYP3A was linked to the genetic polymorphisms and DDIs (Samer et al. 2010b). Clear reduction in the formation of CYP2D6 dependent metabolites oxymorphone and noroxymorphone was detected after quinidine and in the PM genotype.

Lee et al. reported a case study in a patient with osteomyelitis and severe pain (Lee et al. 2006). The infection was treated with rifampicin, and the pain medication consisted of oxycodone and adjuvant pain medication without sufficient pain relief. As the urine screen for opioids was negative, the authors suggested, that oxycodone metabolism was induced by rifampicin. Telithromycin decreased the apparent oral clearance of oxycodone and increased its concentrations, but did not hinder but increased the formation of oxymorphone as the CYP3A inhibition shifted the metabolism of oxycodone towards the CYP2D6-mediated metabolic route (Grönlund et al. 2010a). The inhibition of CYP3A mediated metabolism of intravenous and oral oxycodone was studied in healthy volunteers (Saari et al. 2010). Itraconazole increased the exposure to intravenous oxycodone by 51% and oral oxycodone by 144% and decreased the formation of noroxycodone. Furthermore, the formation of oxymorphone increased specifically after oral oxycodone. Itraconazole increased the oral bioavailability of oxycodone from 55% to 82%.

Table 3. RCTs involving the drug-drug interactions between oxycodone and CYP inhibitors

Interacting drug	N	Enzyme inhibited	Change in the AUC of OXY	Change in the AUC of OXM	Ref.
Quinidine	10	CYP2D6	NS	> 90% ↓	Heiskanen et al. 1998
Telithromycin	11	CYP3A and CYP2D6	80% ↑	91% ↑	Grönlund et al. 2010a
Paroxetine	20	CYP2D6	20% ↑	67% ↓	Lemberg et al. 2010
Itraconazole	12	CYP3A	iv: 51% ↑ po: 144% ↑	iv: 159% ↑ po: 359% ↑	Saari et al. 2010
Quinidine or Ketoconazole or both	10	CYP2D6 or CYP3A or CYP3A + CYP2D6	50% ↑ 80% ↑ 200% ↑	40% ↓ 250% ↑ NS	Samer et al. 2010b
Paroxetine or Itraconazole + Paroxetine	11	CYP2D6 CYP3A + CYP2D6	NS 188% ↑	44% ↓ 22% ↑	Grönlund et al. 2010b

RCT = randomized, controlled trial; N = number of participants; OXY = oxycodone; OXM = oxymorphone; ↑ = increase; ↓ = decrease; NS = not significant change; iv = intravenous administration of oxycodone; po = oral administration of oxycodone

Tramadol and oxycodone are substrates of CYP2D6 and CYP3A and may thus compete for these metabolic routes, but a single dose tramadol did not affect the clearance of oral oxycodone (Curry et al. 2007). Also amitriptyline has been studied in combination with oxycodone, but 10–50 mg doses did not affect the pharmacokinetics of oxycodone (Pöyhiä et al. 1991). Grant et al. studied the effects of 5 mg oral oxycodone doses given four times at intervals of four hours, on the pharmacokinetics of fluoroquinolones gatifloxacin and levofloxacin. No changes in AUC or C_{max} of gatifloxacin or levofloxacin were observed (Grant et al. 2001, Grant et al. 2002).

The pharmacodynamic interactions of oxycodone have been described in case reports. Dangerous or even fatal toxic interactions have been described during concomitant use of oxycodone and alcohol or benzodiazepines (Cone et al. 2003). A case report presented a possible interaction between repeated doses of controlled-release oxycodone and increasing doses of carisoprodol (Reeves and Mack 2003). Carisoprodol is a skeletal muscle relaxant, which is metabolized to meprobamate and has the potential for respiratory depression, abuse and dependency. Toxic symptoms were reversed by intravenous naloxone and the patient recovered completely. Oxycodone is not considered as an inhibitor serotonin reuptake, but a few cases with serotonin syndrome symptoms have been reported after concomitant use of oxycodone and antidepressants such as fluvoxamine or sertraline (Rosebraugh et al. 2001, Karunatilake and Buckley 2006).

3. AIMS OF THE STUDY

The general objective of these studies was to investigate the interactions of a widely used analgesic oxycodone with medicinal, herbal and nutritional products which modulate the activity of CYP3A enzymes.

The specific aims of these studies were:

1. To investigate the effect of the CYP3A inducers rifampicin and St. John's wort on the pharmacokinetics and pharmacodynamics of oral (rifampicin and St. John's wort) and intravenous (rifampicin) oxycodone (Studies I and III).

2. To determine the oral bioavailability of oxycodone, and to evaluate the influence of rifampicin on the bioavailability of oxycodone (Study I).

3. To investigate the effects of the CYP3A inhibitors voriconazole, grapefruit juice, and ritonavir or lopinavir/ritonavir on the pharmacokinetics and pharmacodynamics of oral oxycodone (Studies II, IV and V).

4. MATERIALS AND METHODS

4.1 Subjects

Altogether 55 healthy volunteers were enrolled in Studies I–V. One of the volunteers participated in Studies II and IV, two in Studies III and V and one in Studies II, IV and V. The demographic details are shown in Table 4.

12 healthy, non-smoking volunteers, with no medication including hormonal contraception were enrolled in each study via internet advertisements directed to university students. After reading the announcement, subjects interested in the study contacted the investigator by phone or e-mail and received more information about the study. If they still were willing to participate in the study, they were invited to a personal visit.

Table 4. Characteristics of volunteers (mean (range)) in Studies I-V.

	Sex F/M	Age years (range)	Weight kg (range)	BMI kg/m^2 (range)	EM	PM	IM	UM
I	7/5	23 (20–31)	72 (53–110)	23 (19–32)	11	1		
II	6/6	22 (19–25)	70 (50–95)	23 (18–30)	11			1
III	6/6	23 (19–35)	70 (53–86)	23 (19–27)	10	1		1
IV	5/7	23 (18–27)	65 (47–76)	22 (19–25)	10		1	1
V	4/8	23 (18–26)	69 (52–84)	23 (19–27)	9		1	2

F = female; M = male; BMI = body mass index; EM = extensive metabolizer; PM = poor metabolizer; IM = intermediate metabolizer; UM = ultrarapid metabolizer

During the preliminary visit, volunteers were informed about the study protocol in detail, and they were ascertained to be in good physical health through medical history and clinical examination including blood pressure in a sitting position. The risk of participants to develop opioid addiction was estimated low as assessed by the Abuse Questions (Table 5, Michna et al. 2004).

All subjects gave their written informed consent. A 12-lead electrocardiogram and routine laboratory tests including complete blood count (hemoglobin, hematocrit, differential white blood cell count and platelet count), serum aspartate aminotransferase, alanine transferase, alkaline phosphatase, blood urea nitrogen and creatinine were in normal limits. Urine tests for glucose, proteins, drugs with addiction potential (amphetamine, cannabis, cocaine, opioids, phencyclidine, methadone, dextropropoxifen and benzodiazepines), and a pregnancy test for women were negative. Participants were instructed to avoid all products with known effects on CYP enzyme activity, including grapefruit juice 4 weeks prior to and during the study. The subjects were trained to do the tests.

Table 5. Abuse Questions by Michna 2004 (Michna et al. 2004).

1. Is there a history of alcohol or substance abuse in your family, even among your grandparents, aunts, or uncles?
2. Have you ever had a problem with drugs or alcohol or attended an Alcoholics Anonymous or Narcotics Anonymous meeting?
3. Have you ever had any legal problems or been charged with driving while intoxicated or driving under the influence?

4.2 Study design

All studies were performed in the Institute of Clinical Medicine, Department of Anaesthesiology, Intensive Care, Emergency Care and Pain Medicine, University of Turku and Turku University Hospital, Turku, Finland and the Institute of Biomedicine; Department of Pharmacology, Drug Development and Therapeutics, University of Turku, Turku, Finland.

The data was collected between March 2007 and April 2009. All studies were of randomized, balanced, placebo-controlled, cross-over design. In Study I, a four-session, paired design was used, whereas Studies II–IV consisted of two and Study III of three phases. In all studies, there was a four week drug free period between the phases.

Pre-treatments of Studies I–V

The volunteers were instructed to take the pre-treatment drugs at home according to the protocols. Compliance was followed with mobile phone text messages sent by the volunteers after taking each dose. If the investigator was not contacted in due time by the volunteer, she reminded the subject to take the dose with a text message. Voriconazole concentrations in Study II and ritonavir and lopinavir concentrations in Study V were determined. The dosing schedule of the pre-treatments is shown in Table 6.

In Study I, rifampicin (Rimapen® 600 mg, Orion, Finland) or placebo (Capsugel®, Oriola, Finland) was administered on an empty stomach for 7 days. In Study II, voriconazole tablets (Vfend® 200 mg, Pfizer, Sandwich, UK) or placebo capsules were ingested on an empty stomach. The pre-treatment used in Study III was St. John's wort 300 mg tablets (LI 160, Jarsin® 300mg, Klosterfrau, Berlin, Germany; dry extract of St. John's wort 3–6:1, extraction solvent methanol 80%, hyperforin range 2–6%) or placebo t.i.d. In Study III, pre-treatment was instructed to be taken with a meal, as advised by the manufacturer.

Table 6. Design of Studies I-V.

Study	Pre-treatment		Oxycodone hydrochloride
	CYP3A inductor or inhibitor	***Dosing schedule***	***Dosing schedule***
I	**A) Intravenous part** **Phase 1** Rifampicin 600 mg tabl **Phase 2** Placebo caps **B) Oral part** **Phase 3** Rifampicin 600 mg tabl **Phase 4** Placebo caps	1 tabl/caps at 8 P.M. for 7 days 1 tabl/caps at 8 P.M. for 7 days	0.1 mg/kg IV at 8 A.M. on day 6 15 mg P.O. with 150 ml warm water at 8 A.M. on day 6
II	**Phase 1** Voriconazole 200 mg tabl **Phase 2** Placebo caps	2 tabl/caps at 7 P.M. on day 1, 2 tabl/caps at 7 A.M. and 1 tabl/caps at 7 P.M. on day 2 1 tabl/caps at 7 A.M. and 1 tabl/caps at 7 P.M. on days 3–4.	10 mg P.O. with 150 ml warm water at 8 A.M. on day 3
III	**Phase 1** St. John's wort 300 mg tabl **Phase 2** Placebo caps	1 tabl/caps at 9 A.M., 4 P.M., 10 P.M. for 15 days	15 mg tabl with 150 ml warm water at 8 A.M. on day 14
IV	**Phase 1** Dose: grapefruit juice 200 ml **Phase 2** Dose: water 200 ml	1 dose at 7 A.M., 1 P.M., 7 P.M. for 5 days	10 mg P.O. with 150 ml of grapefruit juice (Phase 1) or water (Phase 2) at 8 A.M. on day 4
V	**Phase 1** Dose: 3 ritonavir 100 mg caps + 1 placebo caps **Phase 2** Dose: 2 lopinavir/ritonavir 200/50 mg tabl + 2 placebo caps **Phase 3** Dose: 4 placebo caps	1 dose at 7 A.M. and at 7 P.M. for 4 days	10 mg P.O. with 150 ml warm water at 8 A.M. on day 3

In Study IV, the subjects ingested either 200 ml of grapefruit juice (Valio Ltd, Helsinki, Finland) or water t.i.d. Pre-treatment in Study V was administered as 4 capsules or tablets b.i.d. The components of each dose were packed into sealed opaque coded envelopes containing either 2 lopinavir/ritonavir 200/50 mg tablets (Kaletra®, Abbott Laboratories Ltd, Queenborough, UK), and 2 placebo capsules, or 3 ritonavir 100 mg capsules (Norvir®, Abbott Laboratories Ltd, Queenborough, UK) and 1 placebo capsule, or 4 placebo capsules.

Oxycodone dosing

The study days of oxycodone dosing were spent in the study facility located in the Department of Pharmacology, Drug Development and Therapeutics, from 6.30 A.M. to 8.30 P.M. The subjects returned to the study facility the next two mornings to give samples at 24 h and 48 h for the determination of oxycodone, noroxycodone, oxymorphone and noroxymorphone concentrations.

The subjects fasted for 8 hours before the administration of oxycodone, and were given a standard meal 4 hours and a light meal 8 hours after the intake of oxycodone. Drinking of water or raspberry juice was allowed only during the meals. No cola drinks, alcohol, coffee, tea, or grapefruit juice was allowed during the test days except for grapefruit pre-treatment in Study IV.

Oxycodone hydrochloride (Oxynorm®, Mundipharma, Bard Pharmaceuticals Ltd, Cambridge, UK) 0.1 mg/kg (free base 0.0894 mg/kg) was administered intravenously as a 2-minute manual injection in the first part of the Study I. In the second part, a dose of oral oxycodone hydrochloride, 15 mg containing free base 13.41 mg (Oxynorm® 5 mg and 10 mg capsules, Mundipharma, Bard Pharmaceuticals Ltd, Cambridge, UK), was used. The dose of oral oxycodone hydrochloride was 10 mg (free base 8.94 mg) in Studies II, IV and V and 15 mg (free base 13.41 mg) in Study III. The dosing schedule of oxycodone is introduced in Table 6.

Pharmacokinetic and pharmacodynamic assessments

Blood samples were collected for 48 hours to enable the pharmacokinetic calculations of oxycodone and its metabolites noroxycodone, oxymorphone and noroxymorphone. Behavioral and experimental pain tests were performed before and 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours after oxycodone administration. Behavioral measurements consisted of recording subjective drug effects with visual analogue scales, the Maddox Wing Test (MWT), Cogan's pupillometer and digit symbol substitution test (DSST). The cutaneous heat-pain threshold and tactile sensitivity were assessed in Studies I and II, and the cold pain test was used in all studies. The order of the tests performed was standardized as follows: subjective scales, pupil size, MWT, DSST, tactile sensitivity (Study I and II), heat pain threshold (Study I and II) and the cold pressure test. Blood samples were collected before experimental pain tests.

4.3 Blood sampling, determination of plasma drug concentrations and genotyping

Blood sampling

In the beginning of each study day, a forearm vein was cannulated for the venous blood sampling. In the first part of Study I, another cannula was inserted into the opposite side for oxycodone dosing. Timed blood samples (10 ml in each) were drawn into

ethylenediaminetetraacetic acid tubes (EDTA; BD Vacutainer®) before and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, and 48 h after oxycodone administration for the analysis of oxycodone and its metabolites. An additional sample was collected 0.25 h after the intravenous administration of oxycodone in Study I. A sample before each oxycodone dosing was drawn to enable the determination of possible pre-treatment concentrations. Two EDTA samples (6 ml in each) were taken in the beginning of each study for the genotyping analysis. A total of 632 ml of blood was collected from each volunteer in Study I; 312 ml in Studies II–IV and 462 ml in Study V.

The blood samples for determining concentrations of oxycodone and its metabolites were centrifuged for 10 minutes at room temperature at 2100 g within 30 minutes of sampling. Plasma was separated into two PP-tubes (Sarstedt®). The plasma samples were stored at –70 °C until the determination and the whole blood samples for genotyping at –30 °C.

Determination of oxycodone, noroxycodone, oxymorphone and noroxymorphone

The plasma drug concentrations were analyzed in the Department of Clinical Pharmacology, at the University of Helsinki, Helsinki, Finland.

Analysis was performed with a validated sensitive and specific liquid chromatography–tandem mass spectrometric method (LC–MS/MS) (Neuvonen and Neuvonen 2008). Following solid-phase extraction, the analytes were separated on a reverse-phase column by gradient elution. The mobile phase consisted of 5 mM ammonium format at pH 9.4, adjusted with 25% ammonium hydroxide solution and methanol. Total chromatographic run time was 25 minutes. The detection and quantification of the components was performed in a tandem mass spectrometry according to the molecular weight. The lower limits of quantification (LLQ) were 0.1 ng/mL for oxycodone and oxymorphone and 0.25 ng/mL for noroxycodone and noroxymorphone. The interday coefficients of variation were less than 15% for all analytes at representative plasma concentrations.

Voriconazole

After solid phase extraction, the plasma concentrations of voriconazole were determined by high performance liquid chromatography (HPLC), as described earlier (Gage and Stopher 1998, Pennick et al. 2003). Fluconazole analogue (UK 54373) was used as the internal standard. The lower limit of quantification (LLQ) for voriconazole was 20 ng/ml. The interday coefficients of variation (CV) were 9.7% at 1,031 ng/ml (n=6) and 1.6% at 9,654 ng/ml (n=6).

Ritonavir and lopinavir/ritonavir

Plasma ritonavir and lopinavir analysis was performed with a validated, sensitive and specific liquid chromatography–mass spectrometric method (LC–MS), as described recently using saquinavir as the internal standard (Gage and Stopher 1998, Pennick et al. 2003, Rezk et al. 2009). After solid phase extraction, the analytes were differentiated by the HPLC method. The detection and quantification of the components was performed in the mass spectrometry. LLQ for ritonavir was 0.1 μg/ml and for lopinavir 0.4 μg/ml.

The interday CV was less than 5.4% for ritonavir and less than 7.0% (n=3) for lopinavir at representative concentrations.

Genotyping

The *CYP2D6* genotypes were determined in the Laboratory of Molecular Genetics, HUSLAB, Helsinki University Central Hospital, Helsinki, Finland.

Genotyping was performed using a 2-step multiplex primer extension method, which allows the detection of 11 of the most relevant polymorphic positions, the assessment of whole-gene deletion and duplication, and the allele composition of gene duplication (Sistonen et al. 2005).

4.4 Pharmacokinetic analysis

The pharmacokinetic data were analysed by the use of the pharmacokinetic program WinNonlin (version 4.1, Pharsight Corporation, Mountain View, CA, US). The peak plasma concentrations (C_{max}) of oxycodone and its metabolites and corresponding times to peak concentrations (t_{max}) were derived directly from the data. For each subject, the terminal log-linear phase of the oxycodone, noroxycodone, oxymorphone or noroxymorphone plasma concentration-time curve was identified visually, and the elimination rate constant (k_e) was determined by regression analysis. The elimination half-life ($t_{½}$) was then calculated from the equation: $t_{½} = \ln 2 / k_e$.

The area under concentration–time curves of oxycodone and its metabolites ($AUC_{0-\infty}$) were calculated using the trapezoidal method. The extrapolation of the AUC to the infinity was done using the k_e value. The linear trapezoidal rule was used when the successive concentration values were increasing and the logarithmic trapezoidal rule when the concentration values were descending after the peak concentration value. Individual metabolite-to-parent drug area under plasma concentration–time curve (AUC) ratios (AUC_m/AUC_p) for the oxycodone metabolites were determined to describe the activity of the various metabolic routes. After intravenous administration of oxycodone, plasma clearance (CL), and the steady state volume of distribution (V_{ss}) of oxycodone was calculated using noncompartmental methods based on the statistical moment theory. The oral bioavailability (F) of oxycodone was calculated as follows: $F = [AUC_{0-\infty\ oral} \cdot Dose_{intravenous}]/[AUC_{0-\infty\ intravenous} \cdot Dose_{oral}]$. After oral administration of oxycodone, the apparent clearance (CL/F) and the apparent volume of distribution of oxycodone during elimination (V_z/F) were calculated.

The dose adjusted $AUC_{0-\infty\ control}$ and the dose adjusted ratios ($AUC_{0-\infty\ treatment}/AUC_{0-\infty\ control}$) of oxycodone were calculated to compare the effect of gender on the $AUC_{0-\infty}$ of oral oxycodone and on the difference between the treatment effects on AUC in Studies I-V.

4.5 Assessment of experimental pain

Tactile sensitivity

Tactile sensitivity was examined to estimate the effects of oxycodone on tactile stimuli mediated by Aβ-fibres (Park et al. 2001). It was assessed by using a set of five von Frey hairs (Semmes-Weinstein Aesthesio meter kit, Stoelting Co., Wood Dale, IL, US). Each hair was presented 8 times in randomized order. The hairs produced forces of 0.23, 0.27, 1.63, 4.0 and 11.8 mN. Each hair and placebo (= no hair) was applied to the distal phalanx of the index finger at an interval of approximately five seconds. The place differed slightly. The subjects were lying comfortably in the bed, eyes blind-folded during the examination. They were requested to tell if they felt the stimulus (rating 2), did not feel it (rating 0) or were unsure (rating 1). Ratings 1 and 2 were combined. The tactile threshold was profiled from the psychometric curve as the 50% detection rate of stimuli (Martikainen et al. 2005). The change in the tactile threshold before and two hours after dosing of oxycodone was calculated.

Cutaneous heat-pain threshold

The cutaneous heat pain threshold was determined to describe the effects of oxycodone on contact heat pain stimuli. A contact heat pain stimulus with a feedback-controlled computer-driven contact termode (TSA-2, Medoc Inc., Rehovot, Israel) was used. This device has been widely used in clinical and experimental settings (Koltzenburg et al. 2006, Schaffner et al. 2008, Scherens et al. 2009). A 30x30 mm thermode was placed on the right forearm. Three measurements were done at 30 s intervals and the testing site on the skin differed slightly to avoid sensitization. The thermode began to warm up slowly 1 °C/s from 32 °C until the volunteer pressed a button as a sign of the first feeling of pain. This temperature was registered in °C, after which the temperature returned back to the baseline. A security limit was set at 50 °C, which was not exceeded in the experiments. The mean value of three repeated measurements was used in the analysis. The difference between the average values before and one hour after the dosing of oxycodone was calculated.

Cold pain sensitivity

A cold pressor test was performed to estimate the cold pain threshold, the cold pain intensity and cold pain unpleasantness (De Jalon et al. 1985, Posner et al. 1985, Jones et al. 1988, Koltzenburg et al. 2006). The subjects immersed their left hand into ice-cold water (0.5–2 °C) up to the wrist. The volunteers kept their hand immobile and reported when the cold sensation became painful. This time point was recorded as the cold pain threshold in seconds. The cold pain intensity (CPI) (Studies I–V) and unpleasantness (CPU) (Study I) were separately assessed at 30 seconds (CPI_{30}, CPU_{30}) and 60 seconds (CPI_{60}, CPU_{60}) following immersion with a numerical scale of 0–100 (0 = no pain or unpleasantness, 100 = the maximal imaginable pain or unpleasantness). $AUEC_{0\text{-}12}$ (area under the effect curve from zero to 12 hours) was determined using the linear trapezoidal rule.

4.6 Other pharmacodynamic measurements

Behavioral measurements were carried out in Studies I–V. For each variable, the $AUEC_{0\text{-}12}$ was determined by the trapezoidal rule.

Subjective effects

Subjective effects were evaluated with the 100 mm visual analogue scale (Bond and Lader 1974) on the following items: alert/drowsy, good/poor performance, no/strong drug effect and unpleasant /pleasant feeling. In addition, item no/extreme nausea or vomiting was recorded in Studies II–V.

Digit symbol substitution test

The DSST was used to determine the psychomotor function of the volunteers (Stone 1984). In DSST, the subjects substituted simple digits with symbols using a pencil and paper. A model of nine digits with corresponding symbols was presented at the top of the paper. A total of 300 digits (9 different) were arranged in 12 rows on each paper in a randomized order. The number of digits correctly substituted in 3 minutes was recorded. The order of the symbols was changed at each testing time point to prevent the effects of learning.

Maddox Wing Test

The MWT was used to measure co-ordination of the extraocular muscles (Hannington-Kiff 1970). When subjects looked into the Maddox Wing apparatus, oblique and vertical wings divided their visions. The right eye saw an arrow and the left eye a horizontal numbered scale. Divergence of the eyes resulted in an image like the arrow would move. The number, which the arrow seemed to point at when stopping, indicated diopters and this was registered.

Cogan's pupillometer

Cogan's pupillometry was used to measure pupil size as a measure of opioid effect (Cogan 1941). In stable light conditions, subjects looked through a black, plastic sheet (Cogan's pupillometry) with one eye. There was a column of small hole-pairs at increasing distances of 0.5 mm from each other on the pupillometer. The volunteers looked through the holes and found the first hole-pair where a separate view of each hole could be seen. This distance of hole-pairs in millimetres represented pupil size.

4.7 Statistical analysis

Based on previous studies (Pöyhiä et al. 1991, Pöyhiä et al. 1992, Grönlund et al. 2010a, Saari et al. 2010), it was estimated that 10 subjects would be required to detect a 30% difference in the $AUC_{0\text{-}\infty}$ of oxycodone at a power of 80% and level of significance $P < 0.05$. Enrolling 12 subjects allowed for about a 20% dropout rate. Balanced Latin-

square randomization was used. The Pharmacy of Turku University Hospital supplied the pre-treatment drugs and placebos in coded envelopes to be delivered to volunteers.

The results were expressed as mean ± SD, except for t_{max}, where median values with minimum and maximum were reported. Figures were expressed as mean values ± SEM in Studies II–V and as mean values ± SD in Study I. Data was evaluated for normality of distribution using probit plots and the Shapiro-Wilk's W-test. Nonnormal data were log transformed for analysis but reported as nontransformed results (arithmetic mean ± SD). The paired Student t-test was used to compare $AUC_{0-\infty}$ and $AUEC_{0-12}$ values between pre-treatment and placebo phases in Studies I-IV. Values for heat pain threshold and tactile sensitivity measured before and after oxycodone administration in Studies I and II were compared with analysis of variance for repeated measures (ANOVA). In Study V, ANOVA was used and *a posteriori* testing was performed using Tukey's test. The values were regarded significant, if $P < 0.05$. T_{max} was analysed with the Wilcoxon signed rank test. Ninety-five per cent confidence intervals (95% CIs) for the differences between the pre-treatment and placebo phases were calculated.

Because pharmacokinetic drug interactions can be assessed statistically by the same methods that are standard for the investigation of bioequivalence, geometric mean ratios with 90% CIs for the parameter $AUC_{0-\infty}$ of oxycodone were also calculated. Bioequivalence (i.e., the lack of an interaction) was concluded, if the 90% CI of the geometric mean ratios for oxycodone $AUC_{0-\infty}$ was within the acceptable limit of 0.8 to 1.25 (Van Peer 2010). In Study V, geometric mean ratios were calculated for all pharmacokinetic parameters. Correlation analysis with the Pearson's correlation coefficient was used to study the possible association between oxycodone plasma concentrations and effects. The statistical analyses were carried out using the statistical program SYSTAT for Windows (version 10.2; Systat software, Richmond, CA, US).

5. RESULTS

5.1 Pharmacokinetic results

The mean changes in the pharmacokinetic parameters of oxycodone during pre-treatment with rifampicin, voriconazole, St. John's wort, grapefruit juice, ritonavir or lopinavir/ritonavir with 95% CI are shown in percentages in Figures 11–13.

Effects of CYP3A inducers (I, III)

The CYP3A inducers rifampicin and St. John's wort decreased the plasma concentrations of intravenous (rifampicin) and oral (rifampicin and St. John's wort) oxycodone and increased the formation of CYP3A-dependent metabolites noroxycodone and noroxymorphone. Metabolism via the CYP2D6-mediated route was decreased and the formation of oxymorphone reduced during the rifampicin and St. John's wort phases when compared to the control.

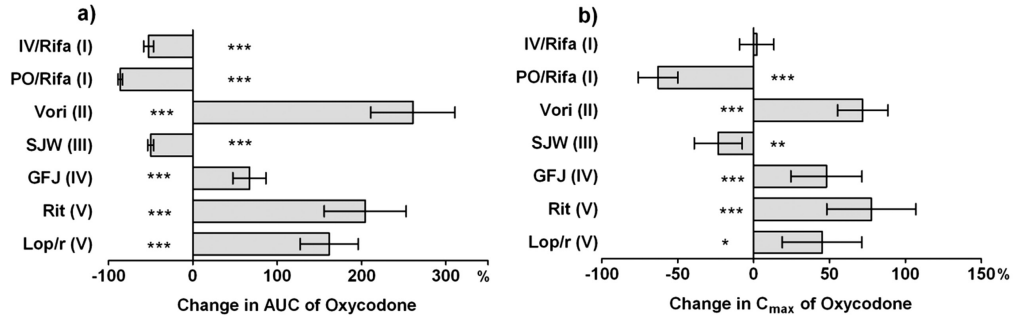

Figure 11. Change in the mean $AUC_{0-\infty}$ (a) and the mean C_{max} (b) of oxycodone with 95% CI during pre-treatment with rifampicin (Rifa), voriconazole (Vori), St. John's wort (SJW), grapefruit juice (GFJ), ritonavir (Rit) or lopinavir/ritonavir (Lop/r) when compared to the controls.

IV = intravenous administration of oxycodone and PO = oral administration of oxycodone. Studies are denoted by Roman numerals in parentheses. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Rifampicin decreased the mean $AUC_{0-\infty}$ of intravenous oxycodone by 53% ($P < 0.001$) and the mean $AUC_{0-\infty}$ of oral oxycodone by 86% ($P < 0.001$) when compared to the control. The mean C_{max} of oxycodone diminished by 63% from 26.1 ± 11.0 ng/ml to 8.3 ± 2.2 ($P < 0.05$) after oral oxycodone, but was not changed after intravenous administration. Rifampicin reduced the oral bioavailability of oxycodone from 69% to 21% (P< 0.001). The mean elimination $t_{½}$ decreased by 34%, from 3.7 h to 2.4 h ($P < 0.001$) after intravenous oxycodone and rifampicin and by 35%, from 3.8 h to 2.4 h ($P < 0.001$) after oral oxycodone. The CL of intravenous oxycodone increased by 2.2-fold during the rifampicin phase, when compared with the control. CL/F of oxycodone

increased by 7.6-fold due to the decreased oral bioavailability and increased CL, but when the individual F was taken into account, the CL of oral oxycodone increased by 2.2-fold similarly to that after intravenous administration.

The AUC_m/AUC_p of noroxycodone increased by 141% ($P < 0.001$) after intravenous oxycodone and by 664% ($P < 0.001$) after oral administration. The formation of oxymorphone was greatly decreased by rifampicin. After rifampicin no detectable oxymorphone was observed in eight subjects during the intravenous administration of oxycodone and in five subjects during oral administration. The mean AUC_m/AUC_p of noroxymorphone increased by 142% ($P < 0.001$) after intravenous oxycodone and by 858% ($P < 0.001$) after oral administration.

St. John's wort decreased the mean $AUC_{0-\infty}$ of oral oxycodone by 50% ($P < 0.001$) and the mean C_{max} from 33.6 ± 14.4 ng/ml to 24.0 ± 8.9ng/ml ($P < 0.01$). The mean $t_{½}$ of oxycodone decreased by 20% from 3.8 h to 3.0 h ($P < 0.001$). The mean AUC_m/AUC_p of noroxycodone increased by 127% ($P < 0.001$). The mean $AUC_{0-\infty}$ of oxymorphone decreased 46% ($P < 0.01$), but the AUC_m/AUC_p of oxymorphone did not differ from that during the control phase. The mean AUC_m/AUC_p of noroxymorphone increased by 155% ($P < 0.001$) after the St. John's wort pre-treatment, when compared with the control phase.

Effects of CYP3A inhibitors (II, IV, V)

CYP3A inhibitors voriconazole, grapefruit juice, ritonavir and lopinavir/ritonavir elevated the plasma concentrations of oxycodone and decreased the formation of the CYP3A-dependent metabolites noroxycodone and noroxymorphone. The metabolism was shifted towards the CYP2D6-mediated route and the plasma concentrations of oxymorphone were increased. Voriconazole seemed to be the most potent inhibitor of the inhibitors used in these studies, followed by ritonavir, lopinavir/ritonavir and grapefruit juice.

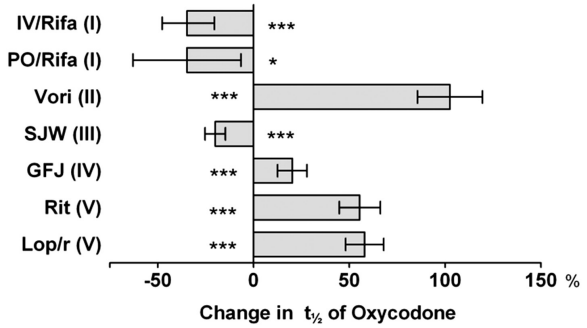

Figure 12. Change in the mean $t_{½}$ of oxycodone with 95% CI when compared to the control during pre-treatment with rifampicin (Rifa), voriconazole (Vori), St. John's wort (SJW), grapefruit juice (GFJ), ritonavir (Rit) or lopinavir/ritonavir (Lop/r).

IV = intravenous administration of oxycodone and PO = oral administration of oxycodone. Studies are denoted by Roman numerals in parentheses. *** $P < 0.001$, * $P < 0.05$.

Voriconazole increased the mean $AUC_{0-\infty}$ of oral oxycodone by 261% ($P < 0.001$) and the mean C_{max} by 72% from 18.1 ± 4.0 ng/ml to 30.5 ± 5.1 ng/ml ($P < 0.001$). The mean $t_{½}$ was prolonged from 3.5 h to 7.1 h ($P < 0.001$). The mean AUC_m/AUC_p of noroxycodone decreased by 92% ($P < 0.001$) during the voriconazole phase. The mean AUC_m/AUC_p of oxymorphone increased by 118% ($P < 0.01$) after oxycodone and voriconazole when compared to the control. After voriconazole, the mean C_{max} of noroxymorphone decreased by 91% ($P < 0.001$), and the mean AUC_m/AUC_p by 88% ($P < 0.001$).

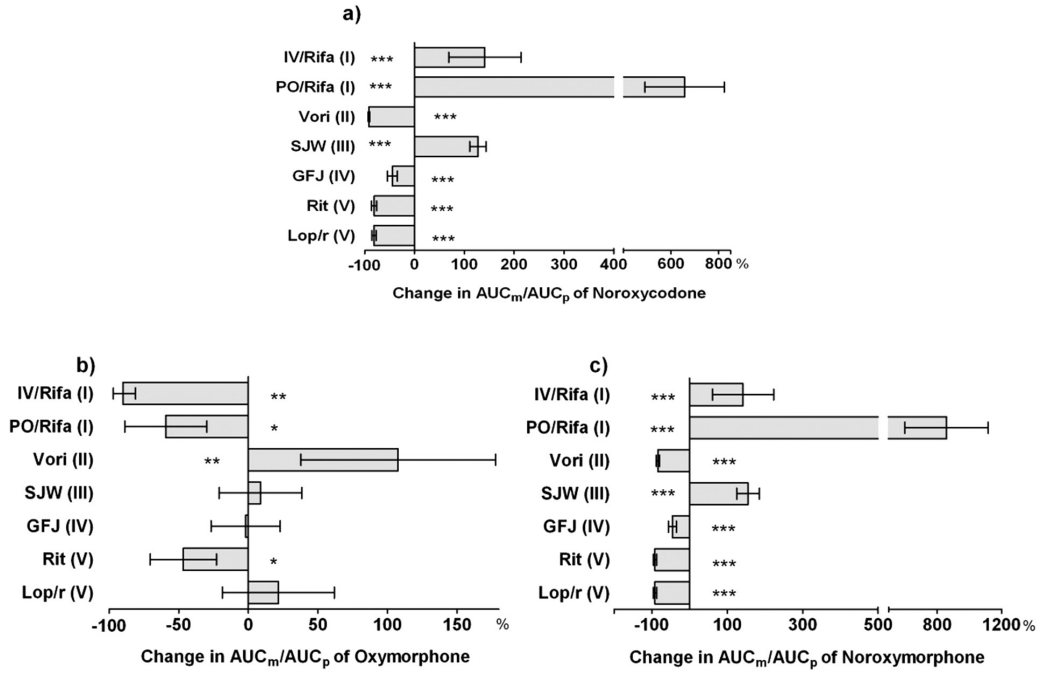

Figure 13. Change in the mean AUC_m/AUC_p of oxycodone metabolites noroxycodone (a), oxymorphone (b) and noroxymorphone (c) with 95% CI after pre-treatment with rifampicin (Rifa), voriconazole (Vori), St. John's wort (SJW), grapefruit juice (GFJ), ritonavir (Rit) or lopinavir/ritonavir (Lop/r) when compared to the control.

IV = intravenous administration of oxycodone and PO = oral administration of oxycodone. Studies are denoted by Roman numerals in parentheses. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Grapefruit juice increased the mean plasma $AUC_{0-\infty}$ of oxycodone by 67% ($P < 0.001$), and elevated the mean C_{max} by 48% from 18.9 ± 4.7 ng/ml to 26.8 ± 4.0 ng/ml ($P < 0.001$). The $t_{½}$ of oxycodone was prolonged by 20% from 3.5 ± 0.5 h to 4.1 ± 0.7 h ($P < 0.001$) during the grapefruit juice phase. Grapefruit juice decreased the mean AUC_m/AUC_p of noroxycodone by 44% ($P < 0.001$), but did not alter the mean AUC_m/AUC_p of oxymorphone. The mean $AUC_{0-\infty}$ of oxymorphone increased by 56% ($P < 0.01$) during the ingestion of grapefruit juice and oxycodone. During grapefruit juice ingestion, the AUC_m/AUC_p of noroxymorphone decreased by 45% ($P < 0.001$).

Ritonavir increased the mean plasma $AUC_{0-\infty}$ of oxycodone by 204% ($P < 0.001$), and lopinavir/ritonavir by 162%, when compared with the control. The mean C_{max} of oxycodone was elevated by 78% ($P < 0.001$) during ritonavir and by 45% during lopinavir/ritonavir ($P < 0.05$). The mean $t_{½}$ of oxycodone was prolonged by 55% from 3.6 h to 5.6 h ($P < 0.001$) during ritonavir and by 58% to 5.7 h ($P < 0.001$) during lopinavir/ritonavir. The mean AUC_m/AUC_p of noroxycodone diminished by 82% ($P < 0.001$) and 81% ($P < 0.001$) during ritonavir and lopinavir/ritonavir, respectively. The mean AUC_m/AUC_p of oxymorphone decreased by 49% ($P < 0.05$) after ritonavir ($P < 0.05$). The mean AUC_m/AUC_p of noroxymorphone diminished 95% ($P < 0.001$) during ritonavir and 91% ($P < 0.001$) during lopinavir/ritonavir.

5.2 Pharmacodynamic results

Effects of CYP3A inducers (I, III)

Pharmacodynamic changes (drug effects) were observed especially after oral administration of oxycodone. After rifampicin and intravenous oxycodone, only the pupillary miosis was significantly reduced ($P < 0.05$), when compared to the control phase. After rifampicin and oral oxycodone, significant reduction was observed in the self-reported drug effect $AUEC_{0-12}$ ($P = 0.001$), drowsiness ($P < 0.01$), and performance competence ($P < 0.05$) when compared to the controls. The heterophoria decreased during the rifampicin phase when compared to the control ($P < 0.05$). The $AUEC_{0-12}$ of the cold pain threshold ($P < 0.01$) was reduced during rifampicin when compared to the values during the control phase, whereas the cold pain intensity ($P < 0.01$) and cold pain unpleasantness ($P < 0.01$) were significantly increased after rifampicin and oral oxycodone (Figure 14 b), when compared to the values after the placebo treatment.

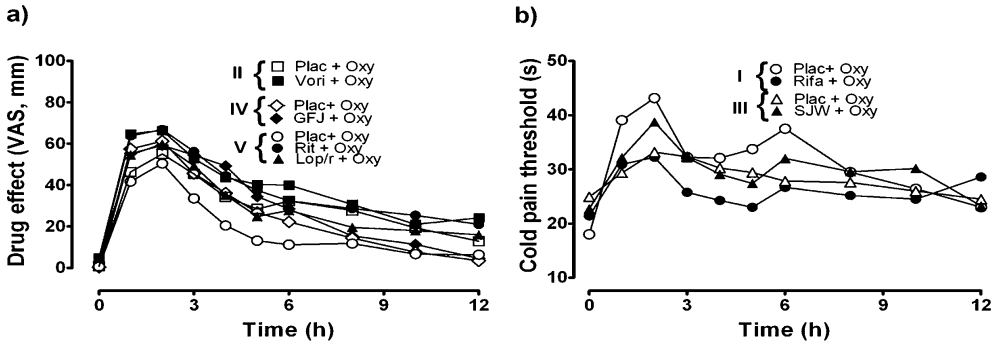

Figure 14. The mean self-reported drug effect of oral oxycodone during pre-treatment with CYP3A inhibitors voriconazole (Vori, Study II), ritonavir and lopinavir/ritonavir (Rit and Lop/r, Study V) or grapefruit juice (GFJ, Study IV), when compared with the control (a). The mean cold pain thresholds are shown during oral oxycodone and CYP3A inducers rifampicin (Rifa, Study I) or St. John's (SJW, Study III) wort, when compared to the control (b).

After oral oxycodone and St. John's wort, the self-reported drug effect $AUEC_{0–12}$ was significantly reduced ($P < 0.01$), when compared with the control. After the placebo and oral oxycodone, the initial value of CPT (18.2 ± 10.2 s) increased to 33.8 ± 17.8 s at 1 hour. During the St. John's wort phase, the initial value of CPT 16.2 ± 6.0 s increased significantly to 23.6 ± 10.2 s ($P < 0.05$). Significant differences between experimental cold pressure pain $AUEC_{0–12}$ s during St. John's wort and oral oxycodone were not observed, when compared with the control.

Effects of CYP3A inhibitors (II, IV, V)

After voriconazole and oral oxycodone, the $AUEC_{0–12}$s of the pupil size decreased ($P < 0.001$), and heterophoria ($P < 0.05$) increased significantly when compared to the control phase. During the placebo and oral oxycodone, the initial values of CPT increased from 22 ± 4 s to 39 ± 6 s ($P < 0.05$) at 1 hour, and during voriconazole and oxycodone from the initial 19 ± 3 s to 33 ± 4 s ($P < 0.05$), but the difference was not statistically significant and did not alter the $AUEC_{0–12}$ of CPT significantly. Deterioration of performance was significantly increased after grapefruit juice and oral oxycodone compared to the values during the water phase ($P < 0.05$). Pre-treatment with CYP3A inhibitors ritonavir and lopinavir/ritonavir increased the self-reported drug effect $AUEC_{0–12}$ ($P < 0.001$), when compared to the controls (Figure 14 a). Ritonavir increased oxycodone-associated nausea and vomiting ($P < 0.01$).

5.3 Other results

During pre-treatment with voriconazole, ritonavir and lopinavir/ritonavir, all the volunteers had measurable values of the corresponding drug, indicating compliance with the dosing schedule.

Genotypes and predicted phenotypes

The genotypes and predicted phenotypes of the volunteers in each study are shown in Table 7.

The PM subjects appeared to have the lowest oxymorphone concentrations during the placebo and oxycodone treatment (Figure 15), although this result is highly speculative due to the small number (n = 2) of PMs in these studies.

Table 7. Genotypes and predicted phenotypes in Studies I–V.

Phenotype	Genotype	N	Study
EM	*CYP2D6*1/*1*	24	I/5, II/6, III/5, IV/6, V/4
EM	*CYP2D6*1/*3*	1	I/1
EM	*CYP2D6*1/*4*	15	I/4, II/4, III/3, IV/3, V/3
EM	*CYP2D6*1/*5*	2	III/1, V/1
EM	*CYP2D6*1/*6,*	2	II/1, IV/1
EM	*CYP2D6*1/*9*	1	I/1
EM	*CYP2D6*1/*10*	1	III/1
EM	*CYP2D6*1/*41*	1	V/1
PM	*CYP2D6*3/*4*	1	I/1
PM	*CYP2D6*4/*4*	1	III/1
IM	*CYP2D6*4/*41*	1	IV/1
IM	*CYP2D6*10/*41*	1	V/1
UM	*CYP2D6*1/*1X2*	4	II/1; III/1 IV/1; V/2

N = the number of the genotype appearing among 55 volunteers;
EM =extensive, PM = poor, IM= intermediate and UM = ultrarapid metabolizer via CYP2D6;
Roman numeral/Arabic numeral = study number/the total number each genotype in the corresponding study.

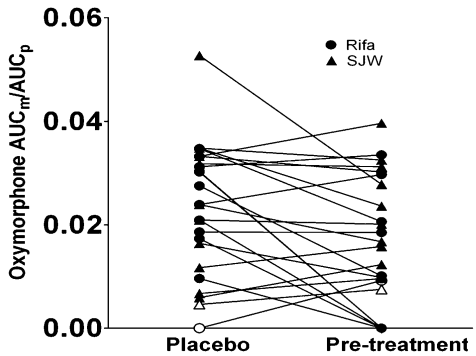

Figure 15. Individual oxymorphone AUC_m/AUC_p after rifampicin (circles) or St. John's wort (triangulars) pre-treatment and oral oxycodone hydrochloride 10 mg. Subjects with PM for CYP2D6 are depicted with open circle and triangle.

Effect of gender on the dose adjusted $AUC_{0-\infty}$ of oxycodone

The dose adjusted AUCs (mean ± SD) during the placebo treatment in Studies I-V were 0.66 ± 0.12 min · µg/ml for women (n = 28) and 0.74 ± 0.27 min · µg/ml for men (n = 27). The difference between women and men was insignificant ($P = 0.14$). The dose adjusted AUC ratio was 1.88 ± 1.55 for women and 1.82 ± 1.28 for men. There were no significant differences between the dose adjusted AUC ratios and the gender after different pre-treatments in Studies I-V ($P = 0.86$).

Adverse effects

The adverse effects remained mild and all subjects completed the studies. Nausea, vomiting, itching, tiredness and dizziness were the most frequently reported adverse events after oxycodone dosing. Five subjects were treated with tropisetron against vomiting during the test days. These cases occurred after a bigger oxycodone dose (15 mg) and placebo, or after the potent CYP3A inhibitors voriconazole, ritonavir and lopinavir/ritonavir. During the voriconazole phase, three subjects reported transient visual adverse events, such as chromatopsia and altered perception of light. During the pre-treatment with protease inhibitors, the adverse events reported were abdominal irritation (n = 6), diarrhoea (n = 5) and nausea (n = 3), which is in line with the product information from the manufacturer.

6. DISCUSSION

6.1 Methodological considerations

Based on power analysis, the sample size of 12 volunteers was sufficient to detect the 30% difference in the $AUC_{0-\infty}$. The use of a small group size was enabled with the use of a crossover study design, where each subject served as their own controls, thus diminishing the influence of interindividual variation.

Altogether 55 individuals found through internet advertisements directed towards university students participated in these studies and all of them were Caucasian and residents of Finland. The recruitment of both genders allowed us to do comparisons also between females and males. Half of the volunteers enrolled were women. Previously, it has been considered, that CYP3A activity might be higher in women as compared to men (Hunt et al. 1992), but this was questioned by Shimada et al., who reported no sex-related differences in the CYP3A activity in the liver microsomes of Japanese and Caucasians (Shimada et al. 1994). Gender-related differences in the metabolism of oxycodone have been reported in a study with rather small group size (Kaiko et al. 1996), but these findings have not been supported in later studies (Liukas et al. 2008). In the present series of studies, dose adjusted AUCs and the dose adjusted AUC ratios ($AUC_{treatment}/AUC_{control}$) of oxycodone for 55 subjects participating in these studies were calculated. No gender related differences in the dose adjusted AUCs of oxycodone or in AUC ratios during the interaction were detected.

A four week interval between the study phases was sufficient to exclude the carry-over effect as the terminal half-lives of the drugs studied were clearly exceeded. It was chosen in all studies to minimize the possible effects of the menstrual cycle on the results of female participants, although the influence of the menstrual cycle on the drug metabolism remains conflicting. The majority of studies reveal no significant differences in the pharmacokinetics of CYP3A substrates during different hormonal stages (Kirkwood et al. 1991, Kharasch et al. 1997).

As this was an academic study, we had no resources to use a completely identical placebo and thus it was impossible to use a double-blind study design. Theoretically, the subjects could define the pre-treatment used, if they wanted to. As the primary endpoints were pharmacokinetic changes and were based on the plasma concentrations of the parent drug and its metabolites determined from coded plasma tubes, the lack of double-blinded circumstances might not have biased the pharmacokinetic results.

Although it would be preferable to reach a steady-state with the inducer or inhibitor before the administration of the substrate, this was not possible in Studies I and V for ethical reasons. It would have been unethical to expose healthy volunteers to long

antimicrobial treatments, as interactions have been described already after short dosing (Backman et al. 1996, Olkkola et al. 1999). St. John's wort was administered during 2 weeks, and it can be considered to have achieved a steady-state, although its half-life is not known. The steady-state of voriconazole is achieved on day two, if the loading dose used in the present study is given, but interindividual variability can result in variations in the achievement of a steady-state. The half-lives of the active substances of grapefruit are not known. Even one glass of grapefruit juice has been reported to inhibit CYP3A maximally (Lundahl et al. 1998), but we used multiple dose ingestion to study the effects of longer exposure.

In the consequence of the autoinduction, the repeated administration of rifampicin reduces its oral bioavailability by 25% and increases the clearance (Loos et al. 1985). Autoinduction has been reported also with ritonavir (Hsu et al. 1997). As the full autoinduction occurs during 2 weeks (Hsu et al. 1997), the results concerning rifampicin and ritonavir were not those during a true steady-state. It can be speculated that without the autoinduction, the interaction at the steady-state might have been more extensive, but autoinduction could diminish this effect to some extent.

The compliance was assured for voriconazole (Study II), ritonavir and lopinavir (Study V) with determination of their concentrations with previously validated methods (Gage and Stopher 1998, Pennick et al. 2003, Rezk et al. 2009), and the observed concentrations were in line with the dosing schedule indicating good compliance. In Studies I, III and IV, adherence to the pre-treatment schedule was controlled only with the SMS tehnology used in all the studies. It was impossible to be absolutely sure about the intake of pre-medication, although it seemed likely as every dose, even the placebo and glasses of water in Study IV were confirmed with text messages. In some cases, the message was delayed for half an hour until the subject was reminded, but this should not bias the results.

Pharmacodynamics was assessed with methods used earlier in pharmacokinetic studies (Manner et al. 1987, Saari et al. 2007, Samer 2010a, Grönlund et al. 2010a). Several VAS-scores, the Maddox Wing Test, and DSST were used to measure the effects of oxycodone. VAS scores are based on a subjective rating, which is not specific only to opioid, as for instance, more sleepiness can be felt in the morning or after lunch also without opioids. Pupillary miosis has been sensitive in the pharmacodynamic studies of opioid effects (Posner et al. 1985, Phimmasone and Kharasch 2001, Baririan et al. 2007). In these studies, technical equipment, such as an infrared pupillometer with a resolution of 0.1 mm, has been used. In the present studies, the measuring was conducted with a Cogan's pupillometer, which has a resolution of 0.5 mm (Cogan 1941). The data collection was performed in a stable, but normal light, as we had no possibility for a constant, dim lightning in the investigating room. The light of the environment can bias the results as it diminishes the baseline diameter of the pupil resulting in smaller changes.

Pain was measured with the cold pressure test, which is a sensitive model for the measurement of opiate-induced analgesia in healthy volunteers (Posner et al. 1985, Jones et al. 1988, Koltzenburg et al. 2006, Eisenberg et al. 2010, Samer et al. 2010a). The cold pressure test is also easily available as the hand is immersed into water with crushed ice and it is repeatable. After oxycodone administration, the cold pressure test was repeated 9 times. It is possible, that some habituation may have occurred during the day, but any possible effect on the results would have been diminished by the cross-over design used in the studies. The limitation of these studies was, that there was not access to stirred water, which has often been used in previous studies (Posner et al. 1985, Eisenberg et al. 2010), thus preventing the water from warming around the hand. However, the temperature was measured and it was kept between 0.5 and 2 ˚C.

In Studies I and II, the effects of oxycodone on the heat pain threshold and tactile sensitivity were measured. This was done to find out whether the analgesic effect of oxycodone in this experimental setting was dependent of the submodality of pain (heat vs. cold), or type of sensory stimulus (pain vs. touch). As oxycodone had no effect on tactile sensitivity and the cold pressor test appearred to be sensitive to opioid analgesia, we decided to simplify the protocol and applied only the cold pressor test to assess pain in Studies III–V.

Pharmacokinetic and statistical analysis was performed with standard methods, which have been used in previous pharmacokinetic studies and were valid for this kind of study design. Most of the pharmacodynamic parameters were analyzed using $AUEC_{0–12}$ determined with the linear trapezoidal rule. It is obvious, that the effect of a single small dose of oxycodone can hardly be quantified for the period of 12 hours, as the $t_{½}$ of oxycodone is less than 4 hours. Although some of the pharmacodynamic parameters seemed to have clear differences during the first 3 hours, the $AUEC_{0–3}$ was not significantly changed. In recent studies, $AUEC_{0–90}$ has been used (Samer 2010b), but this was not possible in our studies, as only two measuring points were included in the first 1.5 hours. After multiple doses, the cumulation of oxycodone might have increased the effect of especially inhibition, but only a single dose was administered to avoid the exposure of healthy volunteers to multiple doses of opioids. For the pharmacodynamic analysis, pharmacokinetic-pharmacodynamic modelling might have given more information about the relationship between the plasma concentrations and pharmadynamics of oxycodone.

6.2 Pharmacokinetic considerations

When initiating these studies, no previous, randomized studies had been published about the effects of CYP3A inducers or inhibitors on the pharmacokinetics or pharmacodynamics of oxycodone. The findings of Study I showed, that concomitant administration of rifampicin and oral oxycodone accelerated the first-pass metabolism of oxycodone in the intestine and liver and decreased the bioavailability. After intravenous

administration of oxycodone, no first-pass elimination occurred and C_{max} remained unchanged. The elimination phases were accelerated equally after the intravenous and oral administration of oxycodone and an approximately 35% reduction in the $t_{½}$ and 2.2-fold increase in CL was observed. However, it was less substantial when rifampicin was used together with intravenous oxycodone, since the first-pass metabolism was not involved.

Rifampicin was a more potent CYP3A inducer than St. John's wort. Following oral oxycodone, the first-pass metabolism in the intestine and liver further diminished the concentrations of oral oxycodone and decreased the $AUC_{0-\infty}$ of oral oxycodone by 86% whereas the corresponding value during St. John's wort was 50%. These results are in line with the previous studies concerning the interaction between oral midazolam and rifampicin or St. John's wort. The concomitant administration of rifampicin and midazolam has led to an approximately 95% reduction in the plasma concentrations and to total loss of effectiveness (Backman et al. 1996, Backman et al. 1998), whereas the extent of induction during St. John's wort was linked to hyperforin content. High hyperforin containing extract LI 160, used also in the present studies, decreased the concentrations of midazolam by 79%, whereas low hyperforin extract caused only a 21% reduction in its AUC_{0-12} (Mueller et al. 2006).

The induction of CYP3A-mediated metabolism shifted the metabolism of oxycodone towards the CYP3A-mediated route. AUC_m/AUC_p ratios of CYP3A-dependent metabolites noroxycodone and noroxymorphone increased significantly after both rifampicin and St. John's wort. The concentrations of CYP2D6-dependent active metabolite oxymorphone decreased during rifampicin, but St. John's wort did not alter its concentration. This can be explained by the higher potency of rifampicin to shift the metabolism towards noroxycodone, and accelerate the metabolism of oxymorphone via CYP3A to noroxymorphone. The findings of Studies I and III are in positive agreement with previous studies on the effect of rifampicin on the pharmacokinetics of midazolam and triazolam (Backman et al. 1996, Villikka et al. 1997), and the effect of St. John's wort on the pharmacokinetics of midazolam (Rengelshausen et al. 2005, Mueller et al. 2006) and alprazolam (Markowitz et al. 2003).

Voriconazole was the most potent inhibitor of CYP3A studied, affecting both the first-pass metabolism and elimination of oxycodone and increased the exposure to oxycodone by 3.7-fold. Ritonavir and lopinavir/ritonavir influenced the pharmacokinetics almost similarly, although the dose of ritonavir was three times higher during the ritonavir phase, thus suggesting almost complete inhibition already with the lower dose of ritonavir. After ritonavir and lopinavir/ritonavir the $AUC_{0-\infty}$ of oxycodone increased by 3.0-fold and 2.7-fold, respectively. The almost equal alteration in the concentrations of oxycodone might be explained with the additional inhibitory effect of lopinavir, which has been shown *in vitro* (Weemhoff et al. 2003). Concomitant administration of voriconazole and oral oxycodone increased the concentrations of oral oxycodone clearly, but much less than the increase of 10.3-fold after voriconazole and oral midazolam (Saari et al. 2006b).

Based on *in vitro* studies, it has been suggested, that CYP2C19 could contribute also to the formation of noroxycodone and oxymorphone (Lalovic et al. 2004). Voriconazole inhibits also the CYP2C9-and CYP2C19-mediated metabolism *in vitro* (Jeong et al. 2009), and it can be suggested that the presence of CYP2C19 inhibition could explain the higher increase of oxycodone concentrations by voriconazole. However, at least 80% of the formation of both noroxycodone and oxymorphone are mediated by CYP3A and CYP2D6 *in vitro,* thus leaving the quantitative effect of CYP2C19 minor (Lalovic et al. 2004).

The inhibition of the CYP3A-mediated metabolism of oxycodone by voriconazole shifted the metabolism towards CYP2D6, and increased the concentration of oxymorphone similarly to the other studies with oxycodone and CYP3A inhibitors (Saari et al. 2010). After ritonavir and lopinavir/ritonavir, the concentration of oxymorphone varied widely, partly because of the *CYP2D6* polymorphism. After ritonavir, the AUC_{0-48} of oxymorphone increased insignificantly by 1.6-fold (range 1.3–3.9-fold), and after lopinavir/ritonavir by 3.3-fold (range 0.9–7.4-fold). Due to the increased parent oxycodone concentration, the AUC ratios of oxymorphone/oxycodone decreased after ritonavir by 49%, but after lopinavir/ritonavir no significant changes were seen. It can be speculated that the higher dose of ritonavir was a more potent inhibitor of CYP2D6 than that in lopinavir/ritonavir. Previously, already low doses of ritonavir have inhibited CYP2D6 (Wyen et al. 2008). Both ritonavir and lopinavir/ritonavir can also induce CYP2C19 and glucuronidation, which theoretically could bias our results. Their role in the metabolism of oxycodone and its metabolites is of minor importance and if present, would decrease the values in the Study V.

Oxymorphone is a potent opioid and its role in the analgesic effect of oxycodone has been studied recently (Lalovic et al. 2006, Zwisler et al. 2009b, Lemberg et al. 2010, Samer et al. 2010a). However, only about 10% of oxycodone is metabolized via the CYP2D6-mediated route to oxymorphone (Lalovic et al. 2004). The concentrations of oxymorphone have remained low, thus suggesting a minor role for oxymorphone in the effects of oxycodone (Kaiko et al 1996, Heiskanen et al. 1998, Lalovic et al. 2006). This was supported by the findings of the present Study I, where intravenous oxycodone produced no detectable oxymorphone concentrations in two subjects after the placebo, and in eight subjects after rifampicin. After oral oxycodone and rifampicin, five subjects had analyzed oxymorphone concentrations below the LLQ. Although oxymorphone can contribute to the analgesic effects of oxycodone (Lalovic et al. 2006, Zwisler et al. 2009, Lemberg et al. 2010, Samer et al. 2010a), parent oxycodone seems to be mainly responsible for most of its effects (Kaiko et al. 1996, Heiskanen et al. 1998, Lalovic et al. 2006).

Grapefruit juice is considered to principally inhibit the intestinal CYP3A, as the pharmacokinetics of intravenously administered CYP3A4 substrates has remained unaltered (Ducharme et al. 1995, Lundahl et al. 1997). The inhibition is probably caused by the degradation of intestinal CYP3A (Lown et al. 1997, Lundahl et al. 1997). In Study IV, repeated ingestion of grapefruit juice increased the exposure to oxycodone by

1.7-fold and elevated its peak concentration by 50%. The interaction remained moderate as principally only the intestinal CYP3A was inhibited and the liver remained mainly untouched. Grapefruit juice has not usually changed the $t_{½}$ (Kantola et al. 1998, Culm-Merdek et al. 2006), but after repeated ingestion the elimination half-life of atorvastatin, cisapride and triazolam have increased (Lilja et al. 1999, Kivistö et al. 1999, Lilja et. al 2000a). Also in Study IV, $t_{½}$ of oxycodone was increased slightly but significantly in every subject during the grapefruit phase, resulting in a 20% prolongation after the repeated ingestion of grapefruit juice. This indicates that some ingredients of grapefruit juice alter the elimination of oxycodone during repeated ingestion, probably by inhibiting liver CYP3A or increasing the enterohepatic circle.

In Study IV, a commercial grapefruit juice brand available in supermarkets and used in previous studies was used (Lilja et al. 1998b, Lilja et al. 2000a). The CYP3A inhibition caused by grapefruit juice has been strongest with drugs of a low oral bioavailability, as the reduction of the pre-systemic metabolism results in increased concentrations, such as a 15-fold elevation in lovastatin AUC (Kantola et al. 1998). There is a great variation in the constituents of commercial grapefruit juice brands and lots (Ho et al. 2000). Oxycodone was administered concomitantly with grapefruit juice during the grapefruit juice phase, which can be speculated to delay the absorption of oxycodone. This is not supported by the present findings, as grapefruit juice did not decrease the C_{max} or prolong the t_{max} of oxycodone.

A considerable interindividual variation in the extent of induction or inhibition of CYP3A was seen in all Studies I–V. During the CYP3A inducers, the AUC of oxycodone decreased most after oral administration. After rifampicin, the AUC of oral oxycodone decreased from 78% to 91% and after St. John's wort from 37% to 57%. Voriconazole increased the AUC of oxycodone in one subject by 6-fold, ritonavir over 4-fold and lopinavir/ritonavir by 3-fold. Explanations for these variations have been the individual changes in the intestinal and liver CYP3A content (Shimada et al. 1994), or differences in the expression of CYP3A5 (Lown et al. 1994, Koch et al. 2002)

The role of P-gp in the pharmacokinetics of oxycodone in humans still remains unclear. From the compounds investigated in these studies, rifampicin and St. John's wort can induce P-gp (Greiner et al. 1999, Dürr et al. 2000), and ritonavir and lopinavir/ritonavir can inhibit it (Ding et al. 2004, Wyen et al. 2008). Grapefruit juice does not seem to inhibit P-gp (Parker et al. 2003), although its role in the function of P-gp is controversial. If oxycodone was a P-gp substrate, the induction would activate P-gp leading to parallel effects, compared to CYP3A induction, thus reducing the concentrations of oxycodone. The inhibition of P-gp would inhibit its function as an efflux transporter, thus leading to increasing concentrations of oxycodone similar to the effects of CYP3A inhibition. This was not supported by our studies. The pharmacokinetic parameters altered greatly, but the most extensive inhibition of oxycodone was observed after the pure CYP3A inhibitor voriconazole.

6.3 Pharmacodynamic considerations

The differences in the pharmacological effects of oxycodone during the interaction remained moderate. After rifampicin and oral oxycodone, the analgesic effects of oxycodone decreased significantly as assessed by the means of the cold pain threshold, cold pain intensity and cold pain unpleasantness $AUEC_{0–12}$. During the other Studies II–V, no significant changes in pain measurements were detected. Similar results after oxycodone and CYP inhibitors have been reported by other investigators (Grönlund et al. 2010a,b, Saari 2010), whereas some groups have suggested differences in the analgesic effect of oxycodone depending on the oxymorphone concentrations based on rather small sample sizes (Zwisler et al. 2009, Samer et al. 2010a).

The lack of significant differences in the analgesic effects between oxycodone and the combination (oxycodone and inducer/inhibitor) can be explained with rather small sample sizes and high interindividual variation in pharmacodynamic measurements. In analgesia, 10-fold differences between the subjects were found. As only single small dose of oxycodone was administered, it hardly affects the pharmacological responce during the whole observation period of 12 hours.

The effects of CYP3A4 inhibition (ketoconazole) and/or CYP2D6 inhibition (quinidine) on the pharmacodynamics of oxycodone have been studied recently in 10 healthy volunteers (Samer et al. 2010a). The findings of this study suggested that CYP2D6 and CYP3A4 inhibition resulted in the higher pharmacologic effects of oxycodone. In that study, an equal method was used in the cold pressure test when compared to those of the Studies I–V, but they assessed the pain only during 6 hours, used $AUEC_{0–90}$ values and administered antidote naloxone 1.5 hours after oxycodone. As only the first four time points (before, 0.5, 1, 1.5 hours after oxycodone) were included into the AUEC calculations, the plasma concentrations remained high and more differences could be found. Sample calculations were not shown in the study.

Miosis has been a specific pharmacodynamic measurement of opioid effects in earlier studies (Posner et al. 1985, Phimmasone and Kharasch 2001, Baririan et al. 2007). In the present studies, significant differences in miosis were detected during rifampicin and intravenous oxycodone (Study I), where the miotic effect of oxycodone decreased. Voriconazole increased the oxycodone-induced miosis, but this effect was minor as the baseline of the two phases differed.

The self-reported drug effect was one of the most frequently affected measurement during the present studies. CYP3A inducers decreased the self-reported drug effect and CYP3A inhibitors ritonavir and lopinavir/ritonavir increased it. The self-reported drug effect is not influenced by oxycodone only but also by the pre-treatment drugs. Some of the volunteers reported minor drug effects already after pretreatment, before oxycodone administration, during rifampicin, voriconazole, ritonavir or lopinavir/ritonavir. The

highest VAS value was 25 mm in one subject during the ritonavir phase and it was increased to 85 mm after the oxycodone dose.

In Studies II–V, the moderate pharmacodynamic results can not be concluded to mean a missing interaction. A more probable explanation is the lack of the statistical power of the studies to demonstrate significant differences in the pharmacodynamic effects of oxycodone.

6.4 Pharmacogenetic considerations

Fifty-five different volunteers participated in these studies. The subjects were not included in these studies according to their genotypes and the number of genotype variants was too low (Table 7) to make overall conclusions about the effects of the CYP2D6 genotype on the metabolism of oxycodone. Both PMs had low AUC ratios of the CYP2D6 dependent metabolites oxymorphone and noroxymorphone, which is in line with the findings of Zwisler et al. (Zwisler et al. 2010a). They concluded that the CYP2D6 genotype did affect the formation of oxymorphone, but did not alter the postoperative intravenous analgesia. In Studies I–V, the interindividual variation in the pharmacodynamics made it impossible to make any conclusions on the pharmacodynamic differences based on these few PMs. It has been reported that EMs had better analgesia after oxycodone in experimental single electrical sural nerve stimulation and the cold pressure test (Zwisler et al. 2009). This was supported by the results from Samer et al. (Samer et al. 2010a). Based on two subjects, they suggested, that CYP2D6 UMs experienced 1.5- and 6-fold increase in the analgesic effects of oxycodone. In the present studies, the UMs did not differ from the overall variation in the pharmacodynamic parameters.

The frequency of UMs was higher (5%) than 1%, which has been reported earlier in the Swedish population (Dahl et al. 1995). One explanation for this is that one of our volunteers participating in more than one study happened to represent the genotype precipitated as UM. The formation of oxymorphone was highest among UMs in the Studies II and IV; but did not differ substantially compared to EMs in Studies III and V. The frequency of CYP2D6 PMs (4%) was almost similar to those reported in Finnish population (3.4%) (Arvela et al. 1988). Although the formation of oxymorphone was lowest among the PMs, the impairment in the analgesic effect was not clear. Despite these findings, it is impossible to draw conclusions about the pharmacokinetic or pharmacodynamic differences based on single subjects.

6.5 Clinical aspects

Experimental pain differs from clinical pain in many aspects. In experimental models, the analgesic drug is administered before the noxious stimulus is applied, which can influence the findings. In acute or chronic pain, the somatosensory system and central

processing are activated by pain, which exists before the drug is administered. In a clinical situation, several factors, such as physiological, psychological and psychosocial factors, as well as other diseases and their treatments, may influence the clinical effects of analgesics. As all of the volunteers in the present studies were healthy, non-smoking adults with no medication and no clinical pain, the results of these studies must be carefully extended to patients or the elderly. The cold pressor test used in the present studies, however, has been shown to induce descending noxious inhibitory control (Granot et al. 2008) and thus, it may mimic tonic clinical pain more closely than some other pain models. Considering these limitations, it is noteworthy that in situations when interactions are too strenuous to be studied in patients, findings of studies conducted in healthy volunteers can and should be taken advantage of in clinical work.

The use of CYP3A inducers concomitantly with oral oxycodone decreased the concentrations of oxycodone and can diminish its analgesic efficacy requiring optional treatment. Unfortunately, the choice of optional medication can be difficult since most of the opioids are metabolized by CYP3A. Fentanyl, sufentanil, alfentanil, methadone and buprenorphine are CYP3A substrates and thus prone to interactions (Tateishi et al. 1996, Saari et al. 2006a, Liu et al. 2007b, McCance-Katz et al. 2007). Morphine, hydromorphone and oxymorphone are metabolized principally by glucuronidation, but glucuronidation is induced also by rifampicin and a decrease in the effectiveness of morphine has been reported (Fromm et al. 1997, Armstrong and Cozza 2003). Epidural analgesia can be an advisable choice when rifampicin treatment occurs during acute pain, if it is suitable for the pain condition. Intravenous oxycodone administration can be considered if the pain relief remains unsatisfied, but dose adjustment can be needed.

Clinicians should be aware of the possibility that patients can use a herbal drug such as St. John's wort, decreasing the exposure to oral oxycodone. Other CYP inducers that can interact with oxycodone are carbamazepine, phenytoin, phenobarbital and dexamethasone (Pelkonen et al. 2008). Although carbamazepine use in neuropathic pain has decreased due to its adverse events, it is used, for example, in trigeminal neuralgia and it is probable that the effect of oxycodone is decreased similarly as shown here with rifampicin. In a recent study, dexamethasone combined with paracetamol was shown to decrease the consumption of oxycodone after orthopaedic surgery, but this is explained by the anti-inflammatory effect of the combination (Mattila et al. 2009).

During CYP3A inhibitors, the concentrations of oxycodone are increased and harmful adverse effects can be present without dose adjustment, but no improvement in the analgesic effect was seen in these studies. Voriconazole, grapefruit juice, ritonavir and lopinavir/ritonavir inhibited the metabolism of oral oxycodone, resulting in 3.6-, 1.7-fold, 3.0-fold and 2.6-fold increase in the exposure to oxycodone. In previous studies, itraconazole increased the concentrations of intravenous oxycodone by 1.4-fold, and those of oral oxycodone by 2.4-fold (Saari et al. 2010) and telitromycin by 1.8-fold (Grönlund et al. 2010a). Furthermore, oxycodone concentrations have been greatly increased during the concomitant administration of CYP3A and CYP2D6 inhibitors ketoconazole and

quinidine (Samer et al. 2010b), or itraconazole and paroxetine (Grönlund et al. 2010b). CYP3A inhibition can increase the adverse effects of oxycodone since its therapeutic index is rather narrow. Most of the studies have been performed with single doses of oxycodone. The repeated administration of oxycodone together with CYP3A inhibitors further increases the risk of oxycodone-related adverse effects. Fatal consequences have been reported after an oxycodone overdose or abuse (Dhalla et al. 2009).

6.6 Ethical considerations

In Studies I–V, healthy volunteers were exposed to the effects of opioid oxycodone. The risk of developing opioid abuse was assessed as low, using the Finnish translation of the Abuse questions (Michna et al. 2004). A "yes" answer to any one question resulted in the exclusion of the subject (Table 5). In addition, a commercially available urine screen for drugs with addiction potential was required before participation. Three volunteers were excluded because of a positive answer to the Abuse Questions, and one because of an abnormal laboratory test. The doses of oxycodone used in these studies were equal to the small doses used in postoperative pain management. In Study I, subjects received four single doses of oxycodone, in Studies II–IV two doses and in Study V three single doses at an interval of one month. The subjects were instructed not to participate in any other drug study involving opioids during the same year. The drugs used as pre-treatments in these studies have been on the market for a long time. The doses used were equal to those used clinically.

Pain tests were used to investigate the effects of DDIs on the analgesic effect of oxycodone. Experimental pain tests used in the studies have been used in numerous previous studies and are non-invasive and well tolerated by healthy subjects (De Jalon et al. 1985, Jones et al. 1988, Staahl et al. 2006a, Staahl et al. 2009). Participants were aware of transient pain caused by the experimental pain tests and venous cannulation before writing the informed consent, and had right to exit from the studies whenever they wanted without giving any reason for it.

Volunteers were exposed to the drug effects used in Studies I–V. The adverse events reported by the participants were transient and not severe. The study facilities were equipped with first aid and resuscitation instruments. The investigators were prepared to treat the adverse effects of the opioids with tropisetron or opioid antagonist naloxone. The duration of pre-treatments were intended to be kept as short as possible.

Although there were risks in Studies I–V, such as transient pain, opioid exposure and drug adverse effects of drugs, they were considered to be well in line with the information obtainable from the studies.

6.7 Implications of future studies

Recently, many new investigations have been published about the pharmacology and interactions of oxycodone (Lemberg et al. 2006, Zwisler et al. 2009b, Grönlund et al. 2010a, Lemberg et al. 2010, Saari et al. 2010, Samer et al. 2010b). The present studies introduce results on the importance of the CYP3A-mediated route in the metabolism and the interactions of oxycodone. Further randomized, double-blind controlled studies in pain patients are needed to fully understand the clinical importance of CYP3A induction or inhibition on the analgesic effect of oxycodone in patients with persistent pain.

During these studies, the results of altogether 55 healthy volunteers were collected. These data could be used in further analysis with pharmacokinetic-pharmacodynamic modelling, which could give more information about the relationship between oxycodone plasma concentrations and pharmacodynamic parameters.

The role of P-gp in the pharmacokinetics of oxycodone in humans still remains unclear. Theoretically, the use of the specific P-gp inhibitor valspodar (PSC833) might enable the investigation of the role of P-gp in the pharmacokinetics of oxycodone in humans. This compound is in phase III in clinical studies targeting against multi-drug resistance in chemotherapy regimens, and has earlier been used when studying the role of P-gp in oxycodone pharmacokinetics in rats (Boström et al. 2005).

Despite the recent steps forward in understanding the pharmacokinetics and pharmacodynamics of oxycodone, it still remains unclear what is the pharmacological action of oxycodone in the human brain. Previously, positron emission tomography (PET) has been used to study the relationship between the plasma concentrations of the opioid receptor antagonist nalmefene and its central μ-receptor occupancy by using a radiolabelled tracer, [^{11}C]carfentanil as an opioid receptor agonist (Ingman et al. 2005). In the future, it would be interesting to explore the relationship between oxycodone pharmacokinetics and pharmacodynamics with μ-opioid receptor occupancy in the human brain.

7. SUMMARY AND CONCLUSIONS

1. Rifampicin decreased the exposure to intravenous and oral oxycodone by 53% and 86%, respectively. After rifampicin and oral oxycodone, the elimination half-life of oxycodone was decreased from 3.7 to 2.4 h. As the CYP3A-mediated route to noroxycodone was induced, the formation of noroxycodone increased by 664% after oral oxycodone, and the formation of oxymorphone decreased clearly. After St. John's wort, the concentrations of oral oxycodone decreased by 50%, and the mean elimination half-life decreased by 20%. The self-reported drug-effect of oral oxycodone decreased after rifampicin and St. John's wort. Although rifampicin increased the cold pain as compared to the placebo, on the average, CYP induction had a minor effect in experimental pain models.

2. Rifampicin decreased the oral bioavailability of oxycodone from 69% to 21%, indicating that first-pass metabolism was greatly involved in the interaction.

3. Voriconazole was the most potent inhibitor of CYP3A investigated in these studies. It increased the exposure to oral oxycodone by 261%, and increased the maximal concentrations of oxycodone by 72%. The mean elimination half-life of oxycodone was prolonged from 3.5 to 7.1 h. When the CYP3A-mediated route was inhibited, the concentrations of noroxycodone decreased by 92%, and those of oxymorphone increased by 118% as the metabolism of oxycodone shifted towards the CYP2D6-mediated route.

   Grapefruit increased the exposure to oxycodone by 67%, as it affected mainly at the intestinal level.

   The effect of ritonavir and lopinavir/ritonavir was almost equal, although the ritonavir dose was three times higher during the ritonavir phase. The exposure to oxycodone was increased by 204% and 162%, respectively.

   Compared to the placebo, the inhibition of CYP3A resulted in a moderate change of the oxycodone-associated behavioural effects. The self-reported drug effect was increased after ritonavir and lopinavir-ritonavir. CYP inhibition did not alter the experience of experimental pain.

   As a conclusion, the clinical importance of the interaction between rifampicin and specifically oral oxycodone is of great importance, and can lead to ineffectiveness of oxycodone and a significant impairment of analgesia. Concomitant use of rifampicin and oral oxycodone should be avoided. Clinicians should be also aware of the common use of herbals, such as St. John's wort, and its ability to decrease the exposure and effects of oxycodone.

CYP3A inhibitors voriconazole, grapefruit juice, ritonavir and lopinavir may enhance the adverse events of oxycodone. Dose adjustment of oral oxycodone and careful monitoring of drug effects are necessary, if oxycodone is used concomitantly with CYP3A inhibitors. If other antimicrobials are available, voriconazole should be replaced with an antifungal not inhibiting the CYP3A.

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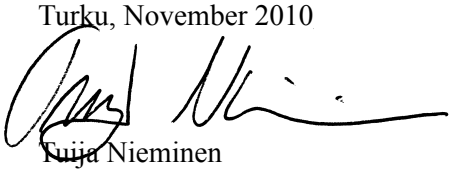

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