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EFFECT OF CYTOCHROME P450 2D6 AND 3A ENZYME INHIBITION ON THE METABOLISM OF OXYCODONE

by

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ABSTRACT

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Effect of Cytochrome P450 2D6 and 3A Enzyme Inhibition on the Metabolism of Oxycodone

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Cytochrome P450 (CYP) enzymes are important for the metabolism of many drugs. The inhibition or induction of the CYP enzymes has been found to be a major mechanism behind pharmacokinetic drug-drug interactions. Oxycodone is an opioid analgesic used increasingly in the treatment of moderate and severe pain in inpatient and outpatient care. Since oxycodone is extensively metabolized by CYP2D6 and CYP3A enzymes, it might be prone to drug interactions. In the present studies, the effect of CYP2D6 and CYP3A inhibitors on the pharmacokinetics and pharmacological effects of oxycodone were studied by using a randomized, placebo-controlled crossover study design in clinical trials carried out with healthy volunteers.

Although the inhibition of CYP2D6 enzyme by paroxetine considerably decreased the metabolism of oxycodone to oxymorphone, it did not lead to increased concentrations of the parent drug oxycodone. However, the CYP3A inhibition by itraconazole increased the exposure to oxycodone by 1.5- and 2.4-fold after the intravenous and oral administration of oxycodone, respectively. Concomitant treatment with paroxetine and itraconazole increased the exposure to oxycodone by 2.0- and 2.9-fold after the intravenous and oral administration of oxycodone, respectively. A short course of telithromycin increased the exposure to oxycodone by 1.8-fold whereas the use of miconazole oral gel resulted in a 1.6-fold increased exposure to oxycodone. Although these pharmacokinetic interactions clearly increased the achieved plasma concentrations of oxycodone, the used PD models were not able to detect clinically relevant changes in the pharmacological effects of oxycodone after a single low dose to healthy volunteers.

The results of these studies demonstrate that the inhibition of CYP2D6 has most likely no clinical relevance for the metabolism of oxycodone, if the function of the CYP3A dependent pathway is normal. On the contrary, the inhibition of CYP3A may lead to clinically relevant increases in oxycodone concentrations. In addition, it was noted that the role of the CYP2D6-dependent metabolism of oxycodone is emphasised if the CYP3A4 pathway is inhibited. Although the concomitant use of oxycodone with telithromycin or with miconazole oral gel increased the achieved oxycodone concentrations only moderately, these interactions might have clinical significance in the prolonged use of oxycodone.

Keywords: oxycodone, drug interaction, cytochrome P450, CYP2D6, CYP3A4, opioids

TIIVISTELMÄ

Juha Grönlund

Sytokromi P450 2D6- ja 3A-entsyymien estäjien vaikutukset oksikodonin aineenvaihduntaan

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Sytokromi P450 (CYP)-entsyymit ovat tärkeitä monien lääkeaineiden aineenvaihdunnalle. CYP-entsyymien toiminnan estymisen (inhibitio) tai kiihtymisen (induktio) on todettu olevan keskeisiä mekanismeja farmakokineettisten lääkeyhteisvaikutuksien syntymiselle. Oksikodoni on opioidi-ryhmän kipulääke, jota käytetään lisääntyvästi keskivaikean ja vaikean kivun hoidossa, niin sairaaloissa kuin myös avohoidon potilailla. Koska oksikodonin aineenvaihdunta tapahtuu pääasiassa CYP2D6- ja CYP3A-entsyymien välityksellä, se voi olla altis CYP-entsyymi välitteisille lääkeyhteisvaikutuksille. Tässä tutkimussarjassa selvitettiin CYP2D6- ja CYP3A-entsyymien estäjien vaikutusta oksikodonin aineenvaihduntaan ja lääkevasteeseen käyttämällä satunnaistettua plasebo-kontrolloitua vaihtovuoroista koejärjestelyä terveillä vapaaehtoisilla tehdyissä kliinisissä tutkimuksissa.

Vaikka CYP2D6-entsyymin esto paroksetiinilla vähensi huomattavasti oksikodonin metaboloitumista oksimorfoniksi, se ei lisännyt oksikodonin pitoisuuksia. Itrakonatsolin aiheuttama CYP3A-inhibitio sen sijaan lisäsi suonensisäisesti annostellun oksikodonin altistusta 1.5-kertaiseksi ja suun kautta annostellun oksikodonin altistusta 2.4-kertaiseksi. Paroksetiinin ja itrakonatsolin yhteiskäyttö nosti suonensisäisesti annostellun oksikodonin altistusta 2-kertaiseksi ja suun kautta annostellun oksikodonin altistusta 2.9-kertaiseksi. Telitromysiini nosti oksikodonialtistusta 1.8-kertaiseksi ja mikonatsoli suugeelin käyttö puolestaan 1.6-kertaiseksi. Vaikka nämä farmakokineettiset interaktiot selkeästi lisäsivät oksikodonipitoisuuksia, oksikodonin lääkevasteessa ei terveille vapaaehtoisille annettujen pienten kerta-annosten jälkeen todettu merkittäviä muutoksia.

Tämän tutkimussarjan tulokset osoittavat, ettei CYP2D6-entsyymin estolla liene kliinistä merkitystä, jos oksikodonin CYP3A-välitteinen aineenvaihdunta toimii normaalisti. CYP3A-entsyymien esto sen sijaan voi johtaa kliinisesti merkittäviin oksikodonipitoisuuksien nousuihin. Lisäksi todettiin, että CYP2D6-entsyymin kautta tapahtuvan oksikodonin aineenvaihdunnan merkitys korostuu CYP3A-reitin toiminnan estyessä. Vaikka telitromysiinin ja toisaalta mikonatsolisuugeelin käyttö lisäsi oksikodonipitoisuuksia vain kohtalaisesti, näillä interaktioilla voi olla kliinistä merkitystä, kun oksikodonia annostellaan toistuvasti.

Avainsanat: oksikodoni, lääkeyhteisvaikutus, sytokromi P450, CYP2D6, CYP3A4, opioidit

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ABBREVIATIONS

ANOVA	analysis of variance
$AUC_{(0-t)}$	area under plasma concentration-time curve from zero to t hours
$AUEC_{(0-t)}$	area under effect-time curve from zero to t hours
AUC_m/AUC_p	metabolite-to-parent drug area under plasma concentration-time curve ratio
BBB	blood-brain barrier
CI	confidence interval
CL	plasma clearance
CL/F	apparent oral clearance
C_{max}	peak plasma concentration
CV	coefficient of variation
CYP	cytochrome P450
DDI	drug-drug interaction
DSST	digit symbol substitution test
EDTA	ethylene-diamine-tetra-acetic acid
EM	extensive metabolizer
EMA	European Medicines Agency
F	oral bioavailability
FDA	Food and Drug Administration
GMR	geometric mean ratio
IM	intermediate metabolizer
k_{el}	elimination rate constant
Ki	inhibition constant
LC-MS	liquid chromatography-mass spectrometry
LC-MS/MS	liquid chromatography tandem mass spectrometry
LLQ	lower limits of quantification
NADPH	nicotinamide adenine dinucleotide phosphate
P-gp	p-glycoprotein
PM	poor metabolizer
SPC	summary of product characteristics
SSRI	selective serotonin reuptake inhibitor
$t_{1/2}$	elimination half-life
t_{max}	time to peak concentration
UM	ultrarapid metabolizer
VAS	visual analogue scale
V_{ss}	steady-state volume of distribution
VZ/F	apparent oral volume of distribution during elimination

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text by the Roman numerals I-V. These original works have been reproduced with the permission of the copyright holders.

I Grönlund J, Saari T, Hagelberg N, Martikainen IK, Neuvonen PJ, Olkkola KT and Laine K. Effect of telithromycin on the pharmacokinetics and pharmacodynamics of oral oxycodone. *J Clin Pharmacol* 2010; 50: 101-108.

II Saari TI, Grönlund J, Hagelberg NM, Neuvonen M, Laine K, Neuvonen PJ and Olkkola KT. Effects of itraconazole on the pharmacokinetics and pharmacodynamics of intravenously and orally administered oxycodone. *Eur J Clin Pharmacol* 2010; 66: 387-397.

III Grönlund J, Saari TI, Hagelberg NM, Neuvonen PJ, Olkkola KT and Laine K. Exposure to oral oxycodone is increased by concomitant inhibition of CYP2D6 and 3A4 pathways, but not by inhibition of CYP2D6 alone. *Br J Clin Pharmacol* 2010; 70: 78-87.

IV Grönlund J, Saari TI, Hagelberg NM, Neuvonen PJ, Laine K and Olkkola KT. Effect of Inhibition of Cytochrome P450 Enzymes 2D6 and 3A4 on the Pharmacokinetics of Intravenous Oxycodone: A Randomized, Three-Phase, Crossover, Placebo-Controlled Study. *Clin Drug Investig* 2011; 31: 143-153.

V Grönlund J, Saari TI, Hagelberg N, Neuvonen PJ, Olkkola KT and Laine K. Miconazole oral gel increases the exposure to oral oxycodone by inhibition of CYP2D6 and CYP3A4. *Antimicrob Agents Chemother* 2011; 55: 1063-1067.

1 INTRODUCTION

The use of several drugs concomitantly i.e. polypharmacy is increasingly common in modern medicine. As a consequence, the risk of receiving potentially interacting drugs simultaneously has also increased (Åstrand et al. 2007). Especially the elderly patients with many diseases and with decreased tolerability to adverse effects are at particular risk. The inhibition or induction of hepatic drug metabolizing cytochrome P450 (CYP) enzymes is found to be the major mechanism behind pharmacokinetic drug-drug interactions (DDIs) (Dresser et al. 2000, Pelkonen et al. 2008). The inhibition of CYP enzyme typically results in the increase of plasma concentrations of the drug, enhanced drug effect and increased risk for adverse effects. Although the mechanisms behind many DDIs are well known, interacting drugs are still quite often administered concomitantly in the clinic (Tirkkonen and Laine 2004). Since many of the clinically occurring DDIs are predictable, the adverse drug effects because of DDI are at least partly avoidable if the patients' medication is planned carefully (Pirmohamed et al. 2004).

Oxycodone is an opioid analgesic, synthesized originally from the naturally occurring opium alkaloid thebaine. It was introduced to clinical use almost one hundred years ago. Although oxycodone is an old drug, its basic pharmacology has been poorly investigated, and the new aspects of its metabolism have been discovered only some years ago (Kalso 2005, Olkkola and Hagelberg 2009). After the introduction of immediate- and extended-release oral oxycodone into the market in the 1990s, the global use of oxycodone has increased rapidly (Coluzzi and Mattia 2005, Leong et al. 2009, Hamunen et al. 2009). These new oxycodone preparations have made it possible to use oxycodone in the treatment of chronic pain also in outpatient care settings.

Oxycodone is metabolized mainly in the liver by CYP enzymes. CYP3A and to a lesser degree, CYP2D6 are responsible for the oxidative metabolism of oxycodone (Lalovic et al. 2004). Due to its CYP-mediated metabolism, oxycodone might be prone to drug-drug interactions. Since strong opioids like oxycodone have potential to cause dangerous adverse effect, e.g. respiratory depression, DDIs with oxycodone may have harmful clinical consequences. Because pain patients often have multiple medical problems requiring the use of concomitant medications they are at increased risk for DDIs. Before this study series, the effects of CYP 2D6 and 3A inhibition on the pharmacokinetics of oxycodone have not been systemically studied. Therefore, it was considered important to evaluate the effects of known CYP2D6 and CYP3A inhibitors on the pharmacokinetics and pharmacological effects of oral and intravenous oxycodone.

2 REVIEW OF THE LITERATURE

2.1 Drug metabolism

During the evolution, the human body has developed several mechanisms to protect itself against foreign and possibly harmful xenobiotics. Most drugs used in modern medicine are foreign to the body, and are therefore processed through detoxification processes. These defence mechanisms act at several levels. Firstly, the absorption of the xenobiotics into the body may be restrained by biological membranes or by active efflux transporters. Xenobiotics may be excreted unchanged to the bile or urine and more importantly, they may be metabolized into less harmful compounds that could eventually be more easily excreted out of the body (Nebert and Dieter 2000, Gonzalez et al. 2011).

In order to be effectively absorbed and distributed to the body, many drug molecules are lipophilic. Since the body's excretion mechanisms of lipophilic compounds are rather ineffective, the biotransformation of the drug molecules to more water-soluble forms is needed before they can be excreted effectively. This biotransformation process can be divided in two phases. Phase I reactions are functionalisation reactions where a functional group is added to the parent drug. Phase I reactions include oxidation, reduction and hydrolysis (Testa and Krämer 2006). Of these three reaction types, the oxidation is clearly the most important and is mainly catalysed by cytochrome P450 (CYP) enzymes (Guengerich 2007, Gonzalez et al. 2011).

After a suitable functional group is formed to the substrate by Phase I reactions, the hydrophilicity of the molecule may be further increased in Phase II conjugation reactions by anchoring an endogenous water-soluble molecule like glucuronic acid, glutathione or methyl group to the drug or metabolite molecule (Testa and Krämer 2008). Usually Phase I and Phase II reactions occur in series, but sometimes conjugation can occur without a prior Phase I reaction.

Liver is clearly the most important organ in drug metabolism and concentrations of drug metabolizing enzymes are remarkably high in liver tissue (Pelkonen et al. 2008). Significant amount of drug metabolizing enzymes are also found in the gut wall where they function as a barrier against drugs and other xenobiotics (Kato 2008, Pelkonen et al. 2008). As orally administered drugs have to pass through the gut wall and liver before they can enter into the systemic circulation, a considerable amount of the drug may be metabolised already during this first-pass phase resulting in reduced oral bioavailability of the given drug. This phenomenon is called the first-pass metabolism (Buxton and Benet 2011). Although small amounts of drug metabolizing enzymes are also found in other tissues like brain, kidneys and lungs, the extrahepatic drug

metabolism except the one occurring in gut wall has a minor role in the systemic metabolic clearance of most drugs (Pelkonen et al. 2008).

Metabolites usually have lower activity than parent substrates. However, it is well known that with some drugs the metabolites built up during biotransformation processes are causing a part of the overall drug effect and adverse effects. For example, a strong opioid, morphine, is converted into glucuronide conjugates, morphine-3-glucuronide and morphine-6-glucuronide, the latter being an active metabolite causing some of the noted opioid effects (Lötsch 2005). The opioid effects of commonly used weak opioid codeine are instead dependent on enzymatic metabolism to morphine as codeine itself is nearly inactive (Desmeules et al. 1991).

2.2 Cytochrome P450 (CYP) enzymes

Cytochrome P450 enzymes (CYPs) are a large and diverse superfamily of haem-containing microsomal enzymes found throughout in the nature. Human beings have 57 different CYP genes arranged into 18 families (Nebert and Russell 2002). These genes code for enzymes that have several vital physiological roles. CYP enzymes are identified by family number followed by a capital letter indicating subfamily and a number of the individual enzyme within the subfamily. CYP enzymes are classified into different families and subfamilies on the basis of their similarities in aminoacid sequence (Nebert and Russel 2002). CYP enzymes can exist in different allelic variants causing significant pharmacogenetic heterogeneity between individuals. The allelic variant of the individual CYP enzyme is denoted by adding an asterisk followed by a number for each allelic variant after the enzyme name (www.cypalleles.ki.se).

Catalyzing the biotransformation reactions of drugs and other xenobiotics is undoubtedly the best known function of CYP enzymes. CYPs involved in drug metabolism are concentrated in the endoplasmic reticulum of the liver and in a lesser degree in the enterocytes in the gut wall. Several CYP-enzymes are also expressed in the lung, kidney, skin, brain, adrenal gland and gonads. The expression pattern of CYPs in these different tissues has important physiological roles (Seliskar and Rozman 2007, Pelkonen et al. 2008). In addition to the drug metabolism, CYP enzymes play a central role in several essential processes like the synthesis of fatty acids, cholesterol, bile acids, vitamins and steroid hormones (Nebert and Russel 2002). There are also several CYP enzymes with so far unknown function. It is possible that these enzymes have some kind of a role during the embryogenesis or the fetogenesis (Nebert and Russel 2002).

The CYP enzymes are largely responsible for oxidation, the most important Phase I reaction in drug metabolism. CYP-catalyzed oxidative reaction consists of several steps (Figure 1). At first, the substrate binds to the active site of the enzyme, in close proximity to the ferric form haem group. NADPH-P450 reductase transfers one

electron from NADPH to the complex reducing the ferric haem iron to the ferrous state. The following step is binding of molecular oxygen (O_2) to the ferrous CYP-substrate complex. After the second electron has been transferred from NAPDH to the complex, the substrate is oxidized and the other oxygen atom is reduced to water. Finally, oxidized substrate i.e. the formed metabolite is released. After the substrate has been released from the active site, the enzyme returns to its original state (Lin and Lu 1998, Guengerich 2007). Impairment of some of these aforementioned steps could lead to inhibition of enzyme activity (Lin and Lu 1998).

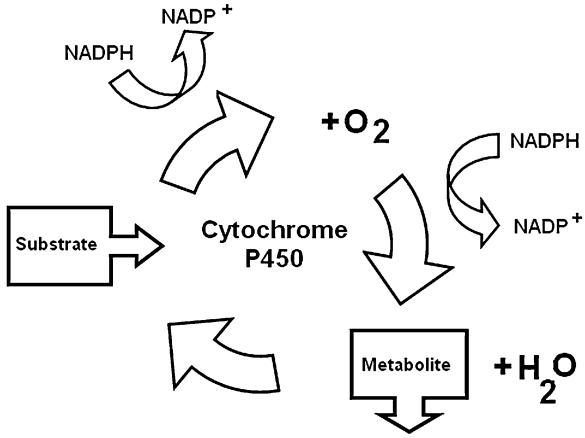


Figure 1. Simplified scheme of the CYP enzyme catalyzed oxidative reaction. NADPH = nicotinamide adenine dinucleotide phosphate.

Only CYP enzymes belonging to the families 1, 2 and 3 are important in the oxidative drug metabolism (Pelkonen et al. 2008). Although each of these enzymes shows some degree of unique substrate specificities there is also considerable overlapping. Therefore, the same substrate may be metabolized by more than one CYP enzyme. Overall, it is estimated that these enzymes are responsible for 75% of all drug metabolism (Guengerich 2008).

2.2.1 CYP1 family

There are three members in the CYP1 family: CYP1A1, CYP1A2, and CYP1B1. CYP1A1 and CYP1B1 are found in varying amounts in different extrahepatic tissues, and are efficient in metabolizing polycyclic aromatic hydrocarbons such as those found in cigarette smoke and charcoal grilled food (Nebert and Russel 2002). CYP1A2 is found most abundantly in the liver and it accounts for more than 10% of total liver CYP content (Pelkonen et al. 2008). CYP1A2 substrates include e.g. caffeine (Faber et

al. 2005), lidocaine (Wang et al. 2000) and melatonin (Härtter et al. 2001). CYP1A2 is inhibited, for example, by fluvoxamine (Brøsen et al. 1993b). Cigarette smoking is known to induce the CYP1A2 enzyme (Zevin and Benowitz 1999).

2.2.2 CYP2 family

The CYP2 family is a large group of CYP enzymes divided into 13 subfamilies (Nebert and Russel 2002). The substrate and tissue specificities of these enzymes differ considerably. Subfamilies CYP2A, CYP2B, CYP2C, CYP2D and CYP2E together constitute over 40% of total liver CYP content (Pelkonen et al. 2008). Enzymes CYP2C9, CYP2C19 and CYP2D6 are important for drug metabolism and it is estimated that they metabolise altogether over 40% of the commonly used drug molecules (Johnson 2008, Guengerich 2008). Clinically significant genetic CYP polymorphisms exist for CYP2B6, CYP2C9, CYP2C19 and CYP2D6 enzymes (Kirchheiner and Seeringer 2007, Zanger et al. 2008).

CYP2B6

The role of CYP2B6 in drug metabolism was earlier thought to be minor. However, today it is known that, for example, bupropion, methadone and ketamine are mainly or partially metabolized by CYP2B6 (Turpeinen et al. 2006, Pelkonen et al. 2008). The known inhibitors of CYP2B6 include clopidogrel and ticlopidine (Turpeinen et al. 2005).By using bupropion as a probe drug, rifampicin and carbamazepine have been found to induce CYP2B6 (Pelkonen et al. 2008).

CYP2C8

The importance of CYP2C8 for the metabolism of drugs has also been discovered only recently. CYP2C8 shares many of its substrates with CYP2C9 and CYP3A4. Substrates of CYP2C8 include antidiabetic drugs repaglinide and rosiglitazone and anticancer drug paclitaxel (Totah and Rettie 2005, Pelkonen et al. 2008). The activity of CYP2C8 has been found to be inhibited by gemfibrozil, trimethoprim and induced by rifampicin (Totah and Rettie 2005).

CYP2C9

CYP 2C9 is the predominant CYP2C form in the liver accounting for up to 20 % of liver CYP proteins (Pelkonen et al. 2008). CYP2C9 is responsible for the oxidative metabolism of roughly 16% of all drugs on the market including many clinically relevant drugs like warfarin and phenytoin (Miners and Birkett 1998, Johnson 2008).

Fluconazole and amiodarone are commonly used drugs that act as inhibitors of this enzyme whereas rifampicin is a potent inducer of CYP2C9 (Miners and Birket 1998, Pelkonen et al. 2008). As warfarin has a narrow therapeutic index and potentially dangerous adverse effects, its interactions are clinically very important.

CYP2C19

CYP2C19 participates in the metabolism of several commonly used drugs e.g. diazepam, citalopram, phenytoin, and omeprazole. However, many of these drugs are not specific substrate for CYP2C19 and are also metabolized by other CYP enzymes (Pelkonen et al. 2008). In addition, being a substrate for CYP2C19, omeprazole also acts as an inhibitor of this same enzyme (Funck-Brentano et al. 1997). Fluconazole and miconazole have been shown to be inhibitors of CYP2C19 (Niwa et al. 2005b).

CYP2D6

Although CYP2D6 only accounts for up to 2% of the liver CYP content (Shimada et al. 1994), it has an important role in drug metabolism. It is estimated that it accounts for from 12% up to 25% of all drug metabolism (Ingelman-Sundberg et al. 2007, Johnson 2008, Guengerich 2008). CYP2D6 substrates include ß-adrenoceptor antagonist metoprolol, several antidepressants such as fluoxetine and paroxetine as well as neuroleptics like risperidone (Ingelman-Sundberg 2005, Pelkonen et al. 2008). Regarding opioids, tramadol (Poulsen et al. 1996), codeine (Desmeules et al. 1991) and oxycodone (Lalovic et al. 2004) are partly metabolized by CYP2D6. Inhibitors of CYP2D6 include for example some of its own substrates e.g. paroxetine and fluoxetine. Although quinidine is not a substrate for CYP2D6 it is a strong CYP2D6 inhibitor and therefore widely used in interactions studies (Pelkonen et al. 2008). CYP2D6 is the only CYP enzyme not inducible by other drugs.

The genetic polymorphism of CYP2D6 causes a wide variability in CYP2D6 enzyme activity and has great importance for the metabolism of substrate drugs of this enzyme (Ingelman-Sundberg 2005, Kirchheiner and Seeringer 2007). Over 70 different functional CYP2D6 gene variants have been described (www.cypalleles.ki.se). Variant alleles are divided into alleles related to increased (ultrarapid metabolizer, UM), normal (extensive metabolize, EM), decreased (intermediate metabolizer, IM) or severely impaired (poor metabolizer, PM) enzyme activity (Ingelman-Sundberg et al. 2007, Sistonen et al. 2007). The most important alleles causing the PM genotype are CYP2D6*4 and CYP2D6*5 whereas CYP2D6*10, CYP2D6*17 and CYP2D6*41 are related to the clearly reduced enzyme activity. Alleles carrying multiple active gene copies cause ultrarapid enzyme activity (UM) (Ingelman-Sundberg et al. 2007). There are considerable interethnic differences in CYP2D6 distribution. In Western Europe approximately 7% of the population is classified as PMs and 5.5% as UMs

(Eichelbaum et al. 2006). However, in Scandinavians the frequency of UMs is only 1% (Dahl et al. 1995) whereas in North Africa and Oceania frequency of UMs is even over 20% (Ingelman-Sundberg et al. 2007, Sistonen et al. 2007). In UMs the metabolism of the substrate drug is accelerated and may lead to ineffective therapy (Dahl 2002, Kawanishi et al. 2004). On the contrary, extensive bio-activation of prodrugs like codeine and tramadol may lead into increased exposure to active metabolite and cause adverse effect as evidenced by several case reports (Gasche et al. 2004, Stamer et al. 2008, Ciszkowski 2009). In PMs there is a risk of accumulation of substrate drugs or ineffective treatment with prodrugs needing bio-activation to active metabolite (Zhou 2009). Similar effect can be seen in EMs with the concomitant administration of drugs inhibiting CYP2D6 activity (Laugesen et al. 2005).

The determination of the subject's CYP2D6 phenotype has traditionally been made by administering the probe drug known to be CYP2D6 substrate and by measuring the ratio of unchanged drug to metabolite from collected urine or blood samples (McElroy et al. 2000). As an alternative to this time consuming method, a genotyping method, which allows the detection of the most relevant polymorphic positions, is available and can be used to predict the subject's phenotype (McElroy et al. 2000, Sistonen et al. 2005). Development in the gene chip technology has made it possible to determine the subject's CYP2D6 genotype rapidly enough to be utilized also in clinical practise (Heller et al. 2006).

2.2.3 CYP3 family

The CYP3 family contains only one subfamily (CYP3A) with four members (CYP3A4, CYP3A5, CYP3A7 and CYP3A43) (Nebert and Russel 2002, Pelkonen et al. 2008).
CYP3A7 is expressed mainly in fetal liver (Nebert and Russel 2002). However, it is now apparent that CYP3A7 is expressed in a varying degree also in adult liver (Sim et al. 2005, Daly 2006). Since CYP3A7 has a high catalytic activity for some endogenous substrates like retinoic acids it might play an important role in fetal development (Daly 2006). It is not known if CYP3A7 found in some adult livers contributes to CYP meditated drug metabolism significantly. Expression of CYP3A43 in the liver is very low, but there is a notable extrahepatic expression in the prostate and testis (Daly 2006). The physiological role of CYP3A43 in these tissues is not yet fully understood but it is believed that it might be a non-functional enzyme (Westlind et al. 2002). In any case, it has no significant role in drug metabolism.

CYP3A4 and 3A5

CYP3A4 accounts for 30% of the hepatic CYP content and is therefore the most abundantly expressed hepatic CYP enzyme (Shimada et al. 1994). It is estimated that

CYP3A4 is responsible for up to 50% of all drug metabolism (Johnson 2008, Guengerich 2008). Besides being the major contributor in hepatic drug metabolism, CYP3A4 is the most abundantly expressed CYP enzyme in the small intestine where it contributes markedly to the first pass metabolism of several orally administered drugs (Paine et al. 2006, Kato 2008). The active site of CYP3A4 enzyme is able to bind wide ranges of structurally diverse substrate molecules. Among the substrates of CYP3A4 there are several clinically important drugs e.g. anxiolytics midazolam and alprazolam, lipid-lowering agents simvastatin and atorvastatin, cardiovascular drugs felodipine and nifedipine, as well as the immunosuppressant cyclosporine (Pelkonen et al. 2008). CYP3A4 takes part in the metabolism of strong opioids fentanyl, alfentanil, oxycodene and methadone (Feierman and Lasker 1996, Ferrari et al. 2004, Lalovic et al. 2004, Klees el al. 2005, Trescot et al. 2008). Many drugs have been shown to affect the activity of CYP3A4 enzyme, either through induction or inhibition. The most well studied inhibitors of CYP3A4 include azole antifungal agents and some macrolide antibiotics (Pelkonen et al. 2008). Many clinically significant drug interactions have been reported after concomitant use of CYP3A4 substrates and inhibitors (Olkkola et al. 1993, Backman et al. 1998, Rowan et al. 2009). CYP3A4 is also known to be inducible. The most important inducers include antiepileptic drugs phenytoin and carbamazepine and antibiotic rifampicin (Liu et al. 2007). In addition to concomitantly administered drugs, the CYP3A4 activity may be induced or inhibited by some herbal products (e.g. St. John's wort) and food substances (e.g. grapefruit) (Kupferschmidt et al. 1995, Lilja et al. 1998, Wang et al. 2001). Although over 20 different CYP3A4 variant proteins have been described, they are found with so low a frequency that they can not be responsible for the clearly more commonly seen interindividual differences in CYP3A4 activity (Ingelman-Sundberg et al. 2007). Therefore, other genetic, environmental and physiological factors must be contributing.

CYP3A5 shows 84% amino-acid sequence similarity with CYP3A4 and substrate specificities of these enzymes are highly overlapping (Williams et al. 2002). CYP3A5 hepatic expression is polymorphic and it is found only in 10-20% of human livers (Westlind-Johnsson et al. 2003, Daly 2006). In livers where CYP3A5 is expressed, it accounts for at least 10-20% of total CYP3A content, although different studies have shown rather conflicting results (Kuehl et al. 2001, Westlind-Johnsson et al. 2003, Daly 2006). CYP3A5 is also expressed in considerable amount in several extrahepatic tissues like small intestine and kidney (Daly 2006). There are several in vivo studies demonstrating that the dose requirements of the immunosuppressant drug tacrolimus are influenced by genotype-dependent CYP3A5 expression levels (Daly 2006). On the other hand, in vivo studies with midazolam, a standard probe drug for CYP3A activity, have shown opposite results (Floyd et al. 2003, Kharasch et al. 2007). Thus, the contribution of CYP3A5 to overall drug metabolism is not clear.

2.3 Inhibition of CYP enzymes

The inhibition of CYP enzyme may be reversible, quasi-irreversible or irreversible. Reversible inhibition is probably the most common type of enzyme inhibition in drug interactions. Reversible inhibition can be roughly divided further into competitive, non-competitive and uncompetitive (Hollenberg 2002). Competitive inhibition occurs when two or more substrates of the same enzyme compete with each other for binding to the active site of the CYP enzyme. The available drug amounts and the affinity of drugs to the enzyme determine the intensity of inhibition. *In vitro* determined inhibitory constant (Ki) can be used to describe the potency of the inhibitor. The lower the Ki, the more potently the drug acts as an inhibitor. To achieve enzyme inhibition *in vivo*, the achieved concentrations of the inhibitor must be higher than Ki (Thummel and Wilkinson 1998). In non-competitive inhibition the inhibitor and the substrate are binding to different locations in the CYP enzyme, whereas in uncompetitive inhibition the inhibitor is able to bind only to the enzyme-substrate complex (Hollenberg 2002, Pelkonen et al. 2008). The onset of reversible enzyme inhibition is rapid and inhibition is only transient ceasing when the inhibitor has been eliminated from body (Lin and Lu 1998, Hollenberg 2002).

Quasi-irreversible inhibition is caused by the formation of metabolic intermediate complex where an inhibitor molecule is tightly bound to the active site of the CYP enzyme. Although the quasi-irreversible inhibition is reversible *in vitro,* in *in vivo* conditions these intermediate complexes are so stable, that enzyme activity can be restored only by the synthesis of new enzymes. In clinical context, the inhibition is therefore irreversible (Lin and Lu 1998, Hollenberg 2002).

Irreversible inhibition is often called mechanism-based inhibition. In this type of inhibition the inhibitor molecule is bound to the enzyme in irreversible manner or it is causing permanent alterations to enzyme structure thereby inactivating the enzyme. The resulting inhibition is longlasting and is not reversed until new active enzymes are synthesized. When the inhibition mechanism is quasi-irreversible or irreversible, the inhibition is possible at significantly lower plasma concentrations than the Ki, and Ki value correlates therefore poorly with the inhibitory potency of the inhibitor (Lin and Lu 1998).

2.4 Induction of CYP enzymes

During a long-term exposure to some xenobiotics the body may enhance metabolic activity by inducing CYP enzymes involved, in order to eliminate the xenobiotic faster from the body. The CYP enzyme induction is usually mediated by a group of ligand-activated transcription factors resulting in an increase in the synthesis of the CYP enzymes (Pelkonen et al. 2008). However, the amount of the metabolizing enzymes

can also be increased by stabilizing the enzyme protein leading to longer life spans of CYP enzymes (Hollenberg 2002). Due to its mechanism, the induction is a slow process, and clinically relevant induction is seen only after multiple doses of the inducing compound (Lin and Lu 1998). It may take up to two weeks to reach the maximum induction. In a similar way, the normalization of the CYP enzyme activity after the withdrawal of inducer may take several days or weeks. Well known CYP inducers include rifampicin, carbamazepine and phenytoin (Pelkonen et al. 2008).

2.5 CYP mediated drug-drug interactions

The inhibition or induction of the CYP enzymes has been found to be the major mechanism behind pharmacokinetic drug-drug interactions (DDIs) (Dresser et al. 2000, Pelkonen et al. 2008). The examination of prescription databases has revealed that the substrates and inhibitors of the CYP enzymes are co-prescribed rather commonly (Tirkkonen and Laine 2004, Molden et al. 2005). Although the inhibition of the CYP enzymes usually results in an increased drug effect, the situation is not so simple when active metabolites or more than one metabolic route are present. The clinical effects of pro-drugs are fully due to active metabolites and the inhibition of their formation may therefore cause a decreased or totally absent drug effect. Besides the resulting change in the exposure to the active moiety of the substrate drug, the clinical significance of the CYP mediated DDIs depends on the therapeutic index of the substrate drug (Dresser et al. 2000).

Prediction of drug-drug interactions

As new and more precise techniques have been developed for studying enzyme inhibition and induction *in vitro*, the interest is increasing in models able to predict *in vivo* interactions based on *in vitro* data. Although these methodologies are very tempting, they contain several simplifications and inaccuracies leading to false predictions in some situations. Predictions of *in vivo* interactions are generally based on *in vitro* determined Ki value. However, there is considerable variability in reported Ki values in literature depending on, for example, the used substrate and inhibitor concentrations and the mechanism of inhibition studied. In addition, the true clinically achieved free inhibitor concentration in the site of action (i.e. in hepatocytes and enterocytes) can not be deduced from *in vitro* studies. Predictions are even more complicated and prone to errors with drugs that have notable first pass metabolism by intestinal CYP enzymes, or have active metabolites or are substrates for transport proteins (Bachmann 2006, Hisaka et al. 2010). Therefore, albeit *in vitro* studies are an invaluable tool in screening for potential interactions of new drugs and in allocating further studies to the most relevant enzymes, the *in vivo* drug interaction studies are still needed to form a precise and quantitative picture of the DDI.

Guidelines on investigation of CYP mediated drug-drug interactions

Guidelines on the investigation of the CYP mediated drug interactions have been published by European Medicines Agency (EMA) as well as by Food and Drug Administration (FDA) (EMA 2010, Huang et al. 2007). According to these guidelines the CYP enzymes responsible for the metabolism of an investigational drug should be first identified *in vitro*. Negative findings from *in vitro* studies can eliminate the need for further studies. If some metabolic route is responsible for more than 25% of the total clearance of drug, *in vivo* DDI studies should be conducted (EMA 2010). *In vivo* drug interaction studies are also needed when active metabolites formed by some metabolic pathway are assumed to be significantly contributing to the overall pharmacological activity of the investigational drug (EMA 2010). To study the possible interaction *in vivo,* it is recommended that the strongest inhibitors or inducers should be used to reach the maximum effect on the metabolism of investigational drug. The list of recommended inhibitors and inducers for specific enzymes are given in these guidelines (Huang et al. 2007, EMA 2010). If the results are negative in studies conducted with the recommended strong inhibitor or inducer, then the absence of a clinically important metabolic DDI is demonstrated. However, if the study results are positive then further clinical studies with less potent inhibitors or inducers will be valuable. Drugs that may often be co-administered in the clinic will be of special interest.

2.6 Oxycodone

2.6.1 Background

Oxycodone is a semisynthetic opioid analgesic, synthesized originally from the naturally occurring opium alkaloid thebaine. It was introduced into clinical use in 1917 in Germany (Kalso 2005). There have been considerable differences in the use of oxycodone between the countries. While in the Northern Europe oxycodone was used mainly for the treatment of acute severe pain, in Australia and the United States it was first used as a combination drug with acetaminophen for the treatment of moderate pain (Pöyhiä et al. 1993, Coluzzi and Mattia 2005, Kalso 2005). After the introduction of immediate- and extended-release oral oxycodone into the market in the 1990s the global use of oxycodone has increased rapidly (Coluzzi and Mattia 2005, Leong et al. 2009, Hamunen et al. 2009). These new preparations made it possible to use oxycodone for large groups of patients formerly treated mainly with morphine. As a result of this sharp increase in oxycodone consumption also the abuse of oxycodone has increased (Cicero et al. 2005). According to the recent studies, the overall opioid consumption is increasing. This rise has been associated with clear change in opioid prescription pattern showing an increased consumption of oxycodone and a decreased consumption of morphine (Leong et al. 2009, Hamunen et al. 2009). Some studies have reported fewer hallucinations, itching and nausea with oxycodone compared with morphine, which was formerly considered a golden standard among strong opioids

(Heiskanen and Kalso 1997, Mucci-LoRusso et al. 1998). However, also other factors like aggressive marketing of certain opioids have probably contributed to the change in prescription patterns of opioids (Olkkola and Hagelberg 2009, Hamunen et al. 2009, Lemberg et al. 2009, Bandieri et al. 2009). In Finland, oxycodone has been the most widely used strong opioid for decades (Pöyhiä 1994a, Hamunen et al. 2009).

2.6.2 Pharmacology and pharmacokinetics

Oxycodone is a weak base with the dissociation constant (pKa) of 8.5. The lipid solubility of oxycodone and morphine are considered almost equal, although a somewhat higher calculated Log Octanol / water partition coefficient (ClogD) values indicating higher lipid solubility is reported for oxycodone (Pöyhiä et al. 1993, Pöyhiä and Seppälä 1994b, Lemberg et al. 2008). Commercially oxycodone is available as hydrochloride salt. Orally oxycodone can be administered as immediate- or extended-release tablets and capsules or as oral mixture. The parenteral route of administration may be used by giving oxycodone as intravenous and intramuscular injections or as subcutaneous and intravenous infusions.

After the oral ingestion of immediate-release oxycodone the peak concentration (C_{max}) is achieved in 1-1.5 hours (Leow et al. 1992, Pöyhiä et al. 1992, Reder et al.1996, Lalovic et al. 2006). With extended-release oxycodone the time to reach the peak concentration (t_{max}) is moderately longer (t_{max} 2.3-3.2 h) (Benziger et al. 1996, Reder et al.1996, Heiskanen et al.1998). Oxycodone has an oral bioavailability of 60% to 87% (Pöyhiä et al. 1992, Leow et al. 1992, Nieminen et al. 2009). In validation studies for extended-release oxycodone the administration of 5mg of immediate-release oxycodone every 6 hours was found to be bioequivalent with the administration of 10 mg of extended-release oxycodone every 12 hours (Reder et al. 1996). When above mentioned dosages were used, the steady state was reached in approximately 1 day (Reder et al. 1996). Fatty meals slightly decrease the achieved C_{max} and marginally enhance the oral bioavailability of immediate-release oxycodone while extended-release oxycodone is not affected (Benziger et al. 1996). Bioavailabilities of 62% and 46% have been reported after rectal and nasal administration of oxycodone respectively (Leow et al 1995, Takala et al. 1997).

38-45% of oxycodone is bound to the plasma proteins, mainly to albumin (Leow et al. 1993, Pöyhiä and Seppälä 1994b). The volume of distribution after the single dose of intravenous oxycodone is reported to be 2.4-3.0 l/kg (Pöyhiä et al. 1991, Leow et al. 1995, Kirvelä et al.1996). In the mentioned studies the plasma clearance of oxycodone is found to be 0.76-1.1 l/min. The elimination half-life ($t_{1/2}$) of oxycodone is 3.0 -3.8 and there is no difference in the values between oral or intravenous administration (Pöyhiä et al. 1991, Leow et al. 1992, Leow et al. 1995, Lalovic et al. 2006, Nieminen et al. 2009).

2.6.3 Metabolism

Oxycodone is extensively metabolized mainly in liver and only about 10% is excreted unchanged or in conjugated form in the urine (Pöyhiä et al. 1992, Lalovic et al. 2006). The predominant oxidative metabolic pathway of oxycodone is CYP3A mediated N-demethylation to noroxycodone (Heiskanen et al. 1998, Lalovic et al. 2004, Lalovic et al. 2006), (Figure 2). In addition to the liver, CYP3A is found in the gut wall and small part of the CYP3A mediated metabolism of oxycodone takes place in the enterocytes leading to the first-pass effect after oral administration (Lalovic et al. 2004). According to the amount of urinary metabolites, CYP3A accounts for about 45% of the metabolism of a single oxycodone dose (Lalovic et al. 2006). In addition to CYP3A4, the polymorphically expressed CYP3A5 enzyme may be contributing essentially to the noroxycodone formation in some individuals (Lalovic et al. 2004). Approximately 10% of oxycodone is O-demethylated to oxymorphone in reaction catalyzed by CYP2D6 (Lalovic et al. 2004, Lalovic et al. 2006). Oxymorphone may be further converted to the secondary metabolite noroxymorphone by CYP3A4 and CYP2D6 (Figure 2). However, this metabolic route is responsible for only a minority of noroxymorphone formation. The principal route of noroxymorphone formation is O-demethylation of noroxycodone by CYP2D6 (Lalovic et al. 2004), (Figure 2). The relative abundance of different metabolites in circulation may be expressed by using a metabolite-to-parent drug area under plasma concentration-time curve ratio (AUC_m/AUC_p). AUC_m/AUC_p values of 0.97 to 1.19 and 0.01 to 0.05 have been reported to noroxycodone and oxymorphone respectively after a single oral dose of oxycodone (Kaiko et al. 1996, Heiskanen et al. 1998, Lalovic et al. 2006, Nieminen et al. 2009). For the secondary metabolite noroxymorphome the reported AUC_m/AUC_p values are 0.25 to 0.64 (Pöyhiä et al. 1992, Lalovic et al. 2006, Nieminen et al. 2009). Most of the excreted oxymorphone in the urine is in conjugated form whereas noroxycodone is excreted in the urine mainly in unconjugated form. Noroxymorphone is excreted mainly in conjugated form (Lalovic et al. 2006).

In addition to the oxidative metabolism, a smaller fraction of oxycodone undergoes reductive metabolism to α- and β- oxycodol accounting for about 8% of the dose. Similarly, the reduction of primary metabolites noroxycodone and oxymorphone to α- and β-noroxycodol and α- and β-oxymorphol respectively, are possible (Figure 2). Altogether these reductive metabolites in urine account for about 18% of the oxycodone dose (Lalovic et al. 2006).

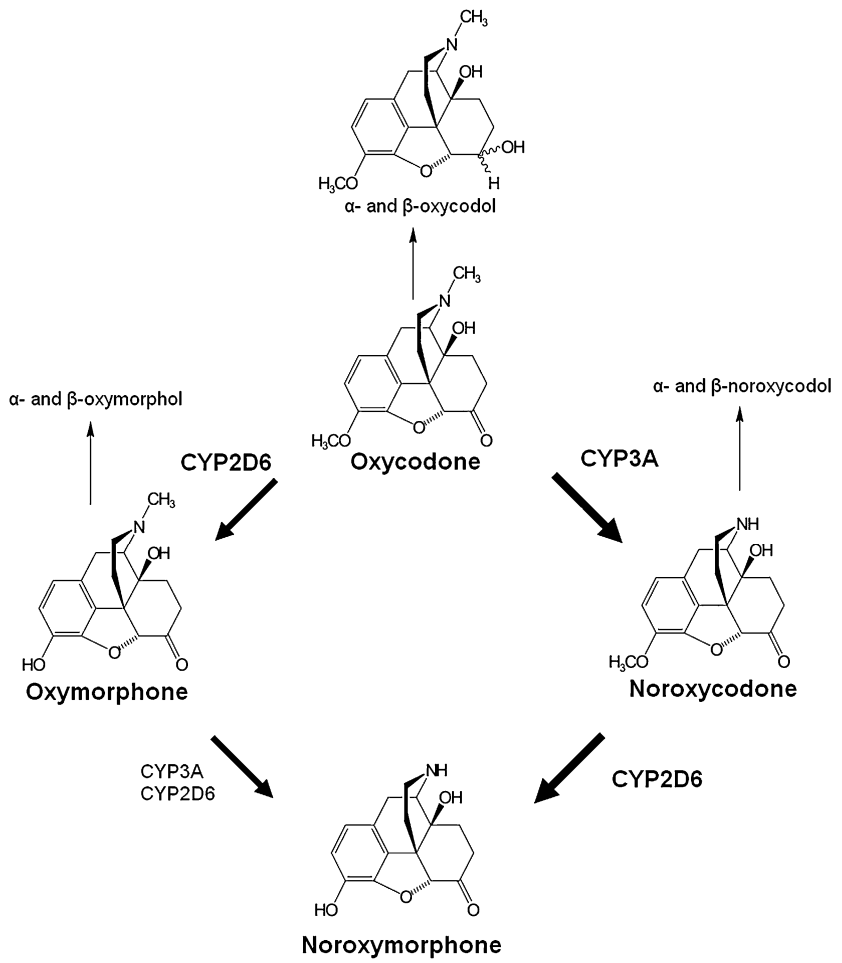


Figure 2. The metabolic scheme of oxycodone. Modified from Lalovic et al. 2006.

2.6.4 The impact of patient characteristics on the pharmacokinetics of oxycodone

The activity of drug metabolizing enzymes is at least partly genetically determined. In addition to the genetics also other ordinary patient characteristics like gender, age and concurrent diseases may contribute to the pharmacokinetic variation. Two studies by Liukas et al. have demonstrated that surgical patients in the age group of 70-90 years have higher exposure to the oxycodone after a single oral or intravenous dose due to the decreased clearance of oxycodone when compared with younger patients (Liukas et al. 2008, Liukas et al. 2011). On contrary, Pesonen et al. (2009) found that the plasma

concentrations of oxycodone were similar in patients older than 75 years and in patients younger than 60 years after cardiac surgery. However, older patients had less pain and were more sedated indicating that they were more sensitive to the opioid effects. In another study with 71 healthy middle-aged (35-55 years) and older (> 65 years) adults, no age related differences were found in pharmacokinetics or pharmacodynamics of a single 10 mg dose of immediate-release oxycodone (Cherrier et al. 2009). In the study by Kaiko et al. (1996) 20 mg of extended-release oxycodone was given to 28 healthy subjects divided into the four groups according age and gender (young and old female and male subjects). Although the mean concentration-time curves for oxycodone suggested that older females would have the greatest exposure to oxycodone, no statistically significant differences were detected between age groups.

The above mentioned study by Kaiko et al. (1996) suggested that clearance of oxycodone would have been about 25% lower in females leading to the higher exposure to the oxycodone. However, the oxycodone dose was not weight-adjusted and the results were not statistically significant. Moreover, neither of two studies by Liukas et al. nor the one by Cherrier et al. observed any gender associated differences in pharmocokinetics of oxycodone (Liukas et al. 2008, Cherrier et al. 2009, Liukas et al. 2011). Several pharmacodynamic studies conducted mainly with morphine have suggested that μ-opioids might have more pronounced effects on women (Niesters et al. 2010).

Elimination of oxycodone is impaired in renal failure since the excretion of its metabolites is severely hindered. Kirvelä et al. (1996) studied the pharmacokinetics of intravenous oxycodone in patients with end-stage renal disease undergoing renal transplantation and found that the half-life of oxycodone was prolonged and inter-individual variation in pharmacokinetics was markedly greater when compared with the healthy control patients. Based on its physicochemical properties, oxycodone is likely to be dialyzable to some extent (Dean 2004, Lee and Cooper 2005).

Hepatic failure impairs the metabolic elimination of oxycodone and increases the exposure to the oxycodone. Kaiko (1997) compared the pharmacokinetics of a single dose of the extended-release oxycodone in patients with hepatic impairment and in matched controls (12 patients per group). It was reported that exposure to oxycodone was 1.9-fold in patients with hepatic impairment and that the elimination half-life was prolonged by two hours. The degree of hepatic impairment was not reported. In the study by Tallgren et al. (1997) the pharmacokinetics of intravenous oxycodone was studied in six patients with end-stage liver cirrhosis before and after liver transplantation. Before the transplantation the clearance of oxycodone was severely impaired and the median elimination half-life was as long as 13.9 hours. Three to four weeks after liver transplantation the pharmacokinetic parameters of oxycodone were similar to the results reported in healthy subjects.

2.6.5 Pharmacodynamics

Oxycodone acts trough specific opioid receptors that also serve as target receptors for the endogenous opioid peptides (e.g. endorphins). Opioid receptors belong to the group of G-protein coupled receptors and they have a common general structure consisting of an extracellular N-terminus, intracellular C-terminus and seven trans-membrane helices. There are three major subtypes of opioid receptors the μ - (mu), δ- (delta) and κ- (kappa) opioid receptors. Multiple mu (Pasternak 2010), delta (Traynor and Elliot 1993, Zaki et al. 1996) and kappa (Traynor 1989) receptor types have been proposed from a number of *in vitro* and *in vivo* experiments. In addition, an opioid receptor-like protein (ORL) has been detected (Waldhoer et al. 2004, Yaksh and Wallace 2011). Although the general structure of opioid receptors is similar, there are also divergent features, particularly in the extracellular terminus resulting in differences in the opioid binding sites of receptors. Therefore, the different opioid receptors have some degree of ligand selectivity but considerable overlapping also exists (Waldhoer et al. 2004, Yaksh and Wallace 2011). There are some experimental pain studies indicating that the divergency in the clinical profile of different opioids may be partly explained by the differences in their receptor subtype specificity (Andresen et al. 2010). The opioid receptors are found in abundance in the brain and in the spinal cord (Yaksh and Wallace 2011). To a lesser degree, the opioid receptors are expressed also in peripheral tissues. Inflammatory states may induce opioid receptor expression in periphery (Stein et al. 2003).

Ligand binding to the opioid receptor leads to conformational changes in G-protein complex, which in turn triggers several intracellular signalling pathways. The major intracellular consequences of opioid receptor activation are inhibition of adenylyl cyclase activity leading to the decrease of intracellular cyclic adenosine monophosphate (cAMP), the reduced opening of voltage gated Ca^{++} channels, the increased K^{+} efflux leading to the hyperpolarization of the cell membrane and the blocking of the release of pain neurotransmitters such as substance P and glutamate. Together these mechanisms attenuate the neuronal activity resulting in analgesia and other opioid effects (Stein et al. 2003, Yaksh and Wallace 2011). Agonists for the different opioid receptors show a slight divergence in their typical effects (Table 1).

Table 1. Pharmacologic actions of opioid receptors (Trescot et al. 2008, Fukuda 2005)

Receptor type	Pharmalogical effects of typical receptor agonist
μ	Analgesia, sedation, vomiting, respiratory depression, euphoria, constipation, physical dependence, pruritus, miosis
δ	Analgesia, dysphoria
κ	Analgesia, sedation, respiratory depression, euphoria, diuresis, dysphoria

Lalovic et al. (2006) clearly demonstrated that oxycodone and all of its metabolites are selective for the μ-opioid receptor. When compared with the μ-opioid receptor, the affinity for the κ-opioid receptor was negligible. Parallel findings of the receptor selectivity of oxycodone have been reported by Narita et al. (2008). By contrast, another study group has suggested that antinociceptive effects of oxycodone are at least partly mediated by κ-opioid receptors (Ross and Smith 1997, Nielsen et al. 2007). Experimental multi-modal pain studies in humans have shown that oxycodone might be more effective than morphine in the treatment of visceral pain (Staahl et al. 2006, Staahl et al. 2007). Authors proposed that the different effects on the κ –opioid receptor might be one explanation for the differences in the analgesic profiles of these two opioids. However, in a recent clinical study, where pancreatic cancer pain was used as a model of clinical visceral pain, oxycodone and morphine provided similar analgesia and adverse effects (Mercadante et al. 2010). In rats the antinociceptive effects of oxycodone have shown to be antagonized by μ-opioid receptor antagonist naloxone, but not by the selective κ- antagonist nor-binaltorphine, indicating that the analgesic effects of oxycodone are not mediated by the κ-opioid receptors (Lemberg et al. 2006). Oxycodone has 5-10 times lower affinity for the μ-opioid receptor when compared with morphine (Lalovic et al. 2006, Narita et al. 2008). The clinical effect profile of oxycodone is generally considered typical for the μ-opioid receptor agonist (Kalso 2007).

The role of metabolites

Since all oxidative metabolites of oxycodone possess some μ-opioid receptor activity, their contribution to the overall opioid effects of oxycodone has been an interesting field of research. Especially the oxymorphone, which is formed by genetically polymorphic CYP2D6 enzyme, has been in special focus. However, according to the present knowledge the parent drug oxycodone seems to be mainly responsible for opioid effects seen after oxycodone administration (Kaiko et al. 1996, Heiskanen et al. 1998, Lalovic et al. 2006, Lemberg et al. 2010)

Oxymorphone

Receptor binding studies have shown that oxymorphone affinity for the μ-opioid receptor is over 40-fold higher than that of parent drug oxycodone and 4- to 9-fold higher when compared with the morphine (Thompson et al. 2004, Peckham and Traynor 2006, Lalovic et al. 2006). Oxymorphone itself is an effective analgesic in humans and it is available for clinical use in many countries (Gimbel and Andieh 2004). However, the plasma concentrations of oxymorphone after administration of oxycodone are very low or even not measurable (Pöyhiä et al. 1992, Kaiko et al. 1996, Heiskanen et al. 1998, Lalovic et al. 2006, Nieminen et al. 2009, Zwisler et al. 2010a, Lemberg et al. 2010). Lipophilicity of oxymorphone is lower than that reported for

oxycodone (Peckham and Traynor 2006). In addition, animal studies have shown that the ability of oxymorphone to penetrate across the blood-brain barrier is poorer than that of oxycodone (Lalovic et al. 2006). Several human studies have suggested that the contribution of oxymorphone to the overall opioid effect of oxycodone is minor (Kaiko et al. 1996, Heiskanen et al. 1998, Lalovic et al. 2006, Lemberg et al. 2010).

Noroxycodone

Noroxycodone is the major metabolite of oxycodone. Its plasma concentrations in the elimination phase are occasionally even higher than that of oxycodone and AUC_m/AUC_p values over unity have been reported (Pöyhiä et al. 1992, Lalovic et al. 2006, Nieminen et al. 2009). In contrast to oxymorphone it is found mainly in unconjugated form. Noroxycodone has four times poorer affinity for the μ-opioid receptor compared with oxycodone (Lalovic et al. 2006). Lemberg et al. (2006) studied the antinociceptive effects of oxycodone and its metabolites after subcutaneous or intrathecal administration to rats and found that noroxycodone has only a poor antinociceptive effect even after a high dose. Similar results have been reported previously after intracerebroventricular administration of noroxycodone to rats (Leow and Smith 1994). According to the brain distribution study in rats, noroxycodone has a low brain-to-plasma ratio indicating poor capability to penetrate across the blood-brain barrier (Lalovic et al. 2006). Therefore, although noroxycodone is abundantly present in the blood circulation, it is unlikely that it would have clinically relevant role in central opioid effects of oxycodone.

Noroxymorphone

In humans, the plasma concentration of secondary metabolite noroxymorphone has been found to be relatively high after orally administered oxycodone. The range for the reported AUC_m/AUC_p values is 0.25 to 0.64 (Pöyhiä et al. 1992, Lalovic et al. 2006, Nieminen et al. 2009). After intravenous administration, the achieved plasma concentrations have been clearly lower (Nieminen et al. 2009). Noroxymorphone has approximately three-fold higher affinity for the μ-opioid receptor when compared with that of oxycodone. Also, the potency to induce intracellular G-protein activation is about two-fold higher compered with oxycodone (Lalovic et al. 2006). Interestingly, Lemberg et al. (2008) found that intrathecally administered noroxymorphone produced effective antinociception in rats whereas subcutaneously administered noroxymorphone was ineffective. Noroxymorphone molecule is rather hydrophilic and its capability to penetrate across the blood-brain barrier is reported to be very low. In a study by Lalovic et al. (2006) the achieved brain tissue concentrations in rats were less than 1% of the plasma concentrations. Therefore, it seems unlikely that noroxymorphone contributes to the central opioid effects of oxycodone.

Reductive metabolites

The role of the reductive metabolites of oxycodone is poorly studied. As mentioned above, oxycodone may directly undergo reductive metabolism to α- and β- oxycodol. Lalovic et al. have shown that affinities of these metabolites for the μ-opioid receptor are 12-fold and 2-fold lower than that of oxycodone, respectively. In the same study, the achieved C_{max} values for these metabolites were about a tenth of the concentrations of oxycodone. In addition, the brain-to-plasma ratio for β-oxycodol in rats was reported to be 0.25. Regarding the reductive metabolites of noroxycodone and oxymorphone the results were parallel. Altogether, reductive derivatives showed lower potency to activate intracellular G-protein and were found at a substantially lower concentration than their mother substances (Lalovic et al. 2006). Therefore, it is unlikely that any of these reductive metabolites would have a clinically relevant role in the opioid effects of oxycodone.

2.6.6 The role of influx and efflux transporters

One of the cell membrane's protective measures against different xenobiotics is the group of transmembrane efflux transporters, which restrict and regulate the uptake of foreign compounds inside the cell. It is known that these transporters substantially alter the pharmacokinetics of many commonly used drugs (Katragadda et al. 2005). P-glycoprotein (P-gp) is the efflux transporter most widely studied. P-gp is found most abundantly in the intestinal epithelium, the hepatocytes, the kidney and in the brain tissue (Katragadda et al. 2005). In central nervous system P-gp functions as a part of the blood-brain barrier (BBB) and restricts the entry of many pharmacological agents into the brain. In the intestinal epithelium, P-gp decreases the absorption of some orally administered drugs by pumping drug molecules back into the gut lumen. In the hepatocytes and in the kidney P-gp takes part in excretion of drugs and other xenobiotics into bile and urine respectively. Since some drugs may act as an inducer or an inhibitor of P-gp, the P-gp dependent drug-drug interactions are possible (Pal and Mitra 2006).

Regarding opioids, for example morphine, methadone and loperamide have shown to be substrates for P-gp in vitro (Wandel et al. 2002, Hassan et al. 2009a). There are also human interaction studies showing that P-gp dependent drug interactions may occur with these opioids (Sadeque et al. 2000, Kharasch et al. 2003, Kharasch et al. 2004, Heiskanen et al. 2008). On the other hand merepidine, oxymorphone and buprenorphine are not substrates of P-gp (Hassan et al. 2009b, Hassan et al. 2009a). The role of P-gp on the pharmacokinetics of oxycodone is controversial. There is one study, where by using in vitro and animal models, it was shown that oxycodone acts as a P-gp substrate with relatively high concentrations (Hassan et al. 2007). However, in another animal study with lower and perhaps more clinically relevant concentrations of oxycodone, the pharmacokinetics and the effects of oxycodone were not affected by the alterations in P-gp activity (Boström et al. 2005). In two human studies, Zwisler et

al. have tried to find out if the antinociceptive effect or the adverse effects of oxycodone are dependent on the subject's P-gp genotype (Zwisler et al. 2010b, Zwisler et al. 2011). In these studies, the subjects were assumed to have different P-gp activities based on the known genetic variability (Hoffmeyer et al. 2000). However, the noted P-gp genotype dependent differences in the frequency of adverse effects or in the strength of antinociceptive effects of oxycodone were minor and inconsistent. Therefore, these studies did not support the idea that effects of oxycodone would be dependent on the P-gp activity of the subjects (Zwisler et al. 2010b, Zwisler et al. 2011). The CYP2D6 inhibitor quinidine is also a well-known inhibitor of P-gp (Sadeque et al. 2000). In a CYP2D6 inhibition study by Heiskanen et al.(1998) the quinidine pre-treatment caused only an insignificant change in the oxycodone exposure. The pharmacodynamic effects of oxycodone were not altered either. Therefore, the results of this study might also indicate that oxycodone is not a P-gp subtrate in humans at clinically relevant concentrations. However, it is also debated that quinidine may not be as strong P-gp inhibitor as for example itraconazole (Kharasch et al. 2003, Heiskanen et al. 2008).

Animal studies have shown that oxycodone is able to concentrate to the central nervous system. The steady state concentration of unbound oxycodone in the rat brain tissue has been found to be three times higher than that in the blood (Boström et al. 2006). These results suggest that there may be some kind of active influx mechanism for oxycodone at the BBB. Based on their in vitro and in vivo studies, Okura et al. have suggested that the active transport of oxycodone through the BBB is, at least partly, mediated by a common transporter with pyrilamine (Okura et al. 2008). On the contrary, the brain to blood concentration ratio for unbound morphine has been found to be 0.29 in rats (Tunblad et al. 2003). Using the microdialysis method in brain trauma patients, the brain to blood AUC ratios of morphine below unity is also shown in humans (Ederoth et al. 2004). Influx and efflux transporter dependent difference in the capability to pass through the BBB may partly explain the discrepancies in vivo and in vitro potency of these two opioids.

2.6.7 Clinical use

Acute pain

Intravenous oxycodone is an effective treatment option for acute postoperative pain. When morphine or oxycodone was administered by patient-controlled analgesia (PCA) for the postoperative period following plastic breast reconstruction or lumbar spinal surgery, similar doses of oxycodone and morphine were needed (Silvasti et al. 1998). In another study, where patients scheduled for the elective abdominal surgery were studied, the intravenous oxycodone provided faster pain relief with smaller doses when compared with morphine (Kalso et al. 1991). According to this study the potency ratio of oxycodone and morphine was 2:3. Similar results were found in a recent randomized double-blind study where intravenous oxycodone or morphine was

administered by PCA system after laparoscopic hysterectomy (Lenz et al. 2009). The efficacy of oral immediate-release oxycodone in acute postoperative pain is concluded in a Cochrane review (Gaskell et al. 2009). Several studies have demonstrated the usefulness of extended-release oxycodone in the early postoperative period (Cheville et al. 2001, De Beer et al. 2005, Ho 2008). After epidural administration to the surgical patients oxycodone has substantially lower potency compared with morphine (Backlund et al. 1997, Yanagidate and Dohi 2004).

Cancer pain

Extended- and immediate-release oxycodone has been shown to provide an equivalent analgesia in the treatment of cancer-related pain (Stambaugh et al. 2001). Randomized controlled trials have confirmed that efficacy and tolerability of extended-release oxycodone are similar to those of controlled release morphine in cancer pain (Heiskanen and Kalso 1997, Mucci-LoRusso et al. 1998, Reid et al. 2006, Mercadante 2010). The potency ratio of oxycodone and morphine in cancer-related pain is found to vary between 2:3 and 1:1.

Chronic non-malignant pain

Chronic non-malignant pain includes chronic pain of nociceptive or neuropathic origin. The development of physical tolerance, opioid side effects and the risk of addiction have restricted the use of opioids in this indication. However, the at least short-term effectiveness of opioids in the management of chronic non-malignant pain has been shown when other treatments have not been able to provide adequate pain relief (Kalso et al. 2004). Randomized controlled trials have indicated that extended-release oxycodone might be an effective and safe treatment option for painful diabetic neuropathy, postherpetic neuralgia and osteoarthritis pain in selected patients (Watson and Babul 1998, Roth et al. 2000, Watson et al. 2003). The mean dose of oxycodone in these studies was about 40 mg per day. Although oxycodone seemed to be safe, durations of these studies were limited to a few weeks and long-term consequences and effectiveness are not therefore definitively established.

Adverse effects

Typical adverse effects of oxycodone like nausea, alterations of mood, sedation, hallucinations, delirium, constipation and physical dependence are common with other opioids (Benyamin et al. 2008). The most feared adverse effect of opioids is the respiratory depression. Without predisposing factors, the clinically significant respiratory depression occurs rarely in chronic opioid use with standard analgesic doses. Still, the respiratory depression is the leading cause of morbidity secondary to

opioid therapy and fatal consequences are also possible (Pattinson 2008, Yaksh and Wallace 2011). The most common adverse effects reported with oxycodone include constipation, nausea and drowsiness (Heiskanen and Kalso 1997). More hallucinations or nightmares have been reported with the use of morphine than with oxycodone in some studies (Heiskanen and Kalso 1997, Mucci-LoRusso et al. 1998). Even when used at very high doses oxycodone does not release significant amounts of histamine (Pöyhiä et al. 2004). This may explain why the incidence of itching may be lower with oxycodone when compared with morphine. Overall, the adverse effect profiles of these two commonly used opioids are rather similar.

Like with other strong opioids, the pharmacological effects of oxycodone include euphoria. In the prolonged use of oxycodone this may lead to the addiction, physical dependence and drug abuse (Benyamin et al. 2008). Decrease of analgesic effect following repeated administration of opioids, i.e. the development of tolerance occurs also with oxycodone. The mechanism of opioid tolerance is complex with several distinct mechanisms involved. Besides changes in opioid receptors and signalling pathways, also alterations in pharmacokinetics of the opioid may be contributing (Dumas and Pollack 2008).

2.6.8 Genetic considerations

There are several genetically determined features that may have an influence on the pharmacokinetics or pharmacodynamics of oxycodone. As previously stated, the activity of CYP2D6 enzyme depends strongly on the genotype of the individual. In Western Europe 7% of the population is classified as poor metabolizers (PM) (Eichelbaum et al. 2006). In these individuals the CYP2D6 dependent metabolism of oxycodone to oxymorphone is assumed to be markedly reduced. In human experimental pain model study, where pharmacokinetic parameters were in general rather poorly reported, Zwisler et al. (2009) found clearly lower oxymorphone concentrations in PMs after oral oxycodone compared with EMs. Their results also suggested that the analgesic effect of oxycodone would have been lower in PMs. In a considerably larger study with 270 genotyped postoperative surgical patients, the same study group demonstrated that although oxymorphone concentrations were substantially lower in PMs, the analgesic effect of oxycodone was similar in both genotypes (Zwisler et al. 2010a).

As the effects of oxycodone are mediated through the μ-opioid receptors, the genetically determined alterations in the expression and function of the μ-receptor may lead to interindividual variability on the pharmacological profile of oxycodone. The human μ-opioid receptor is coded by OPRM1 gene. Several OPRM1 mutations have been detected, from which the 118A>G single nucleotide polymorphism (SNP) is probably the best studied. 118A>G SNP has been found to have allelic frequency of 10-19% in Caucasian population (Lötsch and Geisslinger 2005). It has been shown that

cancer patients homozygous for the variant 118G allele need considerably higher morphine doses for pain relief than wild type patients (Klepstad et al. 2005). It has been suggested that variant allele also protects against opioid adverse effects (Lötsch and Geisslinger 2005). Zwisler at al. (2010b) studied the role of 118A>G SNP on the analgesic effects of oxycodone in healthy volunteers by using experimental pain models. They found that the variant G allele was associated with reduced analgesic effect of oxycodone on electrical nerve stimulation test. The reported adverse effects of oxycodone showed no statistically significant differences between genotypes.

Some non-opioid biological systems may also modulate the opioid effects indirectly. It is known that catecholamines are involved in the modulation of pain. Catechol-O-methyltransferase is an enzyme involved in the inactivation of these catecholamine neurotransmitters (dopamine, epinephrine, and norepinephrine). The COMT gene, coding this enzyme, is associated with allelic variants. It has been shown that a polymorphism in the COMT gene influences the pain sensation and efficacy of opioids in human experimental pain models. There are also studies demonstrating that variability in COMT gene may contribute to the morphine requirements in cancer pain patients (Klepstad et al. 2005, Rakvåg et al. 2008). What role the variation in the COMT gene has in oxycodone induced analgesia is not known.

2.6.9 CYP mediated drug-drug interactions of oxycodone

Due to its CYP-mediated metabolism oxycodone is prone to drug-drug interactions (DDI). Since both CYP2D6 and CYP3A4 are involved in the metabolism of oxycodone as well as in the further metabolism of the primary metabolites, the assessment of possible interactions is not so straightforward. On the other hand, the presence of multiple metabolic pathways may reduce the consequences of possible interaction, because the alternative metabolic route may be preserved. The fact that metabolites of oxycodone possess variable degrees of μ-opioid receptor activity makes the situation even more complicated. Because the knowledge about the metabolism of oxycodone has increased only few years ago, the systematic investigation of the drug interactions of oxycodone has begun only recently.

In an early pharmacokinetic study with oxycodone, Pöyhiä et al. (1992) tested the effect of amitriptyline on the pharmacokinetics of oral oxycodone. Amitriptyline is a tricyclic antidepressant that is currently known to be metabolized mainly by CYP C19 and CYP2D6 (Venkatakrishnan et al. 1998). The pharmacokinetics of oxycodone was not affected by concomintant amitriptyline treatment (Pöyhiä et al. 1992). The inhibition of CYP2D6 pathway has been studied by Heiskanen et al. (1998) in randomized controlled trial where the CYP2D6 pathway was inhibited with quinidine. The plasma concentrations of oxycodone were unchanged whereas the formation of oxymorphone was substantially reduced. The subjective drug effects were unaltered, but the analgesic effect of oxycodone was not assessed. On the contrary, a recent study

by Samer et al. (2010a) suggested that pre-treatment quinidine would cause a 1.4-fold increase in exposure to oral oxycodone. Last year Lemberg et al. (2010) reported that administration of paroxetine concomitantly with oxycodone to the chronic pain patient markedly changed to proportional amounts of metabolites of oxycodone, but the concentrations of oxycodone increased only marginally. Paroxetine had no effects on the analgesic effects of oxycodone (Lemberg et al. 2010).

In a randomized controlled study with healthy subjects, the inhibition of CYP3A4 by voriconazole resulted in a 3.6-fold increase in exposure to oral oxycodone. Also the pharmacological effects of oxycodone were modestly enhanced (Hagelberg et al. 2009). A newly published case series further demonstrates that this interaction is clinically relevant as seven of the reported nine patients treated concomitantly with voriconazole and oxycodone had possible oxycodone induced adverse effect like drowsiness and vomiting (Watanabe et al. 2011). The interaction of oral oxycodone with another antifungal agent, ketoconazole has shown to be somewhat milder and 1.8- to 2.4-fold increases in oxycodone exposure have been reported in recently published randomized controlled trials (Samer et al. 2010a, Kummer et al. 2011).

Tramadol is an opioid analgesic that undergoes metabolism by the same CYP2D6 and CYP3A4 enzymes as oxycodone. Curry et al. (2007) studied the ability of tramadol to impair the metabolism of oxycodone by competition for the same metabolizing enzymes. No changes in pharmacokinetics of oxycodone were detected in this single dose study. In the abovementioned new study by Samer et al. (2010a) the concurrent inhibition of CYP2D6 and CYP3A4 pathways with quinidine and ketoconazole, respectively resulted in 3-fold increase in the exposure to oxycodone.

In a case report, Lee et al. (2006) describe a patient whose pain control with oxycodone was insufficient and urine oxycodone screens were repeatable negative, probably because the rifampicin induced faster metabolism of oxycodone. Nieminen et al. (2009) have conducted a randomized controlled trial where the interaction between rifampicin and oxycodone has been clearly demonstrated. After rifampicin pre-treatment the exposure to the oral oxycodone was reduced by 86%. The interaction also clearly attenuated the pharmacological effects of oxycodone.

There are only very few studies where the effect of oxycodone on the pharmacokinetics of other CYP substrate drugs would have been investigated. Grant et al. have shown that, except for the prolonged time to reach the peak concentration of gatifloxacin, the pharmacokinetics of two antibiotics levofloxacin and gatifloxacin are not significantly affected by concomitantly administered oxycodone (Grant et al. 2001, Grant et al. 2002). One animal study has suggested that prolonged administration of oxycodone may cause variable alterations in the gene expression of several drug metabolizing enzymes. The clinical importance of this finding is not known (Myers et al. 2010).

2.7 Inhibitors studied

2.7.1 Paroxetine

Paroxetine is a selective serotonin reuptake inhibitor (SSRI) commonly used in the treatment of depression. It is also considered a first line treatment option for panic disorder and social anxiety disorder. The clinical effects of paroxetine ensue from enhanced serotonergic neurotransmission (Wagstaff et al. 2002). Commonly used doses are from 20 mg to 40 mg/day. After oral dose paroxetine is almost completely absorbed and a maximum plasma concentration is reached in 5-6 hours. Paroxetine is extensively metabolized in liver mainly by CYP2D6 enzyme into inactive metabolites. The elimination half-life of paroxetine is approximately 21 hours (Bourin et al. 2001, Wagstaff et al. 2002).

Paroxetine's capability to strongly inhibit CYP2D6 mediated drug metabolism has been known for years (Brøsen et al.1993a, Jeppesen et al.1996). There are also reports indicating that these pharmacokinetic interactions between drugs metabolized by CYP2D6 and paroxetine may cause unwanted side effects in clinical situations (Özdemir et al. 1997, Goryachkina et al. 2008). Conventionally paroxetine has been regarded a competitive, reversible inhibitor of CYP2D6. However, recent data have provided evidence for mechanism based on i.e. irreversible inhibition of CYP2D6 (Bertelsen et al. 2003). Due to its strong inhibitor potency, paroxetine is recommended as a standard CYP2D6 inhibitor to be used in DDI studies (Huang et al. 2007, EMA 2010). In vitro studies have revealed that paroxetine is also able to inhibit P-glycoprotein (P-gp) at very high concentrations. However, as clinically achieved paroxetine concentrations are around 250-fold lower than those needed to inhibit P-gp this finding might not have clinical relevance (Weiss et al. 2003).

2.7.2 Itracanazole

Itraconazole is a broad-spectrum triazole antimycotic widely used for the treatment and prophylaxis of both superficial and invasive fungal infections (Boogaerts and Maertens 2001). Although the newer antifungal agents have partly replaced itraconazole for treatment of serious invasive fungal infections, it is still considered a drug of choice for the treatment of superficial fungal infections like onychomycosis (Baran et al. 2008, Chen et al. 2010).

Itraconazole is lipophilic and highly bound to plasma proteins. Only 0.2% of the drug is found in unbound form. Itraconazole has large distribution volume (about 11 l/kg) and it is concentrated to the tissues like skin, liver, kidney, lungs, nails and stomach. This feature has an essential role in the efficacy of itraconazole as the achieved drug concentrations in tissues prone to fungal infections are higher than in

plasma (De Beule and Van Gestel 2001). The oral bioavailability of itraconazole capsules is approximately 55% (Prentice and Glasmacher 2005). Itraconazole is metabolized mainly in the liver and its major metabolite hydroxyl-itraconazole is also a potent antifungal agent. The metabolism of itraconazole is saturated with higher doses. At steady state, the elimination half-life of itraconazole is about 30 hours (De Beule and Van Gestel 2001). Clinically used oral doses of itraconazole are 100 to 400 mg/day.

The antifungal effects of itraconazole are caused by the inhibition of the fungal CYP enzymes involved in synthesis of ergosterol, a vital component of fungal cell membranes. Consequently, itraconazole has inhibitory effects also on the human CYP enzymes albeit these are luckily weaker than seen with fungal CYPs (De Beule and Van Gestel 2001). *In vitro* studies have shown itraconazole to be a potent and specific competitive inhibitor of human CYP3A4 (Niwa et al. 2005a, Niwa et al. 2005b). Hydroxyl-itraconazole, the major metabolite of itraconazole has also shown to be a potent inhibitor of CYP3A4 (Isoherranen et al. 2004, Templeton et al. 2008). The numerous clinical studies have revealed that inhibition CYP3A4 by itraconazole can lead to DDIs with clinical relevance (Olkkola et al.1996, Kantola et al. 1998, Venkatakrishnan et al. 2000, Florea et al. 2003, Gubbins and Heldenbrand 2010). As a result, itraconazole is one of the recommended standard CYP3A4 inhibitors for the *in vivo* DDI studies (Huang et al. 2007, EMA 2010).

Many of the CYP3A4 inhibitors possess inhibitory potency for P-gp. This has been shown also for itraconazole (Wang et al. 2002). Because the substrate as well as inhibitor specificities of CYP3A4 and P-gp are largely overlapping, it is difficult to distinguish to what extent these two mechanisms are contributing to the particular DDI (Katragadda et al. 2005).

2.7.3 Telithromycin

Telithromycin, the first of the ketolide antimicrobials, is a semi-synthetic derivative of erythromycin. It is effective in the treatment of common typical and atypical respiratory tract pathogens and is at present indicated for the treatment of community-acquired pneumonia of mild to moderate severity (Reinert 2004, Nguyen and Chung 2005).

Telithromycin has an oral bioavailability of 57%. After ingestion a typical oral dose of 800 mg the peak plasma concentration of approximately of 2 mg/l is reached in one hour. 60 to 70% of telithromycin is bound to the plasma proteins. Following repeated oral dosing, the elimination half-life is about ten hours. Approximately 50% of the metabolism of telithromycin is mediated by hepatic CYP3A4, but also CYP

independent metabolism exists (File 2005, Nguyen and Chung. 2005, Shi et al. 2005). Metabolites are inactive.

Telithromycin is a strong inhibitor of CYP3A4, but according to the information given by the manufacturer, it has an inhibitory effect also on the CYP2D6 dependent metabolism of metoprolol (File 2005, Shi et al. 2005, Shakeri-Nejad and Stahlmann 2006, Kajosaari et al. 2006, Ketek the summary of product characteristics (SPC) 2007 sanofi-aventis; http://products.sanofi-aventis.us/ketek/ketek.html). Like its close relatives macrolide antibiotics, telithromycin is able to inhibit P-gp *in vitro* (Eberl et al. 2007). One case report has suggested that concomitantly administered telithromycin with digoxin (P-gp substrate) may lead to digoxin toxicity (Nenciu et al. 2006).

2.7.4 Miconazole oral gel

Miconazole is imidazole group antimycotic developed for clinical use at the end of the sixties (Plempel 1979). Similar to other azole antifungals, the action mechanism of miconazole is mediated by the inhibition of fungal CYP enzymes. Miconazole has a broad spectrum of antifungal activity against many clinically important fungi and it was originally used for systemic fungal infections. Due to its limited oral bioavailability (27%), the newer antifungals have replaced miconazole in the treatment of systemic fungal infections (Männistö et al. 1982, Daneshmend and Warnock 1983). However, miconazole is still commonly used as a topical preparation (Oliver et al. 2004, Martinez-Beneyto et al. 2010).

The low oral bioavailability of miconazole ensues from incomplete absorption and hepatic first-pass metabolism. Binding to the plasma proteins is high (95%). Miconazole is metabolized by the hepatic enzymes into inactive metabolites. The metabolism is not altered by repeated administration (Plempel 1979, Daneshmend and Warnock 1983). Miconazole oral gel dosage instruction given by the manufacturer is 2.5 ml (approximately 60mg) every 6 hours. This dosage is reported to lead to peak plasma concentrations of 31-49 ng/ml achieved in two hours post dose (Daktarin oral gel the summary of product characteristics (SPC) 2010 Orion Pharma).

In vitro studies have demonstrated that miconazole is a strong competitive inhibitor of several human CYP enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) (Zhang et al. 2002, Niwa et al. 2005a, Niwa et al. 2005b). Systemically administered miconazole has been shown to be able to inhibit the CYP2C9 mediated metabolism of warfarin in healthy volunteers (O'reilly et al. 1992). A recent review article summarizes numerous case reports where this same interaction has been reported after using miconazole as an oral gel in clinical settings leading to adverse effects (Ariyaratnama et al. 1997, Pemberton et al 2004, Broos et

al. 2010, Miki et al. 2010). One controlled study with healthy volunteers has demonstrated that miconazole oral gel strongly inhibits the CYP3A4 mediated metabolism of etoricoxib (Hynninen et al. 2009). According to *in vitro* studies miconazole is a moderate inhibitor of P-gp. However, based on reported Ki values, it seems that the CYP inhibition is the predominant mechanism in DDI with miconazole (Yasuda et al. 2002).

3 AIMS OF THE STUDY

The global use of oxycodone is rapidly increasing. Although oxycodone has been in clinical use for several decades, its basic pharmacology has been studied rather poorly. Previous in vitro studies have shown, that metabolism of oxycodone is mediated by CYP2D6 and 3A4 enzymes, and it is therefore prone to drug interactions. Before these studies, the effects of CYP2D6 or CYP3A4 inhibitors on the pharmacokinetics of oxycodone in humans have not been studied systematically.

The specific objectives were:

1. To study the effect of the inhibition of CYP2D6 by paroxetine on the pharmacokinetics and pharmacological effects of oral and intravenous oxycodone (Studies III and IV)

2. To study the effect of the inhibition of CYP3A4 by itraconazole on the pharmacokinetics and pharmacological effects of oral and intravenous oxycodone (Study II)

3. To study the effect of the concomitant inhibition of CYP2D6 and CYP3A4 by paroxetine and itraconazole on the pharmacokinetics and pharmacological effects of oral and intravenous oxycodone (Studies III and IV)

4. To study the effect of telithromycin and miconazole oral gel on the pharmacokinetics and pharmacological effects of oral oxycodone (Studies I and V)

4 MATERIALS AND METHODS

4.1 Subjects

Altogether 58 healthy non-smoking volunteers (34 male, 24 female) participated in five separate studies. None of the volunteers participated in more than one study. The demographic data is shown in Table 2. All subjects gave their written informed consent. Before entering the study volunteers were ascertained to be healthy by medical history, clinical examination and laboratory screening. Laboratory tests included complete blood count, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, and for women a pregnancy test. Urine was screened for glucose, proteins and drugs with addiction potential. In addition, a 12-lead electrocardiogram was obtained. The susceptibility of participants to develop opioid addiction was estimated to be low as evaluated by the Finnish modified version of the Abuse Questions (Mincha et al. 2004). The volunteers were not allowed to drink grapefruit juice or take any drugs known to cause CYP-enzyme inhibition or induction for four weeks before the study.

Exclusion criteria in all studies:

1. Previous history of intolerance to the studied drugs.
2. Concomitant drug therapy of any kind for at least 14 days prior to the study.
3. Subjects younger than 18 years and older than 40 years.
4. Existing or recent significant disease.
5. History of haematological, endocrine, metabolic or gastrointestinal disease, including gut motility disorders.
6. History of asthma or any kind of drug allergy.
7. Previous or present alcoholism, drug abuse, psychological or other emotional problems that are likely to invalidate informed consent, or limit the ability of the subject to comply with the protocol requirements.
8. A positive test result for urine toxicology.
9. A “yes” answer to any one of the Abuse Questions.
10. Pregnancy or nursing.
11. Donation of blood for 4 weeks prior and during the study.
12. Participation in any other studies concomitantly or within one month prior to the entry into this study.
13. Smoking.

Table 2. The mean (range) demographics of the volunteers in Studies I-V

Study	Number of subjects	Age, mean (range)	BMI kg/m^2 (range)	CYP2D6 genotype			
				EM	UM	PM	IM
I	11	27 (20-40)	22 (19-25)	9	2		
II	12	22 (22-39)	23 (20-28)	7	1	1	3
III	11	21 (19-25)	23 (20-32)	7	2	2	
IV	12	23 (19-30)	22 (18-25)	10	1	1	
V	12	23 (19-30)	24 (20-31)	10	1	1	

BMI = Body mass index, EM = Extensive metabolizer, UM = Ultrarapid metabolizer, PM = Poor metabolizer, IM = Intermediate metabolizer

4.2 Study design

The clinical parts of these studies were performed between April 2007 and March 2009 in the Departments of Pharmacology, Drug Development and Therapeutics, University of Turku and Anaesthesiology, Intensive Care, Emergency Care and Pain Medicine, University of Turku and Turku University Hospital. A randomized, balanced cross-over study design was used in all studies. Pre-treatments in Studies I-IV were placebo-controlled, whereas the miconazole treatment in Study V was open-label. A four-week wash-out period between the phases was used in all studies. In Studies I-IV a hospital pharmacist, not involved in the study, repacked the study drugs and placebos to daily doses in little paper bags according to a randomization list. The number of capsules per day in different pre-treatments was equalized by the use of additional non-matching placebo capsules.

The volunteers self-administered the pre-treatment drugs at home according to a dosing schedule. To control the adherence to the drug dosing schedule, volunteers were asked to send a mobile phone text message to the investigator after each dose. In addition, plasma concentrations of pre-treatment drugs were measured in the morning of the study day (i.e day of oxycodone administration) to ascertain the use of medication. On the study day the pre-treatment drugs were administered by the investigators in the study facilities. The dosing schedules of pre-treatments are summarized in Table 3. In Studies I, III and IV the volunteers were instructed to take pre-treatment drugs at 8 a.m. and in Study II at 7 a.m. In the Study V the miconazole oral gel was used three times a day (at 7 a.m., 3 p.m. and 11 p.m.).
The volunteers fasted overnight before the study day. One hour after pre-treatment drugs oxycodone was administered orally or intravenously depending on the study phase. The dose of oral oxycodone was 10 mg in Studies I, II, III and V. Intravenous oxycodone dose in Studies II and IV was 0.1mg/kg given as an injection in two minutes. Standard meals were served 4 h and 8 h after the administration of oxycodone. Ingestion of alcohol, coffee, tea, or cola drinks was not allowed during pre-treatment periods and test days. On study days subjects stayed in study facilities from 6.30 a.m. to 8.30 p.m.

Table 3. Structure of Studies I-V

Study	Pre-treatment				Oxycodone dosing
	Phase	Drug	Dose	Duration	
I	Phase 1	Placebo	2 caps. x1	4 days	Oxycodone 10 mg po (Oxynorm®)
	Phase 2	Telithromycin (Ketek®)	800 mg x 1	4 days	
II	Intravenous part				
	Phase 1	Placebo	2 caps x1	5 days	Oxycodone 0.1 mg/kg iv (Oxynorm®)
	Phase 2	Itraconazole (Sporanox®)	200 mg x1	5 days	
	Oral part				
	Phase 1	Placebo	2 caps x1	5 days	
	Phase 2	Itraconazole (Sporanox®)	200 mg x1	5 days	Oxycodone 10 mg po (Oxynorm®)
III	Phase 1	Placebo	3 caps x1	5 days	
	Phase 2	Paroxetine (Seroxat®) + Placebo	20 mg x1+ 2 placebo caps.	5 days	Oxycodone 10 mg po (Oxynorm®)
	Phase 3	Paroxetine (Seroxat®) + Itraconazole (Sporanox®)	20 mg x1 + 200mg x1	5 days	
IV	Phase 1	Placebo	3 caps	5 days	
	Phase 2	Paroxetine (Seroxat®) + Placebo	20 mg x1+ 2 placebo caps.	5 days	Oxycodone 0.1 mg/kg iv (Oxynorm®)
	Phase 3	Paroxetine (Seroxat®) + Itraconazole (Sporanox®)	20 mg x1 + 200mg x1	5 days	
V	Phase 1	No pre-treatment	-	4 days	Oxycodone 10 mg po (Oxynorm®)
	Phase 2	Miconazole oral gel (Daktarin® 2% oral gel)	85 mg x 3; (3.5ml x3)	4 days	

4.3 Blood sampling

On study days, a forearm vein was cannulated and timed venous blood samples were drawn into 10 ml EDTA tubes before and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 hours after oxycodone administration for the determination of plasma concentrations of oxycodone and its oxidative metabolites. In the intravenous phase of Study II an additional sample was drawn 0.25 hours after intravenous oxycodone. In Study IV blood samples were taken before and 0.33, 0.66, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 hours after oxycodone administration. In every study the subjects returned to the clinical laboratory in the two following mornings after the study day to give blood samples also at 24 and 48 hours after oxycodone administration. Plasma was separated within 30 min and stored at −70º C until analysis. For the determination of plasma concentrations of possible treatment drugs, a blood sample was drawn before administration of any drugs in the morning of the each study day (Studies I-IV). In addition two blood samples for the CYP2D6 genotyping were drawn in the morning of the first study day of each study.

4.4 Determination of plasma drug concentrations

Determination of plasma drug concentrations oxycodone and its oxidative metabolites

Plasma concentrations of oxycodone, noroxycodone, oxymorphone and noroxymorphone were determined using a validated liquid chromatography tandem mass spectrometric (LC-MS/MS) method (Neuvonen and Neuvonen 2008). 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 (CV) for oxycodone were < 3% at concentration of 100 ng/mL, < 5% at concentration of 5 ng/ml and < 11% at concentration of 0.1 ng/ml. For oxymorphone the CVs were < 10% at concentration of 5 ng/ml and < 15% at concentration of 0.1 ng/ml. For noroxycodone the CVs were < 5% at concentration of 5 ng/ml and < 12% at concentration of 0.5 ng/ml. For noroxymorphone the CVs were < 10% at concentration of 10 ng/ml and < 11% at concentration of 0.5 ng/ml.

Telithromycin

Plasma concentrations of telithromycin were determined by using a validated liquid chromatography-mass spectrometry (LC-MS) method (Cantalloube et al. 2003). The LLQ for telithromycin was 0.005 mg/l and the interday CV was 2.1% at the concentrations of 0.7 mg/l and 5.6% at the concentrations of 0.07 mg/l.

Itraconazole

Plasma concentrations of itraconazole and its bioactive hydroxyl-metabolite, hydroxyl-itraconazole were determined by using high performance liquid chromatographic (HPLC) with UV detection as described earlier (Gubbins et al. 1998). The LLQ was 10 ng/ml. The interday CVs were 10.9%, 5.7% and 4.1% at 40, 192 and 1200 ng/ml respectively.

Paroxetine

Plasma concentrations of paroxetine were determined with modified version of earlier described LC-MS/MS method where citalopram served as an internal standard (Chu and Metcalfe 2007). The interday CVs were 5.9% at the concentrations of 12 ng/ml and 8.5% at the concentrations of 2.5 ng/ml.

Miconazole

Plasma concentrations of miconazole were determined from the plasma samples drawn 1, 2, and 4 hours after morning dose of miconazole oral gel with liquid chromatography using itraconazole as internal standard (Compas et al. 1996). The LLQ for miconazole was 5 ng/ml and the interday CV was 3.4% at concentration of 25 ng/ml.

4.5 Genotyping

All volunteers participating in studies I-V were genotyped for CYP2D6. Genotyping was performed by using a two-step multiplex primer extension method which allowed the detection of 11 of the most relevant alleles, (i.e. *1, *2, *3, *4, *6, *9, *10, *17, *29, *39 and *41) and the assessment of the whole-gene deletion and duplication and the allele composition of gene duplication (Sistonen et al. 2005).

4.6 Pharmacokinetic calculations

The peak plasma concentrations (C_{max}) and the time to reach peak concentration (t_{max}) of oxycodone and its metabolites were observed directly from plasma concentration data. Individual terminal log-linear phases of the plasma concentration time curves were identified visually. The elimination rate constant (k_{el}) was determined by regression analysis of the log-linear part of the curve. The elimination half-life ($t_{½}$) was calculated by using the equation $t_{½} = \ln 2 / k_{el}$. The areas under plasma concentration time curves (AUC) for oxycodone, noroxycodone, oxymorphone and noroxymorphone

were calculated by using the linear trapezoidal rule when successive concentration values were increasing and by using the logarithmic trapezoidal rule when values were decreasing. For oxycodone and noroxycodone, $AUC_{0-\infty}$ was extrapolated to infinity by using the respective k_{el} -value. Since k_{el} was often impossible to determine due to low plasma concentrations of the oxymorphone and noroxymorphone, the $AUC_{0-48\ h}$ was used instead of $AUC_{0-\infty}$ for these metabolites. After the oral administration of oxycodone, the apparent clearance (CL/F) and the apparent volume of distribution of oxycodone during elimination (VZ/F) were calculated. After the intravenous administration of oxycodone, plasma clearance (CL) and steady-state volume of distribution (V_{ss}) of oxycodone were calculated by the use of noncompartmental methods based on statistical moment theory as follows CL = Dose/AUC and V_{ss} = Dose/ ($k_{el} \cdot$ AUC). The oral bioavailability of oxycodone (F) was calculated as follows $F = (AUC_{0-\infty\ oral} \cdot Dose_{iv})/(AUC_{0-\infty\ iv.} \cdot Dose_{oral})$. The metabolite-to-parent drug AUC ratios (AUC_m/AUC_p) were calculated as indexes of metabolism through different metabolic pathways. The pharmacokinetic data were analyzed with WinNonlin pharmacokinetic program (version 4.1; Pharsight, Mountain View, California,USA).

4.7 Pharmacodynamic measurements

The pharmacological effects of oxycodone were evaluated for 12 hours after oxycodone administration by measuring subjective, psychomotor and physiological effects with commonly used tests discussed below. In addition, the analgesic effects of oxycodone were evaluated by conducting the cold pressor tests and cutaneous heat pain threshold measurements (Table 4). The area under the effect-time curve (AUEC) was determined by trapezoidal rule for 12 hours (Studies I-V) and for the first six hours (Studies III-V) for each variable except the cutaneous heat pain threshold and tactile sensitivity.

Subjective effect

The participants evaluated subjective effects using 100-mm horizontal visual analogue scales (VAS) for the following four items: alert/drowsy, very good performance/very poor performance, no drug effect/very strong drug effect, and worst feeling ever/best feeling ever (Bond and Lader 1974). Although not specific to opioid effects, similar ratings have been used in earlier studies to characterize the effects of opioids (Kaiko et al. 1996, Zacny and Gutierrez 2003, Lalovic et al. 2006). However, the effects of oxycodone are dose dependent and might be evident only when higher oral doses (20-30 mg) are administered (Zacny and Gutierrez 2003).

Pupil size

Opioids are known to induce miosis and opioid effects are therefore commonly evaluated by measuring the changes in the pupil size after opioid administration

(Posner et al.1985, Kaiko et al 1996, Kolzenburg et al. 2006). Oxycodone induced miosis has been reported in several studies (Heiskanen et al. 1998, Lalovic et al. 2006). In the present studies, the pupil diameter was measured using Cogan's pupillometer under steady lighting conditions (Cogan 1941). Cogan's pupillometer is a plastic card where a series of paired holes are arranged in the vertical column starting with a separation of 1 mm. The separation of the holes is increased by 0.5mm ending with a separation of 8 mm. During measurement, the subject looks through the holes and raises or lowers the card until he/she finds the pair that produces luminous discs tangential to each other. The distance between the holes in millimetres in the pair is recorded as the pupil size.

Digit symbol substitution test

The digit symbol substitution test (DSST) was used to assess the effect of oxycodone on cognitive and motor functions (Stone 1984). A set of nine digits with corresponding symbols was shown on the upper part of the paper. The lower part of the paper consisted of 300 digits in randomized order. Volunteers were asked to insert the corresponding symbol below each digit. The number of correct symbols substituted in 3 minutes was recorded. Volunteers were trained to do DSST during the screening visit before entering the study. The sequence of symbols was changed for each time point to prevent the memorising of the code. Oral oxycodone with doses of 20 to 30 mg has been reported to cause psychomotor impairment evident in DSST (Zacny and Gutierrez 2003).

Maddox Wing Test

The Maddox Wing test was used to measure the effect of oxycodone on the extraocular muscle balance. When one looks into the handheld Maddox wing instrument, the vision is divided in a way that a vertical arrow is seen by the right eye and a numbered horizontal scale by the left eye (Hannington-Kiff 1970). The imbalance in extraocular muscle tone produces an image of the arrow apparently moving along the horizontal scale. Subjects were asked to report the number at which the arrow pointed when it ceased moving. The unit in horizontal scale was diopter. Subjects were trained to do the test before making the first measurements.

Cold pressor test

A cold pressor test was used to assess the effect of oxycodone on cold pain sensitivity (Staahl and Drewes 2004, Kolzenburg et al. 2006). This experimental pain model is sensitive to opioid analgesia and it has been used extensively in experimental pain research (Staahl et al. 2009). In this test, the subjects immersed their left hand up to the

wrist in ice water (temperature 0.5 - 2°C) for 1 minute. The latency time (in seconds) from the immersion of the hand to the first sensation of pain was defined as the cold pain threshold. During the immersion, the subjects were asked to report the intensity of the cold pain at the interval of 30 seconds using a numerical rating scale (0 = no pain, 100 = maximal pain). If pain became intolerable, the subject was instructed to withdraw his or her hand from the water. In such a case, the pain intensity after withdrawal was reported as maximal. In study I, the unpleasantness of cold pain was evaluated with similar numerical ratings at the same time points as the pain intensity.

Cutaneous heat pain threshold

The cutaneous heat pain threshold on the forearm skin was determined prior to and 1 hour after oxycodone administration (Kolzenburg et al. 2006, Staahl et al. 2008). A feedback-controlled contact thermode (TSA-2; Medoc Inc, Rehovot, Israel) was used to deliver 3 heat pain stimuli. Heat of the thermode increased slowly (1°C/s) from a baseline temperature of 35°C until the subject reported the first sensation of pain by pressing a button or until the security limit of 50 °C was reached. The interval between the stimuli was 30 seconds. To avoid sensitization, thermode site on the skin was changed between stimuli. The cutaneous heat pain threshold was defined as the mean of these three measurements. Previous studies have reported a pronounced intersubject variability in the response to 15 mg oral oxycodone in cutaneous heat pain (Staahl et al. 2008).

Tactile sensitivity

The effect of oxycodone on sensitivity to tactile stimuli was assessed in study I by using a set of five von Frey monofilaments (Semmes–Weinstein Aesthesiometer Kit; Stoelting Co, Wood Dale, IL). The tactile threshold was determined before and two hours after oxycodone administration. The subject was comfortably lying in the supine position with eyes blindfolded while monofilaments of 5 strengths (0.23 mN, 0.27 mN, 1.63 mN, 3.99 mN, and 11.8 mN) and a sham filament (no stimulus) were applied to the tip of the right index finger. Each stimulus was randomly applied on the skin 8 times at an interval of 5 seconds. The subjects rated each stimulus on a scale of 0 to 2 (0 = no stimulus, 1 = a possible stimulus, 2 = a definite stimulus). For the analysis, scores 1 and 2 were pooled together. Tactile threshold was depicted from a psychometric curve as the 50% detection rate of stimuli.

Table 4. Pharmacodynamic measurements conducted in Studies I-V.

Pharmacodynamic test	Study					Timing
	I	II	III	IV	V	
Subjective effects (four VAS-scores)	x	x	x	x	x	before and 1, 2, 3, 4, 5, 6, 8, 10, 12 hours after oxycodone
Pupil size (Cogan's pupillometer)	x	x	x	x	x	"
Digit symbol substitution test	x	x	x	x	x	"
Maddox Wing test	x	x	x	x		"
Cold pressor test	x	x	x	x	x	"
Cutaneous heat pain threshold	x	x				before and 1 hour after oxycodone
Tactile sensitivity (von Frey hairs)	x					before and 2 hours after oxycodone

4.8 Statistical analysis

The number of volunteers needed in Studies I-V was based on pre-study power analysis. The standard deviation values for oxycodone AUC needed in sample size calculations were derived from previous studies (Pöyhiä et al. 1992, Heiskanen et al. 1998). It was calculated that 10 volunteers would be required to detect mean percentage change of 30% in $AUC_{0-\infty}$ of oxycodone with the power of 80% at significance level of 0.05. In order to prepare for dropouts, our aim was to recruit 12 volunteers in each study.

Data were analyzed by use of the SYSTAT for Windows statistical program (version 10.2; Systat Software, Richmond, California) and with GraphPad Prism 5 for Windows (GraphPad Software, San Diego, California). The normality of data was evaluated prior to the statistical analysis by using D'Agostino-Pearson omnibus normality test and the Shapiro-Wilk test (GraphPad Prism 5). In case of marked positive skewness of the distribution, the data were transformed to logarithms to correct for non-normality of the distribution. In the two-phase studies (Studies I, II and V), the pharmacokinetic and pharmacodynamic variables were compared with the Student's paired t-test. In the three-phase studies (Studies III and IV) the analysis of variance (ANOVA) for repeated measures was used, and a posteriori testing was performed with Tukey test. T_{max} was analyzed by the Friedman's test, and the Wilcoxon signed rank test was used for pairwise comparisons. Results were expressed as mean values ± SD except for t_{max} where medians and ranges were given. Differences between phases were regarded statistically significant if $P < 0.05$. In all studies the changes in the percentage relative to the placebo/control phase were calculated individually for each subject, means and ranges of these values were reported. To assess the possible interaction in terms of bioequivalence, we also calculated the geometric mean ratios (GMRs) with 90% confidence intervals (CI) for the pharmacokinetic results in Studies III and V. Bioequivalance i.e. the lack of interaction was concluded if the 90% CI of GMR fell into range of 0.80-1.25. In Study I the GMR was reported with 95% CI. To estimate

the correlation between pharmacodynamic variables and plasma concentrations of oxycodone, the Pearson correlation coefficient was determined in Studies I and II.

4.9 Ethical considerations

All study protocols were approved by the ethics committee of the Hospital District of Southwest Finland and by the Finnish National Agency for Medicines. The studies were registered to the EudraCT clinical trials register. Before the screening visit, the volunteers received written information on the study by email. The study protocol and information on experimental pain tests were talked through during the screening visit before the volunteers gave their written informed consent. Volunteers were told that they have the right to withdrawn from the study at any time they wanted. Drug doses during the pre-treatment periods were similar to those used in the clinic. The dose of oxycodone in all studies was low to ensure the safety of volunteers also in the case of enhanced drug effect because of an interaction with CYP-inhibitor.

5 RESULTS

The mean percentage changes in the pharmacokinetic parameters of oxycodone and its metabolites during the treatment periods of Studies I-V are shown in Figures 3-7.

5.1 Effect of CYP2D6 inhibition by paroxetine (III, IV)

The inhibition of CYP2D6 enzyme by paroxetine decreased the formation of the CYP2D6 dependent primary metabolite oxymorphone by 44% ($P < 0.05$) and by 66% ($P< 0.001$) after oral and intravenous administration of oxycodone, respectively. However, the pharmacokinetics of parent drug oxycodone was essentially unchanged, and the exposures to oral or intravenous oxycodone were not affected by paroxetine treatments. The formation of the CYP3A dependent metabolite noroxycodone was increased by 68% ($P < 0.001$) after the oral administration of oxycodone and by 70% ($P < 0.001$) after the intravenous administration of oxycodone. During the paroxetine treatment the formation of secondary metabolite noroxymorphone was drastically decreased after oral (-74%, $P < 0.001$) and particularly after intravenous (-95%, $P < 0.001$) administration of oxycodone. In Study III it was noted that two subjects with UM genotype had the lowest plasma paroxetine trough concentrations. In spite of the low paroxetine concentrations it seemed that the CYP2D6 inhibition in these subjects was even somewhat stronger than in the others. Due to the limited number of subjects (n=2) the statistical significance was not reached.

The effects of oral or intravenous oxycodone on the cold pain threshold were not affected by paroxetine treatment. The subjects reported marginally higher VAS-scores for the deterioration of performance for the first six hours following oral oxycodone administration during paroxetine treatment as the $AUEC_{0\text{-}6h}$ value was increased by 24% ($P < 0.05$) when compared to the placebo phase. Following the intravenous administration of oxycodone the $AUEC_{0\text{-}6h}$ value for the deterioration of performance and drowsiness was also slightly increased (+33% and +39% respectively, $P< 0.05$). Pupil diameter was significantly larger during paroxetine treatments in Studies III and IV when compared to the placebo phases and the oxycodone-induced miosis was therefore masked.

5.2 Effect of CYP3A4 inhibition by itraconazole (II)

Concomitant treatment with itraconazole increased the mean $AUC_{(0\text{-}\infty)}$ of intravenous oxycodone by 51% ($P < 0.001$). The inhibitory effect by itraconazole was clearly stronger on the metabolism of oral oxycodone as the mean $AUC_{(0\text{-}\infty)}$ of oral oxycodone was increased by 144% ($P < 0.001$). The clearance and the apparent oral clearance of intravenous and oral oxycodone during the itraconazole phase were 32% ($P <0.001$)

and 57% ($P < 0.01$) lower, respectively, compared with the placebo phase. The administration of itraconazole increased the mean $t_{1/2}$ of oxycodone from 3.8 h to 5.5 h ($P < 0.05$) and from 4.0 h to 5.9 h ($P < 0.01$) for intravenous and oral oxycodone, respectively. The mean C_{max} of oxycodone after a single oral dose was increased by 45% ($P < 0.01$) by the itraconazole treatment. The oral bioavailability of oxycodone was increased from 55% to 82% ($P < 0.001$).

Itraconazole decreased the formation of the CYP3A4 dependent primary metabolite noroxycodone and the mean $AUC_{(0\text{-}48h)}$ of noroxycodone was reduced by 54% ($P < 0.001$) after intravenous oxycodone and by 49% ($P < 0.001$) after oral oxycodone. Correspondingly, the mean AUC_m/AUC_p of noroxycodone after intravenous and oral oxycodone were reduced by 69% and 78% ($P < 0.001$), respectively. On the contrary, the formation of oxymorphone was drastically increased during itraconazole administration and the mean $AUC_{(0\text{-}48h)}$ of oxymorphone was elevated by 159% ($P < 0.05$) after intravenous oxycodone and by 359% ($P < 0.001$) after oral oxycodone. Itraconazole treatment significantly lowered the plasma concentrations of the secondary metabolite noroxymorphone and the mean $AUC_{(0\text{-}48h)}$ of noroxymorphone after intravenous and oral oxycodone were 84% ($P < 0.01$) and 71% ($P < 0.01$) lower than during placebo phase.

Following the oral administration of oxycodone the subjects reported moderately higher VAS-scores for drowsiness, deterioration of performance and subjective drug effect during itraconazole phase vs. placebo, as the $AUEC_{0\text{-}12h}$ values for these variables were increased by 30-45% ($P < 0.05$). The pharmacological effect of intravenous oxycodone was unaltered by itraconazole treatment (Figure 8). Itroconazole did not enhance the analgesic effect of intravenous or oral oxycodone as measured by the heat or cold pain tests.

5.3 Effect of concomitant inhibition of CYP2D6 and CYP3A4 by paroxetine and itraconazole (III, IV)

When both the CYP2D6 and the CYP3A4 pathways were inhibited the mean $t_{1/2}$ of oral oxycodone was prolonged from 3.6 h to 6.0 h ($P < 0.001$). As a consequence the mean $AUC_{(0\text{-}\infty)}$ of oral oxycodone was increased 2.9-fold ($P < 0.001$). In addition, the mean C_{max} of oxycodone was increased 1.8- fold ($P < 0.001$). After intravenous oxycodone, the changes in pharmacokinetics of parent oxycodone were in parallel, but less prominent. The mean $AUC_{(0\text{-}\infty)}$ of intravenous oxycodone was increased 2-fold ($P < 0.001$) as the clearance was decreased by 48% ($P < 0.001$) during the combination treatment. The mean AUC values for noroxycodone and oxymorphone after oral or intravenous oxycodone changed only moderately by the combination of paroxetine and itraconazole. However, the relative abundance of these metabolites was clearly reduced as the mean AUC_m/AUC_p of noroxycodone and oxymorphone were reduced by 69% and 56%, respectively, after oral oxycodone, and by 56% and 66% after

intravenous oxycodone ($P < 0.001$ for all variables). These findings were result from the markedly increased $AUC_{(0-\infty)}$ of the parent drug oxycodone. When both the CYP2D6 and the CYP3A4 enzymes were inhibited, the concentrations of secondary metabolite noroxymorphone were very low after oral oxycodone and not measurable after intravenous administration of oxycodone.

Following the oral administration of oxycodone in the paroxetine + itraconazole phase, the subjects reported marginally higher VAS-scores for drowsiness, deterioration of performance and subjective drug effect during the first six hours after oxycodone and the $AUEC_{0-6h}$ values for these variables were increased by 20-30% ($P < 0.05$). Parallel changes were noted in the VAS-scores for drowsiness and deterioration of performance after the intravenous administration of oxycodone. However, when comparing the $AUEC_{0-12h}$ values for pharmacodynamic variables after oral oxycodone, no statistically significant differences were observed (Figure 8). The analgesic effects of oral or intravenous oxycodone were not altered by the concomitant treatment with paroxetine and itraconazole.

5.4 Effect of telithromycin (I)

Telithrocycin clearly reduced the formation of noroxycodone and the mean $AUC_{(0-\infty)}$ of noroxycodone was decreased by 46% ($P < 0.001$). On the contrary, the mean $AUC_{(0-\infty)}$ of oxymorphone was increased by 82% ($P < 0.01$) whereas the mean AUC_m/AUC_p of oxymorphone was unaltered by the telithromycin treatment. Thus, it seemed that telithromycin has no inhibitory effect on the CYP2D6 mediated metabolism of oxycodone. Overall, telithromycin increased the exposure to the oral oxycodone 1.8-fold ($P < 0.001$).
Slightly enhanced opioid effects of oxycodone were noted during the telithromycin phase. The oxycodone-induced miosis was marginally more pronounced during the telithromycin phase ($P < 0.01$). In addition, the $AUEC_{0-12h}$ values based on the reported VAS-scores for subjective drug effect and deterioration of performance were moderately increased ($P < 0.05$) (Figure 8). Telithromycin did not alter the effect of oxycodone on the pain threshold times in the cutaneous heat pain test or in the cold pain test.

5.5 Effect of miconazole oral gel (V)

The use of miconazole oral gel produced a strong inhibition of the CYP2D6 mediated metabolism of oxycodone as the mean $AUC_{(0-48h)}$ and the mean AUC_m/AUC_p of oxymorphone were both decreased about 80% ($P < 0.001$). Also the CYP3A pathway was somewhat inhibited in spite of the increased $AUC_{(0-48h)}$ of noroxycodone as the mean AUC_m/AUC_p of noroxycodone was reduced by 20% ($P < 0.01$). Together these changes resulted in a 63% increase in the mean $AUC_{(0-\infty)}$ of oral oxycodone ($P <$

0.001). The pharmacological effects of a single oral dose of oxycodone were not altered by the concomitant use of miconazole.

5.6 Effect of CYP2D6 genotype

The CYP2D6 allele distribution and predicted phenotypes of 58 volunteers participating in Studies I-V are shown in Table 5. The effect of the subject's 2D6 genotype on the pharmacokinetics of oral oxycodone was evaluated by combining the data from the placebo phases of Studies I, II, III and V. As shown in Figure 9, the ultrarapid metabolizers (n=6) had lower oxycodone concentrations when compared with extensive metabolizers (P = 0.02). The mean $AUC_{(0-\infty)}$ of oral oxycodone during the placebo phase were 5.0 and 7.3 µg*min/ml for UMs and EMs, respectively. The ultrarapid metabolizers also seemed to have higher oxymorphone concentrations (The mean $AUC_{(0-48h)}$ for oxymorphone were 0.21 and 0.14 µg*min/ml for UMs and EMs, respectively) but statistical significance was not reached for this finding (P = 0.28).

The poor metabolizers (n=4) appeared to have higher noroxycodone AUC and lower oxymorphone AUC values than the extensive metabolisers. Oxycodone concentrations in PM seemed to be only marginally higher than in EM (Figure 9). Because of the insufficient number of PMs (n=4) these findings have no statistical significance.

Table 5. The CYP2D6 allele distribution and predicted phenotypes of 58 volunteers participating in Studies I-V.

CYP2D6 allele composition	Presumed phenotype	Number of subjects		% appearance among 58 subjects
*1/*1	EM	24		
*1/*3	EM	1		
*1/*4	EM	14	43 EM	74%
*1/*6	EM	1		
*1/*10	EM	1		
*1/*41	EM	2		
*1/*1 duplication	UM	7	7 UM	12%
*3/*6	PM	1		
*4/*4	PM	2	5 PM	9%
*4/*5	PM	1		
*4/*6	PM	1		
*4/*41	IM	3	3 IM	5%

EM = extensive metabolizer, UM = ultrarapid metabolizer, PM = poor metabolizer
IM = intermediate metabolizer

5.7 Used inhibitors

The plasma trough concentrations of the used inhibitors were measured to ascertain the use of pre-medication. All subjects had measurable values of corresponding pre-treatment drugs on the morning of the study day indicating good compliance (Studies I-IV) (Table 6). There was considerably variation in the achieved trough concentrations of paroxetine. The lowest plasma paroxetine concentrations were found in Study III in two subjects with CYP2D6 UM genotype.

Table 6. The concentrations of CYP inhibitors used in Studies I-V.

Study	Phase	Paroxetine C_{trough} ng/ml	Itraconazole C_{trough} ng/ml	Telithromycin C_{trough} µg/l	Miconazole $C_{1h\ after\ dose}$ ng/ml
I	Teli (Oxy po)			24 (15 - 32)	
II	Itra (Oxy po)		81 (38 -138)		
	Itra (Oxy iv)		68 (10 -127)		
III	Paro (Oxy po)	9.7 (0.3 - 28.8)			
	Paro + Itra (Oxy po)	12.3 (0.5 – 33.6)	65 (21 - 199)		
IV	Paro (Oxy iv)	11.0 (1.7 – 27.8)			
	Paro + Itra (Oxy iv)	14.5 (1.0 – 41.5)	73 (18 – 180)		
V	Mico (Oxy po)				60 (12 - 114)

The results are shown as mean value (range). C_{trough} = concentrations measured just before the morning dose on study day.

5.8 Adverse effects

One of the subjects complained of nausea during paroxetine pre-treatment in Study III. There were no other reported adverse effects during pre-treatment periods. Altogether nine subjects suffered from nausea on the test day approximately 2-4 hours after oxycodone administration. One subject received 2 mg of tropisetron for the treatment of nausea.

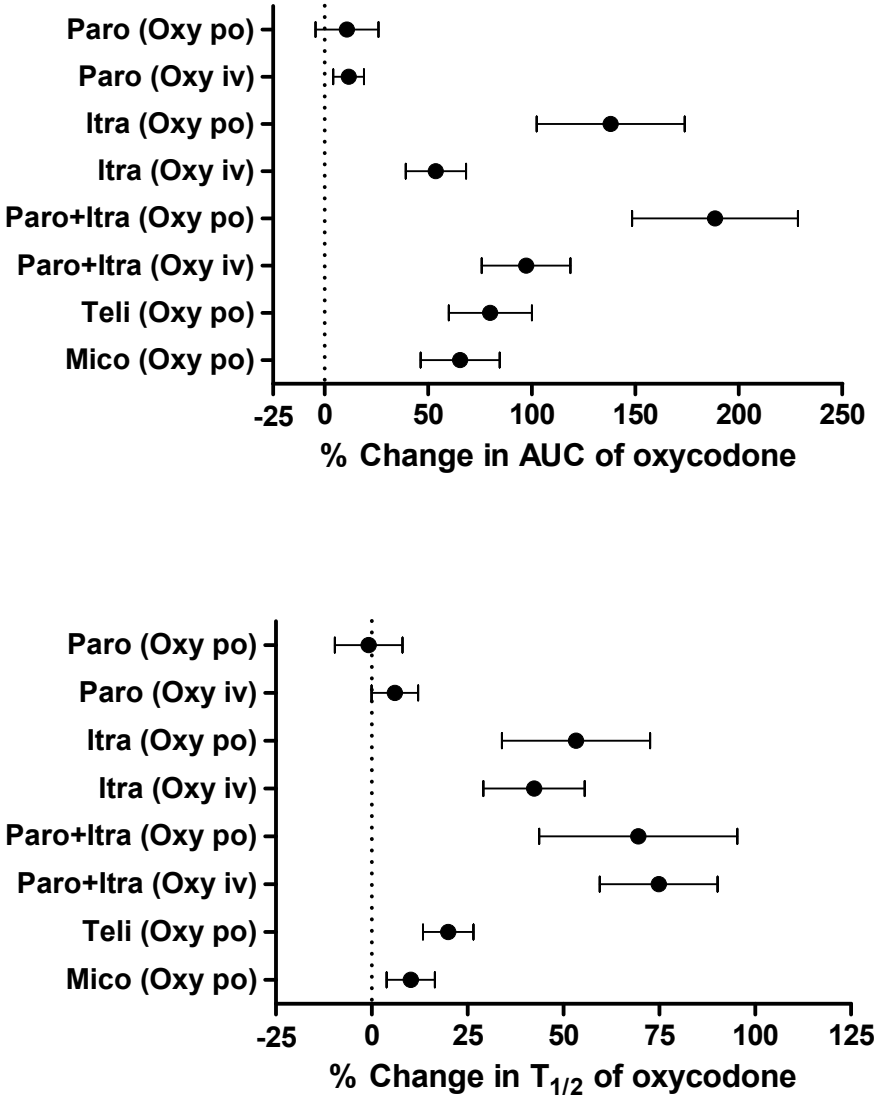


Figure 3. Percent changes in the $AUC_{0-\infty}$ and $t_{1/2}$ of oxycodone with 95% confidence intervals, during the treatment with paroxetine (Paro), itraconazole (Itra), the combination of paroxetine and itraconaxole (Paro + Itra), telithromycin (Teli) and miconazole (Mico) when compared with the placebo/control phases. Oxy = Oxycodone, po = per oral, iv = intravenously.

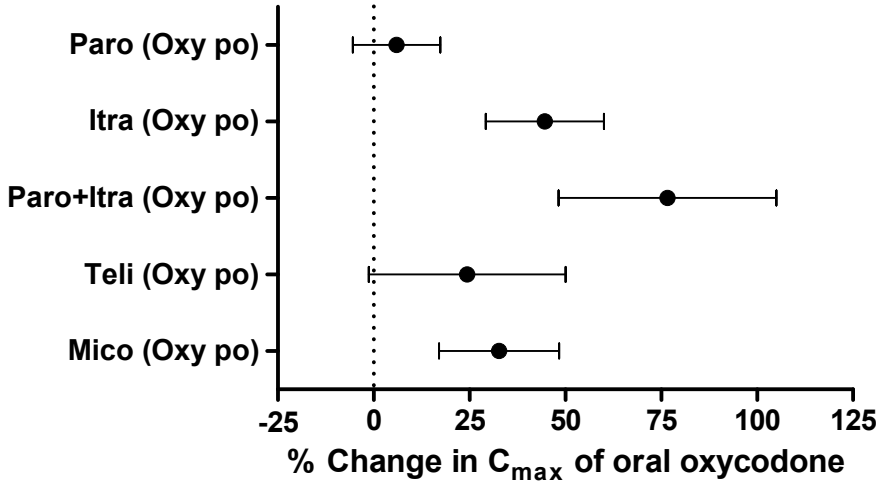


Figure 4. Percent changes in the C_{max} of oral oxycodone with 95% confidence intervals, during the treatment with paroxetine (Paro), itraconazole (Itra), the combination of paroxetine and itraconaxole (Paro + Itra), telithromycin (Teli) and miconazole (Mico) when compared with the placebo/control phases.

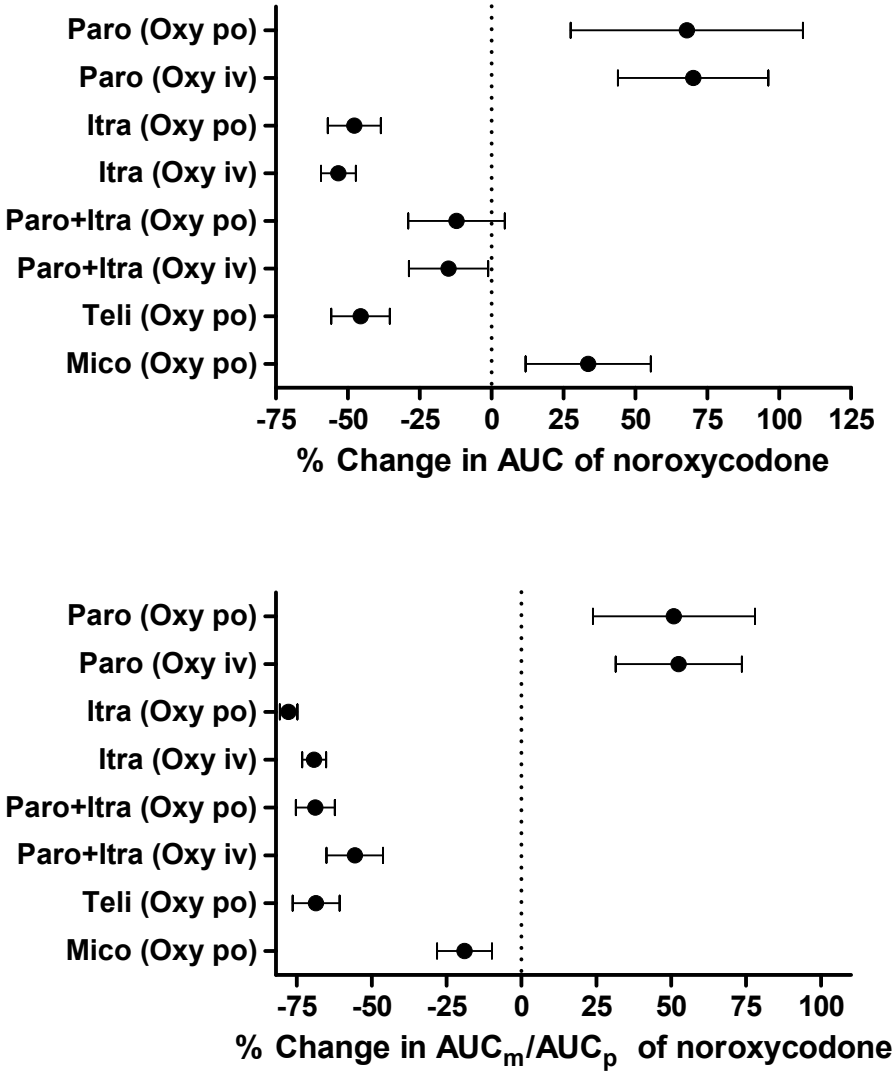


Figure 5. Percent changes in the AUC and AUC_m/AUC_p of noroxycodone with 95% confidence intervals, during the treatment with paroxetine (Paro), itraconazole (Itra), the combination of paroxetine and itraconaxole (Paro + Itra), telithromycin (Teli) and miconazole (Mico) when compared with the placebo/control phases. Oxy = Oxycodone, po = per oral, iv = intravenously.

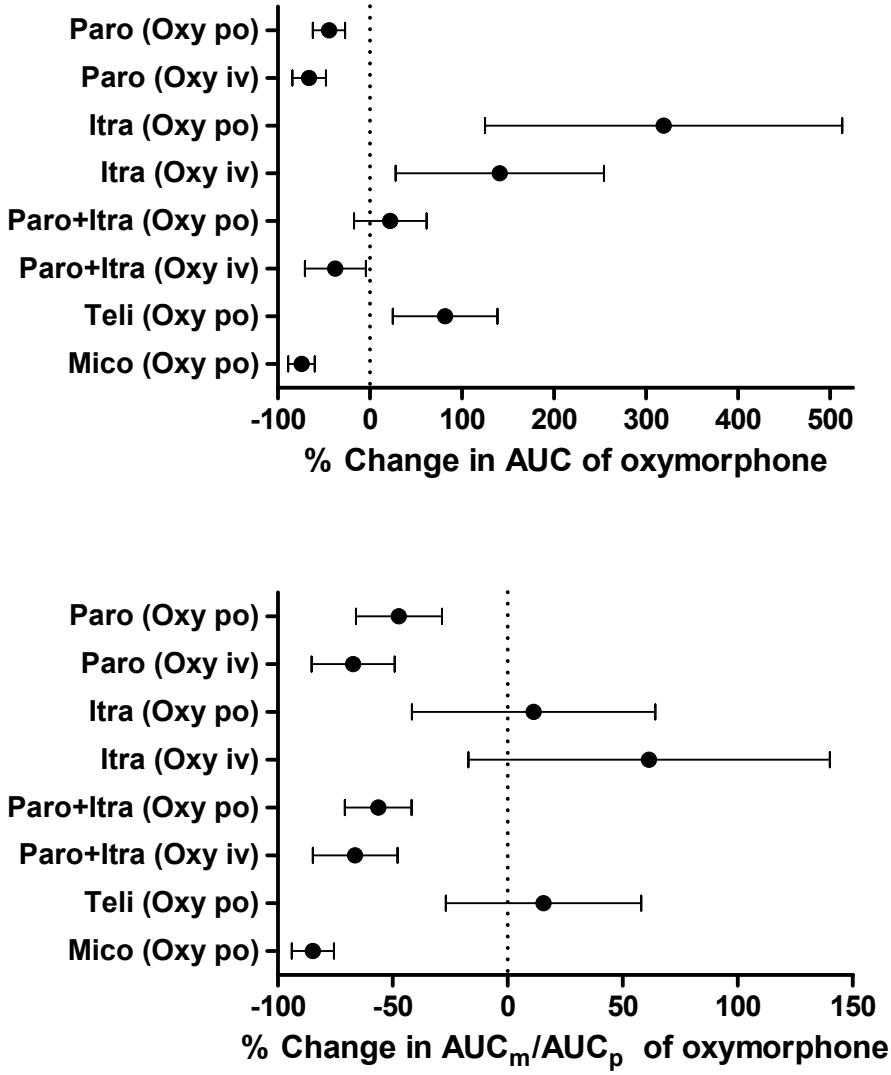
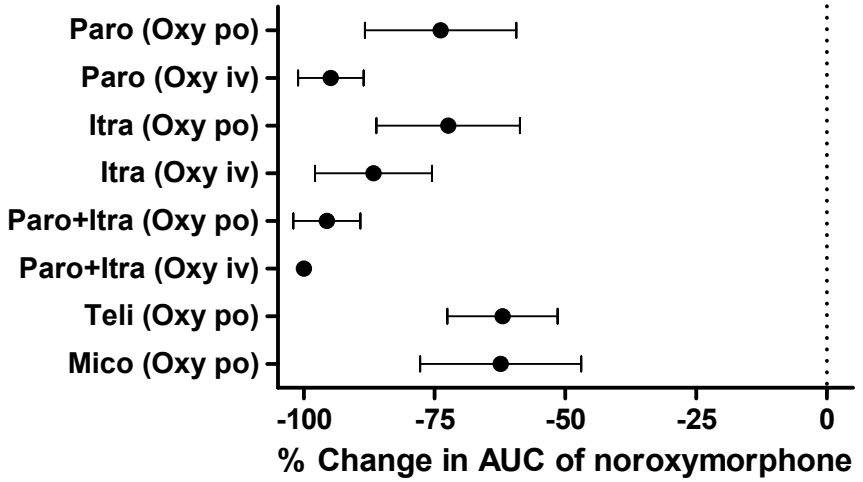


Figure 6. Percent changes in the AUC and AUC_m/AUC_p of oxymorphone with 95% confidence intervals, during the treatment with paroxetine (Paro), itraconazole (Itra), the combination of paroxetine and itraconaxole (Paro + Itra), telithromycin (Teli) and miconazole (Mico) when compared with the placebo/control phases. Oxy = Oxycodone, po = per oral, iv = intravenously.



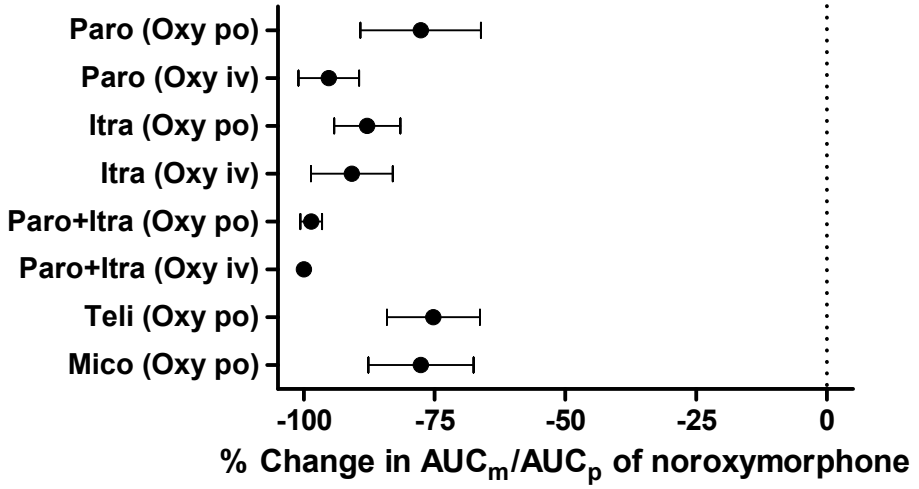


Figure 7. Percent changes in the AUC and AUC_m/AUC_p of noroxymorphone with 95% confidence intervals, during the treatment with paroxetine (Paro), itraconazole (Itra), the combination of paroxetine and itraconaxole (Paro + Itra), telithromycin (Teli) and miconazole (Mico) when compared with the placebo/control phases. Oxy = Oxycodone, po = per oral, iv = intravenously.

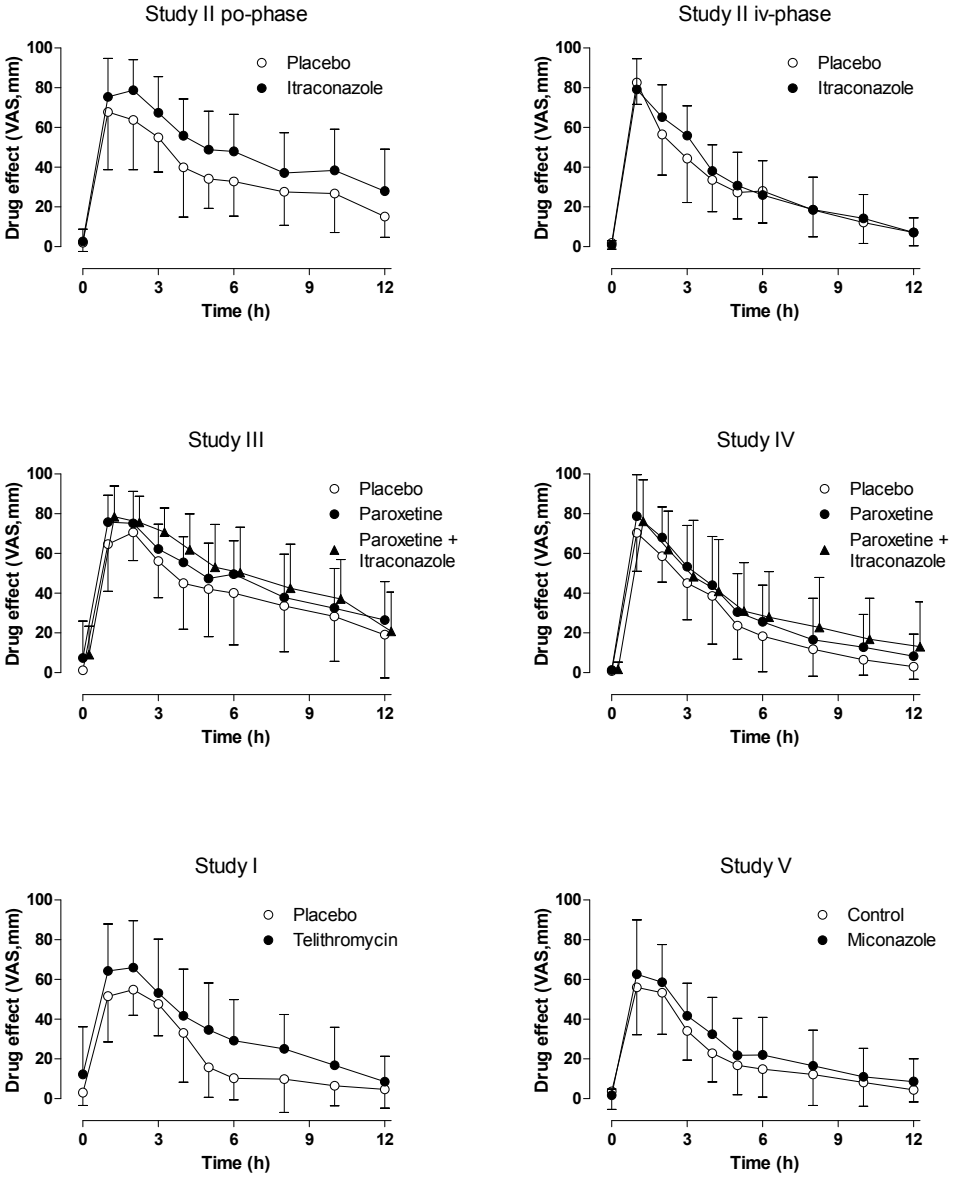


Figure 8. The mean (±SD) self-reported drug effect of oxycodone in Studies I-V during the treatment periods or placebo/control.

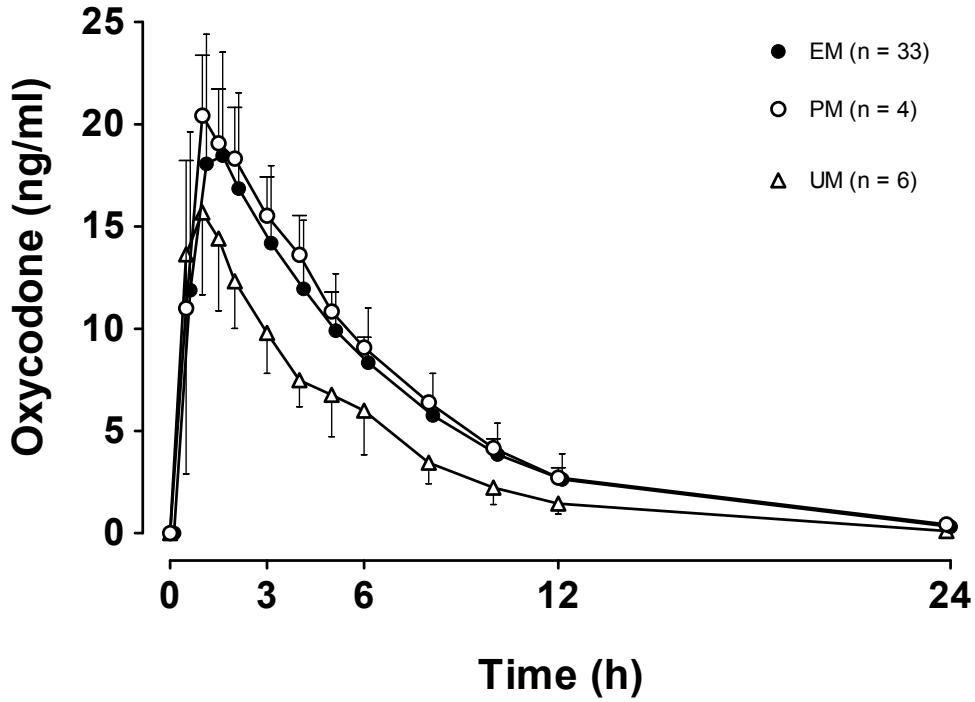


Figure 9. Mean plasma (± SD) concentrations of oxycodone in 46 subjects after 10mg oral dose of oxycodone under placebo phases in the studies I, II, III and V. Symbols for genotypes: EM genotype (●), PM genotype (○) and UM genotype (Δ).

6 DISCUSSION

6.1 Effect of CYP2D6 inhibition

The results with oral and intravenous oxycodone demonstrate that inhibition of the CYP2D6 enzyme with a potent CYP2D6 inhibitor paroxetine has no clinically significant effects on the pharmacokinetics of the parent oxycodone, although it considerably reduces the formation of CYP2D6 dependent metabolite oxymorphone. When the CYP2D6 enzyme was inhibited by paroxetine, the metabolism of oxycodone was shifted more towards the CYP3A pathway as the formation of noroxycodone was increased by about 70%. The results thus demonstrate that normally functioning CYP3A pathway is capable to fully compensate for the reduced activity of CYP2D6 enzyme. Parallel findings have been reported previously with the CYP2D6 inhibitor quinidine and oral extended-release oxycodone (Heiskanen et al. 1998). Recently, another study group has published a study where they evaluated the effect of quinidine on the pharmacokinetics of oral oxycodone (Samer et al. 2010a). In this study, the noted changes in the relative abundances of metabolites were similar to our results with paroxetine, but in addition a 40% increase in $AUC_{0\text{-}24h}$ of oxycodone was reported (Samer et al. 2010a). Just recently, our results were confirmed by Kummer et al. (2011). In their study conducted with healthy volunteers, the plasma concentrations of oral oxycodone were not altered by the paroxetine pre-treatment. Likewise, in a clinical study with chronic pain patients the concomitant use of paroxetine and oxycodone over seven days increased the $AUC_{0\text{-}12h}$ of oxycodone only by 19% (Lemberg et al. 2010). In present studies, paroxetine clearly reduced the formation of secondary metabolite noroxymorphone. In addition to decreased further metabolism of oxymorphone this was mainly result from the reduced CYP2D6 dependent further metabolism of noroxycodone. Overall, the effects of paroxetine on the metabolism of oxycodone were similar for oral and intravenous oxycodone.

Since oxymorphone possesses a strong μ-opioid receptor activity, its role in the pharmacological effects of oxycodone has been debated. Interestingly, it has been shown that in rats intrathecally administered oxymorphone produces a clearly stronger antinociception than oxycodone (Lemberg et al. 2006). However, according to several human studies, the achieved plasma concentrations of oxymorphone after oral and particularly after intravenous administration of oxycodone are very low (Pöyhiä et al. 1992, Kaiko et al. 1996, Heiskanen et al. 1998, Lalovic et al. 2006, Nieminen et al. 2009, Zwisler et al. 2010a, Lemberg et al. 2010). In addition, at least in animals, the ability of oxymorphone to penetrate across the BBB is poorer than that of oxycodone (Lalovic et al. 2006). In the present study series, the mean AUC_m/AUC_p of oxymorphone after oxycodone dosing during the control phase ranged from 0.014 to 0.032 for oral oxycodone and from 0.011 to 0.014 for intravenous oxycodone. Although oxymorphone is a stronger μ-opioid agonist than oxycodone, it is unlikely

that these minor concentrations compared with that of the parent drug oxycodone, have any clinically significant role.

There is some evidence, that CYP2D6 is expressed in certain regions of the human central nervous system (Siegle et al. 2001). In contrast to paroxetine, quinidine penetrates the BBB poorly. Therefore, the possible CYP2D6 dependent metabolism of oxycodone to oxymorphone in the central nervous system might not have been blocked in the abovementioned studies by Heiskanen and Samer. In present studies, paroxetine treatment considerably decreased the achieved plasma concentrations of oxymorphone but the analgesic effect of intravenous or oral oxycodone measured by the cold pressor test was unaltered. Subjects reported marginally higher VAS-scores for drug effect (Study III), drowsiness (Studies III, IV) and deterioration of performance (Studies III, IV) during paroxetine phases. Due to our study design it is not possible to rule out that some of these changes might have been due to the paroxetine itself. However, according to other studies this seems unlikely (Erjavec et al. 2000, Lemberg et al. 2010, Kummer et al. 2011). Our results thus suggest that the opioid effects of oxycodone are not dependent on the formation of oxymorphone. Similar results have been reported with healthy volunteers (Heiskanen et al. 1998, Lalovic et al. 2006, Kummer et al. 2011) and with patient suffering from chronic pain (Lemberg et al. 2010). In contrast, based on the effects of quinidine pre-treatment on the pharmacological effects of oxycodone in 10 healthy volunteers, the opposite conclusion was drawn by Samer et al. (Samer et al. 2010b). The poor metabolizers of CYP2D6 have been assumed to have considerably lower oxymorphone concentrations after oxycodone administration than the extensive metabolizers. Therefore, the importance of oxymorphone for the oxycodone induced analgesia has been evaluated by comparing the analgesic effects of oxycodone in the poor and extensive metabolizers of CYP2D6. A small study using human experimental pain models suggested that analgesic effect of oxycodone might in fact be weaker in poor metabolizers because of lower oxymorphone concentrations (Zwisler et al. 2009) However, a larger clinical study where patients suffering from instant post-operative pain were treated with oxycodone PCA (patient-controlled analgesia), showed no genotype dependent differences in the analgesic effect of intravenous oxycodone (Zwisler et al. 2010a).

In conclusion, the currently available literature and the results of the present studies suggest that the inhibition of CYP2D6 enzyme causes only minimal changes to the exposure to oral or intravenous oxycodone. These minor changes are not reflected to the pharmacological effects of oxycodone. Therefore, the DDI of oxycodone with inhibitors of the CYP2D6 are not clinically important if the function of CYP3A is normal. However, it is possible that in individuals with CYP2D6 UM genotype the consequences of CYP2D6 inhibition may be more prominent than observed in the present studies. Our results also support the concept that in normal conditions oxymorphone has only a minimal, if any role, on the pharmacological effects of oxycodone.

6.2 Effect of CYP3A4 inhibition

At the time when these studies were conducted there was no published information on the effects of CYP3A inhibitors on the pharmacokinetics of oxycodone. Our results showed that the pharmacokinetics of the intravenous and particularly that of the oral oxycodone are markedly affected by the concomitant itraconazole treatment. The exposures to intravenous and oral oxycodone were increased 1.5- and 2.4-fold, respectively. The difference is due to the fact that in addition to the reduced systemic clearance, the increase in the exposure to oral oxycodone was enhanced by increased oral bioavailability as the CYP3A dependent first pass metabolism of oxycodone was inhibited. Due to the increased oral bioavailability the achieved mean C_{max} of oxycodone after oral administration was increased 1.45-fold. Itraconazole caused a marked reduction in the formation of noroxycodone and the metabolism of oxycodone was shifted more towards the CYP2D6 dependent metabolism, as evidenced by the considerably increased concentrations of oxymorphone. In normal conditions, CYP3A is the predominant metabolic pathway for oxycodone and CYP2D6 is accounting only for about 10% of the metabolism of oxycodone (Lalovic et al. 2004, Lalovic et al. 2006). Overall, our results clearly demonstrate that the CYP2D6 pathway has only a limited capacity to compensate alterations in the CYP3A dependent metabolism of oxycodone.

Itraconazole is one of the most commonly used recommended standard CYP3A4 inhibitors for evaluating the interaction potential of substrate drugs in the *in vivo* DDI studies (Huang et al. 2007, EMA 2010). It has been shown previously that the new antifungal agent voriconazole is able to cause extremely strong inhibition of the CYP3A mediated metabolism of midazolam (Saari et al. 2006). The same is true for oxycodone as the concomitant voriconazole treatment increases the exposure to oral oxycodone to 3.6-fold, i.e. clearly more than itraconazole (Hagelberg et al.2009). With clinically used doses, voriconazole thus appears to be stronger inhibitor of CYP3A *in vivo* than itraconazole. *In vitro* data have suggested that CYP enzymes other than CYP2D6 and CYP3A4 may have some minor contribution to the metabolism of oxycodone (Lalovic et al. 2004). Since voriconazole has been shown to be a very strong inhibitor of not only CYP3A4 but also 2C9 and 2C19 (Niwa et al. 2005b), it can be speculated that the observed difference in the magnitude of the interactions may be partly explained by the contribution of these other CYP enzymes inhibited by voriconazole but not by itraconazole. One can also speculate that the role of the CYP2C enzymes may be increased during a strong CYP3A inhibition.

After our studies, two study groups have published their studies on the effect of CYP3A inhibition by ketoconazole on the pharmacokinetics and pharmacodynamics of oral oxycodone (Samer et al. 2010a, Samer et al. 2010b, Kummer et al. 2011). In the study by Kummer et al. (2011), ketoconazole increased the C_{max} and $AUC_{0-\infty}$of oxycodone by 1.8- and 2.5-fold, respectively. These values are close to our results with itraconazole. A somewhat smaller effect by ketoconazole was reported in the study by

Samer et al. (2010a), as the exposure to oxycodone was increased by 1-8-fold. However, it is likely that the maximum inhibition was not reached, as only a single dose of ketoconazole was used in that study.

The induction of CYP3A enzymes by other drugs may also affect the metabolism of oxycodone. In the study by Nieminen et al. (2009), the concomitant treatment with rifampicin reduced the $AUC_{0-\infty}$of oral oxycodone by 86% and attenuated the pharmacological effects of oxycodone. As a result from the rifampicin induced intense increase in CYP3A activity the concentration of noroxycodone and noroxymorphone were drastically increased whereas the concentrations of CYP2D6 dependent metabolite oxymorphone were reduced.

In the present study, itraconazole increased the $AUC_{0-\infty}$of oxycodone in all subjects, and the greatest increase was 3.4-fold. As stated above, the CYP2D6 pathway may partly compensate for the reduced activity of CYP3A. As the activity of 2D6 is genetically determined one could assume that the consequences of CYP3A inhibition are to some degree dependent on the subject's CYP2D6 genotype. In fact, we noted that itraconazole caused smaller increase AUC of oxycodone in single UM subject participating in this study. Because of the small number of subjects, this is highly speculative. Recently, Samer et al. have rather strongly proposed that the magnitudes of the CYP3A mediated drug interactions of oxycodone are dependent on the CYP2D6 genotype of the subjects (Samer et al. 2010a). However, their data included only 10 subjects with two UMs and one PM and the firm conclusions stated in the article may not be justified. When considering the pharmacological effects of oxycodone, the case is even more complicated. In the case of CYP3A inhibition, the PMs might have no compensatory increase in the CYP2D6 mediated metabolism of oxycodone and the interaction would lead to markedly increased exposure to parent oxycodone. On the other hand, in the similar situation the UMs might have somewhat smaller increase in the exposure to oxycodone, but as a consequence of CYP2D6 gene duplication the formation of active metabolite oxymorphone might be remarkably fast. Considering the aforementioned differences in the BBB penetration between oxycodone and oxymorphone, the prediction of the clinical effects of CYP3A inhibition in different genotypes is not so straightforward.

In the present study itraconazole increased the exposure to intravenous oxycodone 1.5-fold but the pharmacological effects were not altered. This finding indirectly supports the minor role of oxymorphone on the pharmacological effects of oxycodone as at the same time the enhanced metabolism through the CYP2D6 pathway increased the exposure to oxymorphone 2.6-fold. Following the oral administration of oxycodone, itraconazole modestly enhanced the subjective drug effect of oxycodone as the reported VAS-scores were somewhat increased. This was most probably resultant from a 2.4-fold increased exposure to parent oxycodone, but one could argue that drastically (4.6-fold) increased exposure to oxymorphone might also have playd some role. By using a sophisticated pupillometry method Kummer et al. (2011) demonstrated that

pre-treatment with ketoconazole made oxycodone induced miosis more pronounced and long lasting. In addition, they were able to show that ketoconazole increased the analgesic effect of oxycodone in the cold pressor test and increased the self-reported drowsiness (Kummer et al. 2011). Parallel findings have also been reported by Samer et al. (2010b). In these studies, the oral oxycodone dose was 0.2 mg/kg i.e. somewhat higher than the dose used in our studies (10mg oral capsule average out 0.15 mg/kg). In a study by Hagelberg et al. (2009) voriconazole increased oxycodone induced miosis, but the analgesic effects of oxycodone were not altered.

6.3 Effect of concomitant inhibition of CYP2D6 and CYP3A4

When the CYP2D6 and CYP3A4 pathways were both inhibited the $AUC_{0-\infty}$ of oxycodone increased 2.0- and 2.9-fold after the intravenous and oral administration of oxycodone, respectively. These changes were seen in all subjects and the biggest increase was more than 4-fold in one subject after oral oxycodone. Although the inhibition of CYP2D6 by paroxetine did not alter the exposure to oxycodone in the present studies, the concomitant administration of paroxetine and intraconazole increased the concentrations of oxycodone clearly more than the administration of itraconazole alone. As the oxidative metabolism of oxycodone is mediated by two CYP enzymes, the compensatory increase in the activity of the remaining pathway may decrease the magnitude of the interaction when only a single pathway is inhibited. Our results clearly demonstrate that although the importance of the CYP2D6 pathway in the metabolism of oxycodone is normally rather limited, its role is emphasised when the CYP3A mediated metabolism of oxycodone is inhibited. Because the mean plasma concentrations of oxycodone, for example, at six hours after oral oxycodone dosing were 2.7-fold higher during combination treatment than in the placebo phase, the accumulation of oxycodone will follow if oxycodone is administered repeatedly.

The strength of CYP2D6 inhibition by paroxetine was not dependent on the route of administration of oxycodone while the CYP3A inhibition by itraconazole was clearly stronger when the oxycodone was administered orally. This is reasonable since unlike CYP2D6, a significant amount of CYP3A4 is found in the small intestine and therefore contributes to the first pass metabolism of orally administered drugs (Kato 2008). Differences in the strength of interactions between the oral and intravenous administration of oxycodone were similar with itraconazole (study II) or with the combination treatment with paroxetine and itraconazole (studies III, IV).

The formation of the secondary metabolite noroxymorphone is dependent on both CYP2D6 and CYP3A enzymes (Lalovic et al. 2004). Concentrations of noroxymorphone were already substantially reduced when either of these enzymes was inhibited. When both enzymes were inhibited simultaneously the formation of noroxymorphone was practically abolished. In normal conditions, the noroxymorphone concentrations after oxycodone administration are relatively high. However, due to the

very low capability to penetrate across BBB its role for the pharmacological effects of oxycodone is assumed to be negligible (Lalovic et al. 2006).

There is wide inter-individual variability in the CYP2D6 activity because of genetic polymorphism. It is estimated that about 7-9% of the population in Western Europe have severely impaired CYP2D6 enzyme activity and are classified as PMs (Eichelbaum et al. 2006, Zwisler et al. 2010a). It could be assumed that in these individuals, the metabolism oxycodone may behave in a similar manner as in the case of strong CYP2D6 inhibition. It has been shown that paroxetine treatment is able to change the subject's CYP2D6 phenotype from EM to PM and from UM to EM or PM (Laine et al. 2001, Žourková and Hadašová 2003). Therefore, our data from the metabolism of oxycodone during the combination treatment with paroxetine and itraconazole could be interpreted also as a situation where the subject with PM genotype is exposed to the CYP3A inhibition. Our results are in line with recent study by Samer et al. (2010a) where the concomitant treatment with quinidine (2D6 inhibitor) and ketoconazole (CYP3A inhibitor) resulted in 3-fold increase in the exposure to the oral oxycodone. In contrast to our result they also reported that this interaction clearly enhanced the pharmacological effects of oxycodone (Samer et al. 2010b). In our studies, where smaller oxycodone doses were used, the noted changes in the pharmacological effect of oxycodone were minor and to some extent biased by the direct effects of paroxetine.

6.4 Effect of telithromycin

Telithromycin is known to be a strong inhibitor of CYP3A4, but it is also stated that it might have some inhibitory effect on CYP2D6 (File 2005, Ketek (SPC) 2007 sanofi-aventis; http://products.sanofi-aventis.us/ketek/ketek.html). Our hypothesis was thus that telithromycin would cause rather strong alterations in the metabolism of oxycodone by inhibiting both of its oxidative metabolic pathways. However, our results showed that the CYP2D6 dependent metabolism of oxycodone was not inhibited by telithromycin. Instead, the concentration of metabolites revealed that the metabolism of oxycodone was shifted more towards CYP2D6 pathway as a consequence of strong inhibition of CYP3A. Overall, telithromycin increased the exposure to oral oxycodone 1.8-fold. Thus the effect of telithromycin on the pharmacokinetics of oxycodone was lower than that of itraconazole (Study II) or voriconazole (Hagelberg et al. 2009). In our single dose study, the opioid effects of oxycodone were slightly enhanced during the telithromycin phase. Considering the risk of the accumulation of oxycodone in repeated administration it should be noted that the mean oxycodone concentration 12 hours after oxycodone dosing was 2.7-fold higher during telithromycin treatment when compared with the placebo phase. The interaction with telithromycin and oxycodone may be clinically relevant if, for example, a chronic pain patient using oxycodone regularly is prescribed a course of telithromycin for the treatment of pneumonia.

6.5 Effect of miconazole oral gel

At present miconazole is used only as topical preparations like an oral gel. Although miconazole oral gel is primarily applied topically on the mucous membranes of the mouth cavity, it is eventually swallowed leading to the intestinal absorption and the systemic effects are therefore possible. It has been demonstrated that *in vitro* miconazole is a strong inhibitor of several human CYP enzymes including the two CYPs important for the metabolism of oxycodone (Niwa et al. 2005a, Niwa et al. 2005b). In the present study, the miconazole oral gel was administered for four days with doses close to those recommended by the manufacturer. With this clinically relevant dosing schedule miconazole treatment increased the $AUC_{0-\infty}$of oxycodone by 63%. The magnitude of the interaction was thus almost in the same order to what was seen with telithromycin and oral oxycodone (Study I). Because of a rather low bioavailability of miconazole, we thought that the inhibition of CYP enzymes by miconazole would be partly mediated by inhibiting the CYP3A enzymes locally in the small intestine. Therefore our hypothesis was that miconazole would cause a stronger inhibition of CYP3A than CYP2D6. In contrast, our results showed that miconazole caused a clearly stronger inhibition of CYP26 than that of CYP3A. Although the capability of miconazole to inhibit the CYP2D6 enzyme has been shown *in vitro*, there is no previous study showing that *in vivo* (Niwa et al. 2005a). Therefore this finding deserves further investigation with a validated substrate drug for CYP2D6.

Since miconazole is used as topical preparations the possibility of systemic adverse effects is often forgotten. Our results add to the previous knowledge and emphasize that in addition to the CYP2C9 and CYP3A inhibition, the inhibition of CYP2D6 by miconazole oral gel is indeed possible *in vivo*. It is advisable to keep this in mind when prescribing miconazole to a patient already taking some drugs metabolized by CYP enzymes (Pemberton et al. 2004, Hynninen et al. 2009, Miki et al. 2010).

6.6 Effects of CYP2D6 genotype

All volunteers participating in Studies I-V were genotyped for CYP2D6. Among the 58 volunteers there were 43 EMs (74%), 7 UMs (12%), 5 PMs (9%) and 3 IMs (5%). The frequency of UM genotype was considerably higher than previously reported for Swedish population (1%) or for the population of Western Europe (5.5%) (Dahl et al. 1995, Eichelbaum et al. 2006). The frequency of PM genotype was at the same level as reported earlier (Eichelbaum et al. 2006, Zwisler et al. 2010a). None of the volunteers participated in more than one study. The diverging frequency of the UM genotype compared with the previous results is most probably only result from change in the rather small material.

At the time of the study inclusion the genotypes of volunteers were not known, as we had no pool of volunteers genotyped beforehand. Therefore, the distribution of

genotypes in different studies was uneven. In these five separate studies, we noted that the CYP2D6 genotype might partly explain the noted inter-individual differences in the pharmacokinetics of oxycodone. However, due to the small number of subjects in the original publications the statistical significance was not reached and any firm conclusion considering the role of CYP2D6 genotype was not possible. Although based on very small study group, similar findings have been reported by others (Samer et al. 2010a, Nieminen et al. 2010). Zwisler et al. (2009) have evaluated the analgesic effect and pharmacokinetics of oral oxycodone in a study with 16 healthy EM and 17 healthy PM volunteers. Their result suggested that PMs would have lower oxymorphone concentrations than EMs. However, it should be noted that they measured the plasma concentrations only from a single plasma sample taken approximately one hour after oral oxycodone administration and then the rate of absorption may bias the result. In a larger scale study with surgical postoperative patients they also measured the oxycodone and oxymorphone concentrations of 24 PM and 246 EM patients about 25 minutes after the intravenous dose of oxycodone. Their results suggested that PMs would have lower oxymorphone concentrations but no difference was noted in the plasma concentrations of oxycodone. It is also possible that there might be some misclassification in their genotype groups as they did not test for the UM genotype (Zwisler et al. 2010a).

By combining the material from the placebo phases of Studies I, II (oral part), III, and V we could gather data from the pharmacokinetics of oral oxycodone in 46 volunteers including 33 EMs, 6 UMs, 4 PMs and 3 IMs. Based on this material we could conclude that UMs had about 32% lower exposure to oxycodone compared with the EMs. In addition, a trend towards higher oxymorphone concentrations among UMs was noted, but statistical significance was not reached for this finding. In contrast, oxycodone concentrations among the PMs were almost equal to those seen in EMs. Oxymorphone concentrations seemed to be lower among PMs, but because of the small number of PMs (n= 4) statistical significance was not reached.

6.7 Pharmacodynamic considerations

The primary endpoint in our studies was the change in the $AUC_{0-\infty}$of oxycodone. Since the power analysis was based on the presumed changes in pharmacokinetics, it was evident that the statistical power of our studies was insufficient for the changes in pharmacological effects as the inter-individual variability was remarkably higher in the pharmacological effects than in pharmacokinetics.

In the present studies, the subjective drug effects, measured by the use of four VAS-scales, seemed to be the most sensitive method in detecting the alterations in the pharmacological effects of oxycodone. The differences between study phases were in most cases detected in VAS-scores of drug effect, the deterioration of performance or drowsiness. However, the detected differences were minor. The use of similar VAS-

scores in evaluating the subjective effects of oxycodone in healthy volunteers has been reported previously (Kaiko et al. 1996, Heiskanen et al. 1998, Zachy and Gutierrez 2003). A small single oxycodone dose used in present studies partly explains why the observed subjective pharmacological effects of oxycodone were rather small. Self-reported drug effects may have been influenced by the pre-treatment drugs and we cannot rule out, for example, the possible influence of paroxetine on reported VAS-scores.

Miosis is a well-known effect of opioids. The measurement of pupil size has been used as a surrogate for the opioid effects of oxycodone in numerous studies (Kaiko et al. 1996, Heiskanen et al. 1998, Zachy et al. 2003, Lalovic et al. 2006, Samer et al. 2010b, Kummer et al. 2011). In the present studies, the pupil diameter was measured with Cogan's pupillometer, which is rather a simple method with resolution of 0.5 mm (Cogan 1941). Other recently published studies have used more sophisticated techniques of pupillometry like static and dynamic pupillometry with infrared camera (Samer et al. 2010b, Kummer et al. 2011). Although the oxycodone-induced miosis was clearly detected in present studies, the differences between study phases were small. In addition, we noted that this pharmacodynamic measurement was hampered by the paroxetine-induced mydriasis in Studies III and IV. The effect of SSRIs on the pupil size has been reported previously (Erjavec et al. 2000). In their recent work, Kummer et al. (2011) reported the blunted oxycodone-induced miosis because of concomitant paroxetine treatment. In other oxycodone interaction studies, where the noted changes in the pharmacokinetics of oxycodone were more prominent than in present studies, the interaction-induced changes on the pupil diameter have been demonstrated (Nieminen et al. 2009, Hagelberg et al. 2009).

In Studies I-V the analgesic effect of oxycodone was evaluated with the feasible cold pressor test where the subjects immersed their hands in ice water (temperature 0.5 -2 ºC). In addition, the more complex measurement of the cutaneuos heat pain threshold was conducted in Studies I and II (Koltzenburg et al. 2006, Staahl et al. 2008). The cold pressor test has been shown to be a sensitive method for studying the analgesic effects of opioids in experimental settings (Posner et al. 1985, Jones et al. 1988, Staahl and Drewes 2004, Koltzenburg et al. 2006, Staahl et al. 2009). In present studies, the administration of oxycodone prolonged the cold pain threshold times and reduced the pain intensity scores in the cold pain test. Similarly, the rise in the heat pain threshold was noted in Studies I and II. However, there were no differences between study phases except for the marginally decreased pain intensity scores in the cold pressor test during the telithromycin phase in Study I. Recently, others have been able to demonstrate that DDI with oxycodone may lead to the alterations in analgesic effect of oxycodone (Nieminen et al. 2009, Samer et al. 2010b, Kummer et al. 2011). Common to these studies were that the used oxycodone doses were higher than used in present study series.

In general, it seems that our rather simple pharmacodynamic measurements were able to measure the opioid effects of oxycodone. However, pharmacokinetic interactions studied were too weak to cause significant changes in pharmacological effects of oxycodone after a single and quite a small dose of oxycodone. In the present studies, the pharmacokinetic differences between study phases were the most evident during the elimination phases when the plasma concentrations as well as the drug effects were already declining. Since immediate-release oxycodone was used, it might have been sufficient to conduct the pharmacodynamic measurements only during the first three to six hours after oxycodone administration. Although the changes in the pharmacological effects of oxycodone in present studies were small or non-existent, it is possible that if multiple doses of oxycodone are administered concomitantly with itraconazole, telithromycin, miconazole or some other CYP3A inhibitor, the accumulation of oxycodone may occur leading to the enhanced opioid effect.

6.8 Limitations of the study

The role of the P-gp in the pharmacokinetics of oxycodone is still under debate. Like discussed above, the animal studies have shown contradictive results (Boström et al. 2005, Hassan et al. 2007). If oxycodone was a P-gp substrate, one could argue that our results could be partly explained by P-gp inhibition, as many of the CYP-inhibitors were also inhibitors of the P-gp. From the CYP-inhibitors used in present studies itraconazole and telithromycin may act as a P-gp inhibitor with clinically used doses, whereas paroxetine and miconazole most probably are not P-gp inhibitors at clinically relevant concentrations (Wang et al. 2002, Yasuda et al. 2002, Weiss et al. 2003, Nenciu et al. 2006, Eberl et al. 2007). If the P-gp inhibition properties of these drugs caused some effect on the pharmacokineticss or pharmacological effects of oxycodone, the alterations would be in the same direction with the effects of CYP inhibition.

To assure the safety of healthy volunteers in the case of markedly inhibited metabolism of oxycodone, the oxycodone dose in present studies was kept rather small. If a larger oxycodone dose had been used, it might be that pharmacokinetic interactions would have been reflected more clearly on the pharmacological effects of oxycodone. In addition, since only a single dose of oxycodone was used the results are not likely to reliable mirror the clinical situations where multiple doses of oxycodone are used. Similarly, it should be noted that these studies were carried out with young healthy volunteers and the results cannot be directly extrapolated, for example, to the elderly or to the patients with marked illness. Overall, for these reasons the interaction potential may have been underestimated.

The dosing of the CYP-inhibitors paroxetine, itraconazole and telithromycin resembled to the clinically used regimens. The dosing schedule of miconazole oral gel in the present study (3.5ml thrice a day) differed slightly from the recommendations given by

the manufacturer (2.5 ml every 6 h). It is unlikely that this minor difference would have affected our results.

Four-day pre-treatments with paroxetine and itraconazole before the administration of oxycodone were too short to achieve the steady state concentrations of these inhibitors, which have the elimination half-life of 21 and 30 hours, respectively (Bourin et al. 2001, De Beule and Van Gestel 2001). However, the inhibitory effect of paroxetine and itraconazole on the CYP enzymes has been previously demonstrated even after short pre-treatments lasting only few days. (Jeppesen et al. 1996, Backman et al. 1998, Kantola et al. 1998, Laugesen et al. 2005). To avoid the unnecessary exposure of healthy volunteers to these drugs, a four-day pre-treatment was considered to be long enough.

6.9 Future research needs

Several animal studies have shown that it is possible that oxycodone concentrates to the central nervous system (Boström et al. 2006, Lalovic et al. 2006, Boström et al. 2008). In contrast, the achieved concentrations of morphine in rat brain tissue have shown to be lower than those in plasma (Tunblad et al. 2003, Boström et al. 2008). Interestingly, the brain to blood AUC ratios of morphine below unity has also been shown in humans by using the quite invasive microdialysis method in brain trauma patients (Ederoth et al. 2004). This finding might help to understand the discrepancies *in vitro* and *in vivo* potencies of these drugs. By using this method, it might be possible to confirm if oxycodone truly concentrates to the brain tissue also in humans as well as the role of oxymorphone vs. the parent in the PD changes due to CYP-inhibition.

The present study series together with several recently published other studies have demonstrated that the inhibition of the CYP3A-dependent metabolism of oxycodone may increase the exposure to the oxycodone considerably (Nieminen et al. 2009, Hagelberg et al. 2009, Samer et al. 2010, Kummer et al. 2011). Because of ethical reasons, strong opioids like oxycodone cannot be administered repeatedly to healthy volunteers. Therefore, further patient studies with multiple oxycodone doses would be worthwhile to evaluate the risk for the accumulation of oxycodone and the clinical significance of CYP3A mediated DDIs with oxycodone. In addition, there is some evidence that CYP3A mediated DDIs of oxycodone might be modulated by subjects CYP2D6 genotype (Samer et al. 2010a, Samer et al. 2010b). Although rather laborious to conduct, a larger scale study with genotyped pain patients would be needed to explore if some individuals are at particular risk for CYP3A mediated DDIs of oxycodone.

In Study V it was noted that miconazole caused a strong inhibition of CYP2D6 dependent metabolism of oxycodone. Although the capability of miconazole to inhibit the CYP2D6 enzyme has been shown *in vitro*, there is no previous study showing that *in vivo* (Niwa et al. 2005a). Therefore this finding deserves further investigation with a validated substrate drug for CYP2D6.

7 CONCLUSIONS

1. The concomitant treatment with the potent CYP2D6 inhibitor paroxetine with intravenous or oral oxycodone had no clinically significant effects on the exposure to parent drug oxycodone or on the pharmacological effects of oxycodone although the formation of oxymorphone was considerably reduced. Our results suggest that DDIs of oxycodone with inhibitors of the CYP2D6 are not clinically important if the function of CYP3A is normal. However, in individuals with CYP2D6 UM genotype taking oxycodone regularly, the effect of CYP2D6 inhibition may be more pronounced than was observed in the present studies.

2. Itraconazole increased the oral bioavailability of oxycodone from 55 to 82%. The inhibition of CYP3A pathway by itraconazole increased the exposure to the parent oxycodone by 51% and by 144% after intravenous and oral oxycodone, respectively. Itraconazole clearly shifted the metabolism of oxycodone more towards CYP2D6-dependent pathway. However, our results demonstrate that the CYP2D6 pathway has only a limited capacity to compensate alterations in the CYP3A dependent metabolism of oxycodone. The effect of CYP3A inhibition is more pronounced when oxycodone is administered orally because the CYP3A mediated first pass metabolism of oxycodone is also inhibited. Itraconazole treatment increased the pharmacological effects of oral oxycodone only modestly, whereas the pharmacological of intravenous oxycodone was unaltered.

   When repeated doses of oxycodone are used concomitantly with itraconazole or some other strong inhibitor of CYP3A, the accumulation of oxycodone may follow leading to the enhanced opioid effects and increased risk of adverse events.

3. When CYP2D6 and CYP3A pathways were both inhibited the $AUC_{0-\infty}$of oxycodone increased 2- and 2.9-fold after the intravenous and oral administration of oxycodone, respectively. In addition, the achieved C_{max} of oral oxycodone was increased 1.8-fold. Despite the markedly increased exposure to oxycodone the alterations in the pharmacological effects after a single dose of oxycodone were minor.

   Present studies demonstrate that although the importance of the CYP2D6 pathway in the metabolism of oxycodone is normally rather limited, its role as a complementary metabolic pathway is emphasised when the CYP3A mediated metabolism of oxycodone is inhibited. Therefore it is possible that the effect of CYP3A inhibition is to some extent also dependent on subject's CYP2D6 genotype.

4. Concomitant telithromycin treatment increased the exposure to oxycodone 1.8-fold. This was mainly a consequence from the inhibition of CYP3A dependent

metabolism of oxycodone. If telithromycin inhibited CYP2D6, the effect was masked by the more forceful inhibition of CYP3A leading to overall increased metabolism through the CYP2D6 pathway. The opioid effects of oxycodone were slightly enhanced during the telithromycin phase.

The short treatment with miconazole oral gel increased the exposure to oxycodone by 63%. Particularly the CYP2D6 enzyme was strongly inhibited by miconazole.

Although the magnitudes of these interactions with telithromycin and miconazole oral gel were rather limited after a single dose of oral oxycodone, they may be clinically relevant when multiple doses of oxycodone are administered.

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