PHARMACOKINETICS OF OXYCODONE AND PARACETAMOL IN THE ELDERLY

A Clinical Pharmacokinetic Study on Orthopaedic Surgical Patients and Healthy Volunteers

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ABSTRACT

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The proportion of elderly people over 65 years of age in Finland is expected to grow to over 25% by the 2025. It has been estimated that elderly people today consume nearly 40% of all drugs. Age brings about number of physiological changes that may affect the disposition, metabolism and excretion of drugs. The function of heart, lungs, liver and kidneys decreases even in healthy people, as they get older. The proportion of total body water decreases and the relative fat percentage increases. Also several other factors such as concurrent diseases, concomitant medication and nutritional factors have an effect on drug therapy in elderly. Age increases the risk of adverse drug reactions, which most often are dose-dependent. Despite all this there are not enough studies involving the elderly people and the elderly are most often excluded from clinical trials.

Oxycodone is a strong opioid analgesic, which is used to treat moderate or severe pain. Paracetamol is a widely used nonopioid analgesic, which has become popular in the treatment of pain in many patient groups. In this series of studies the pharmacokinetics of oral and intravenous oxicodone as well as intravenous paracetamol in the elderly and young adult patients were investigated. Also a study investigating the interaction of oral antibiotic clarityhromycin, a known cytochrome P450 (CYP) 3A4 inhibitor, with oxycodone pharmacokinetics and pharmacodynamics in elderly and young healthy volunteers was carried out.

The pharmacokinetics of oxycodone showed a clear age depency. Patients over 70 years had 50-80% higher mean exposure to oral oxycodone and a twofold greater plasma concentration than young adults 12 h after ingestion of the drug. Elderly patients had 40-80% greater exposure to intravenous oxycodone and patients over 80 years had over twofold greater plasma concentrations 8 h post dose than the young adults. The elderly patients had also greater exposure to intra venous paracetamol compared to young adults. Clarithromycin increased the exposure to oral oxycodone in both young and elderly volunteers. The elderly had marked interindividual variation in the pharmacokinetics and pharmacodynamics when clarithromycin was given concomitantly with oxycodone. Because the pharmacokinetics of oxycodone and intravenous paracetamol depend on the age of the subject, it is important to titrate the analgesic dose individually in the elderly.

Keywords: oxycodone, paracetamol, pharmacokinetics, elderly

TIIVISTELMÄ

Antti Liukas

OKSIKODONIN JA PARASETAMOLIN FARMAKOKINETIIKKA VANHUKSILLA
Kliininen farmkokineettinen tutkimus ortopedisilla leikkauspotilailla ja terveillä
vapaaehtoisilla koehenkilöillä

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Yli 65-vuotiaiden vanhusten osuus Suomessa on arvioitu kasvavan yli 25%:in vuoteen 2025 mennessä. Nykypäivänä vanhukset kuluttavat 40% kaikista lääkkeistä. Ikääntyessä elimistön fysiologia muuttuu, mikä saattaa vaikuttaa lääkeaineiden imeytymiseen, jakautumiseen, aineenvaihduntaan ja eritykseen elimistöstä. Sydämen, keuhkojen, maksan ja munuaisten toiminta heikkenee iän myötä myös terveilläkin ihmisillä. Veden määrä elimistössä laskee ja rasvan suhteellinen osuus lisääntyy. Myös muut tekijät kuten sairaudet, mahdollinen muu lääkitys ja ravitsemukselliset tekijät vaikuttavat ikäihmisten lääkehoitoon. Suurin osa lääkkeiden aiheuttamista haittavaikutuksista on lääkkeen annostuksesta riippuvaisia. Korkea ikä nostaa lääkkeiden haittavaikutusten riskiä. Tästä huolimatta vanhuksia ei ole riittävästi tutkittu ja usein ikäihmiset suljetaan pois kliinisistä tutkimuksista.

Oksikodoni on vahva opiaatti-kipulääke, jota käytetään keskivaikean ja kovan kivun hoitoon. Parasetamoli on yleisesti käytetty kipulääke kaikissa ikäryhmissä. Tässä tutkimuksessa selvitettiin sekä suun kautta annetun että suonensisäisesti annetun oksikodonin ja suonensisäisesti annetun parasetamolin farmakokinetiikkaa nuorilla aikuisilla ja vanhuksilla. Lisäksi selvitettiin sytokromi P450 (CYP) 3A:ta estävän antibiootin, suun kautta annostellun klaritromysiinin vaikutusta oksikodonin farmakokinetiikkaan ja farmakodynamiikkaan sekä iäkkäillä, että nuorilla vapaaehtoisilla.

Tutkimuksessa ikä vaikutti selvästi oksikodonin farmakokinetiikkaan. Yli 70-vuotiailla altistuminen suun kautta annetulle oksikodonille oli 50-80% suurempaa ja heillä oli yli kaksinkertainen oksikodonin plasmapitoisuus nuoriin aikuisiin verrattuna 12 tuntia lääkkeen ottamisesta. Iäkkäillä altistuminen suonensisäisesti annostellulle oksikodonille oli 40-80%suurempaa ja oksikodonin plasmapitoisuus oli yli kaksinketainen 8 tuntia lääkkeen antamisesta nuoriin aikuisiin verrattuna. Myös suonensisäisesti annostellulle parasetamolille altistuminen oli suurempaa iäkkäillä potilailla. Klaritromysiini lisäsi altistumista oksikodonille sekä nuorilla että iäkkäillä vapaaehtoisilla. Iäkkäillä yksilöiden väliset erot olivat suuria oksikodonin farmakokinetiikassa ja farmakodynamiikassa samanaikaisen klaritromysiini-kuurin yhteydessä. Koska potilaan ikä vaikuttaa oksikodonin ja suonensisäisesti annostellun parasetamolin farmakokinetiikkaan ja farmakodynamiikkaan, tulee näidnen lääkkeiden annos arvioida aina yksilöllisesti iäkkäillä potilailla.

Avainsanat: oksikodoni, parasetamoli, farmakokinetiikka, vanhukset

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ABBREVIATIONS

ADR	adverse drug reaction
AM404	N- (4-hydroxyphenyl) arachidonoylethanolamide
ASA	American Society of Anesthesiologists
AUC_{0-t}	area under plasma concentration-time curve from zero to t hours
AUC_m/AUC_p	metabolite to parent drug area under plasma concentration-time curve ratio
BMI	body mass index
CI	confidence interval
CL	plasma clearance
CL/F	apparent clearance
C_{max}	peak plasma concentration
CNS	central nervous system
CPT	cold pain threshold
CYP	cytochrome P450
CV	coefficient of variation
DDI	drug-drug interaction
DSST	digit symbol substitution test
EDTA	ethylenediaminetetra-acetic acid
EM	extensive metabolizer via CYP2D6 enzyme
F	bioavailability of drug
FAAH	fatty acid amide hydrolase
GFR	glomerular filtration rate
HPLC	high performance liquid chromatography
IM	intermediate metabolizer via CYP2D6 enzyme
LC-MS	liquid chromatocraphy-mass spectrometric method
LC-MS/MS	liquid chromatocraphy-tandem mass spectrometric method
LLQ	lower limit of quantification
ln	natural logarithm
MWT	Maddox wing test
NADP	nicotiamide adenine dinucleotide phosphate
NADPH	redused form of NADP
NAPQI	N-acetyl-p-benzoquinoneimine
NSAID	nonsteroidal anti-inflammatory drugs
PACU	post anaesthesia care unit
PM	poor metabolizer via CYP2D6 enzyme
SD	standard deviation
SEM	standard error of mean
SI	international system of units
SpO_2	peripheral arteriolar oxygen saturation
$t_{1/2}$	elimination half-life
t_{max}	time to peak concentration
UM	ultrarapid metabolizer via CYP2D6 enzyme
V_{ss}	steady state volume of distribution
V_z/F	apparent volume of distribution during elimination
VAS	visual analogue scale

LIST OF ORIGINAL PUBLICATIONS

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

I   Liukas A, Kuusniemi K, Aantaa R, Virolainen P, Neuvonen M, Neuvonen PJ, Olkkola KT. Plasma concentrations of oral oxycodone are greatly increased in the elderly. Clin Pharmacol Ther 2008; 84: 462-467.

II   Liukas A, Kuusniemi K, Aantaa R, Virolainen P, Neuvonen M, Neuvonen PJ, Olkkola KT.Elimination of intravenous oxycodone in the elderly: a pharmacokinetic study in postoperative orthopaedic patients of different age groups. Drugs Aging 2011; 28: 41-50.

III Liukas A, Kuusniemi K, Aantaa R, Virolainen P, Niemi M, Neuvonen PJ, Olkkola KT. Pharmacokinetics of intravenous paracetamol in elderly patients. Clin Pharmacokinet 2011; 50:121-129.

IV   Liukas A, Hagelberg N, Kuusniemi K, Neuvonen PJ, Olkkola KT. Inhibition of cytochrome P450 3A by clarithromycin uniformly affects the pharmacokinetics and pharmacodynamics of oxycodone in young and elderly volunteers. J Clin Psychopharmacol 2011; 31: 302-308.

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1. INTRODUCTION

Older people, over 65 years of age are the largest group consuming prescription medication, Elderly are also susceptible to various conditions that require pain medication and the elderly often use concomitantly more than one analgesic drug.

Oxycodone is a semi-synthetic μ opioid agonist analgesic in clinical use since 1916. It has been studied extensively only just since the beginning of the 1990's. Oxycodone can be administered by intravenous, intramuscular, oral and rectal routes. Its main indications are treatment of postoperative and cancer pain (Kalso and Vainio 1990, Kalso et al. 1990, Kalso et al. 1991, Pöyhiä et al. 1991, Pöyhiä et al. 1992). The global use of oxycodone has increased several folds during last decade and it is one of the most used strong opioid for severe pain in Finland (Pöyhiä 1994, Paulozzi 2006, Hamunen et al. 2009, Bell et al. 2009, Bell et al. 2011).

The pharmacokinetic properties of oxycodone in young and middle-aged subjects have been extensively described. Oxycodone has a relatively high oral bioavailability of 55-85%. It is extensively metabolized in the liver and the intestinal wall by cytochrome P450 (CYP) enzymes and less than 10% of a single dose is excreted unchanged in urine. The major oxidative pathway is N-demethylation of oxycodone to noroxycodone via CYP3A, and minor pathway involves O-demethylation of oxycodone to oxymorphone via CYP2D6. These metabolites are further metabolized to noroxymorphone by CYP3A and CYP2D6 (Poyhia et al. 1992, Lalovic et al. 2004, Lalovic et al. 2006).

The interindividual variability in the activity of CYP2D6, CYP3A and the inducible or inhibitable character of CYP3A4, affect the metabolism of oxycodone. Oxidative metabolism of oxycodone via CYP3A4 predisposes individuals with oxycodone therapy to drug-drug interactions, which may increase adverse effects or diminish analgesic effect of oxycodone (Hagelberg et al. 2009, Nieminen et al. 2009).

Clarithromycin is a macrolide antibiotic used in the treatment of respiratory tract infections. It is a potent inhibitor of CYP3A (Gorski et al. 1998) with consequent potential effects on the metabolism of drugs involving CYP3A pathway. Until now there has been no systematic information on the effect of clarithromycin on the pharmacokinetics and pharmacodynamics of oxycodone in humans.

Paracetamol is a widely used nonopioid analgesic, which lacks many of the adverse effects of classic anti-inflammatory drugs (Hyllested et al. 2002). Multimodal analgesia for acute postoperative pain with opioids in conjunction with paracetamol has become very popular after intravenous paracetamol became commercially available in 2002.

Paracetamol is metabolised mainly in the liver by two major hepatic pathways: glucuronic acid conjugation and sulphuric acid conjugation. The sulphuric acid conjugation route is rapidly saturable at doses that exceed the common therapeutic

doses. A small fraction of paracetamol (less than 4%) is metabolised by CYPE1 to a reactive intermediate, N-acetyl benzoquinone imine (NAPQI). NAPQI is rapidly detoxified by reduced glutathione under normal conditions and excreted in the urine after conjugation with cysteine and mercaptopuric acid. However, during vast overdosing, the quantity of this toxic metabolite is increased. 90% of the paracetamol dose is excreted in urine in 24 hours, mainly as glucuronide (60-80%) and sulphate (20-30%) conjugates. Less than 5% of paracetamol is excreted to urine unchanged.

Although both oxycodone and intravenous paracetamol have been studied widely, the pharmacokinetic data is mainly based on studies performed in patients under 70 years of age or in healthy subjects. Nevertheless, a growing number of patients requiring analgesics are much older, up to 90 or even 100 years of age. Old age causes a number of physiological changes that may result in significant alterations in drug pharmacokinetics, which in turn may necessitate dose modifications (Butler and Begg 2008, Klotz 2009).

Oxycodone and paracetamol are widely used analgesics both of which potentially have serious and even life-threatening adverse effects when massively overdosed. Therefore it was considered important to study the pharmacokinetics of oxycodone and intravenous paracetamol in orthopaedic surgical patients of different ages and the inhibitory effect of clarithromycin on the pharmacokinetics of oxycodone particularly in the elderly.

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2. REVIEW OF THE LITERATURE

2.1. Elderly population

To date there are no biological markers to define what is meant by elderly. In the United States and in the United Kingdom the age of 65 has been considered the beginning of the senior years because, until recently in both countries people became eligible to retire with full social security benefits at that age. Indeed, that age is most often considered the starting point of old age. (Herrlinger and Klotz 2001, Cusack 2004, Schwartz 2007, Shi et al. 2008, Klotz 2009). The proportion of population over 65 years of age in Finland in the year 2000 was 14.9% and it is expected to grow to 25.2% by the year 2025 (United Nations 2002). The number of elderly people requiring pain medication is expected to grow even more as elderly people are more likely to have conditions requiring pain treatment than young adults. Elderly people today consume nearly 40% of all drugs (Benedetti et al. 2007). Adverse drug reactions (ADR) were the sixth leading cause of death in the United States in 1994. Age was an independent risk factor influencing the incidence of ADR. Most, over 75% of ADRs were dose-dependent and not caused by idiosyncratic or allergic reactions (Lazarou et al. 1998).

Despite their being the largest group consuming prescription medication, and their susceptibility to conditions such as hypertension and acute coronary syndrome, the elderly are most often excluded from the clinical trials. In 2000, only 3.4% of the total 8945 randomised controlled trials and 1.2% of the 706 meta-analyses included people who were over 65 years old (Nair 2002). As an example in 1995, 37% of patients hospitalized for myocardial infarction in the United States were over 75 years of age. For the clinical trials of acute coronary syndrome published between the 1996 and 2000 more than half failed to recruit patients over 75 years of age (Lee et al. 2001).

2.2. Ageing

As people get older there are a number of progressive physiological changes in multiple organs. The function of heart, lungs, liver and kidneys decreases even in healthy people, as they get older. With age the size and mass of the liver decreases up to 20-30% and hepatic blood flow is also reduced up to 20-50% without diagnosable liver disease (Klotz 2009). This together with other age-related physiological changes e.g. decreased renal blood flow and decreased glomerular filtration rate may contribute to differences in the pharmacokinetics of several drugs. With age the gastric pH increases and gastric emptying is slowed, which may decrease absorption. Also the fraction of total body water decreases and the relative fat percentage increases with age. These changes can affect the absorption, distribution, metabolism and elimination of drugs. Interindividual variability in pharmacokinetics is considerable in the elderly. At advanced age several factors including physiological changes in organ function, concurrent diseases, concomitant medication and nutritional factors may affect the disposition, metabolism and excretion of drugs (Shi et al. 2008, Klotz 2009).

Frailty has been defined as a clinical syndrome by Fried et al in 2001. The diagnosis of frailty can be assessed when three or more of the following criteria are present: unintentional weight loss or loss of muscle mass, weak grip strength or self-reported exhaustion, slow walking speed and low physical activity. Symptoms of frailty are common in the elderly. Frailty is strongly associated with aging but it is not a definite part of advanced age. Frailty involves decreased reserves in multiple organ systems. Frailty can begin by onset of a new disease, malnutrition, stress or simply lack of activity. Condition develops slowly and the over all wellbeing declines stepwise with increments of unfortunate acute events adding to the whole. Frailty involves cachexia, abnormal function of inflammatory as well as neuroendocrine system and poor nutrition or ability to regulate energy. In the frail elderly, the body's ability to maintain homeostasis is impaired during acute stress (Powell 1997, Ahmed et al. 2007, Hubbard et al. 2008, Hubbard et al. 2008, Hubbard et al. 2009). In frail elderly the pharmacokinetics of drugs must be considered separately.

2.3. Pharmacokinetics and ageing

2.3.1. *Drug absorption*

Absorption is dependent on the absorptive capacity of the small bowel where most oral drugs are absorbed. Although there are changes in the gastrointestinal tract with ageing, they don't appear to affect the extent of absorption of drugs significantly (Cusack 2004). The rate of absorption is significant for drugs that require rapid onset of action such as analgesics. No difference in gastric emptying between young and elderly people has been observed when paracetamol was used as a probe (Gainsborough et al. 1993). Body position, however, affects gastric emptying. Lying position has been found significantly delay the gastric emptying compared the sitting position (Ikeda et. al. 2008).

Age may affect the bioavailability of drugs. Bioavailability of many drugs is dependent on presystemic extraction in the bowel mucosa and in liver. For instance, the bioavailability of labetalol is increased in elderly and due to decreased hepatic extraction during first-pass through the liver (Kelly et al. 1982). Bioavailability of some drugs is regulated by presystemic extraction by small bowel CYP3A4 activity and by activity of P-glycoprotein, which is an efflux transporter localized in the apical membrane on the epithelial cells lining the intestinal lumen. Age, however, does not alter the activity of CYP3A4 (Dresser et al. 2000) or P-glycoprotein (Hamman et al. 2001).

2.3.2. *Drug distribution*

The body composition changes with aging. The body fat increases by 35% with people 65-80 years of age when compared to 20 year olds (Klotz 2009). In the elderly population plasma volume decreases by 8%, total body water decreases by 17% and extracellular fluid decreases by 40%. The changes in body fat and total bodywater decreases the volume of distribution of polar drugs and increases the volume of distribution of lipophilic drugs (Hanratty et al. 2000). The distribution is also influenced by protein binding of a drug. Serum albumin levels are known to decrease

with age, yet steady-state concentrations of protein-bound drugs are usually unchanged (Benet and Hoener 2002).

2.3.3. *Metabolism in the liver*

The macroscopical appearance of liver changes with increasing age. The size of the liver is reduced by 20-30% and the color turns from reddish towards brown. The color change in this "brown atrophy" is caused by accumulation of pigmented waste products in lysosomes within hepatocytes (Le Couteur and McLean 1998).

Aging and also cirrhosis of the liver cause structural changes in the sinusoidal endothelium and the perisinussoidal space (space of Disse) of the liver. Collagen buildup and thickening of the endothelium, defenestration of endothelium and the portal-venous and the arterio-venous shunts hinder transfer of drugs into hepatocytes in the elderly (Le Couteur et al. 2005).

Hepatic blood flow decreases markedly with age up to 20-50% (Klotz 2009). Although some studies have shown even greater fall in liver blood flow, the impact on liver perfusion is not equally great since the mass of the liver is also reduced with increasing age (Wynne et al. 1989). There are number of changes in liver with aging witch affect the pharmacokinetics of drugs, especially those with flow limited metabolism or those that undergo phase I metabolism in liver (McLean and Le Couteur 2004).

2.3.4. *Renal elimination*

Kidneys get smaller with age. The kidney mass is reduced by approximately 20 to 25% between age of 30 and 80 years (Beck 1998). Interstitial fibrosis, tubular atrophy and arteriosclerosis are increased (Beck 1998, Melk and Halloran 2001). After the age of 50 years, glomerulosclerosis increases at an accelerated rate in both men and women without apparent renal disease (Neugarten et al. 1999).

Glomerular filtration rate (GFR) and renal tubular secretion are reduced with advancing age. Creatinine clearance (CL_{CR}) is expected to decrease from 140 ml/min at the age of 30 to 97 ml/min at the age of 80 (Rowe et al. 1976). Because of muscle mass reduction with increasing age, serum creatinine level can't be used alone to determine renal function. Measuring the glomerular filtration rate by 24-hour urine collection is the most reliable way to determine the renal function. As 24-hour urine collection is fairly complicated task so GFR has been estimated by using the Cockcroft-Gault formula (Cockcroft and Gault 1976):

$$CL_{CR} \,(\text{ml/min}) = \frac{(140 - \text{age}) \times \text{bodyweight (kg)}}{72 \times \text{serum creatinine (mg/dl)}}$$

For women, this equation is multiplied by 0.85. However the Cockcroft-Gault formula has been found to systematically underestimate GFR and the National Kidney

Foundation practice guidelines for chronic kidney disease published a better validated "modification of diet in renal disease (MDRD)" equation (Levey et al. 2003):

GFR = 88.4^{-1} x $186^{-1.154}$ x Serum Creatinine$^{-1.154}$ (µmol/l) x Age$^{-0.203}$ (years) x (0.742 if female) x 88.4^{-1} and x 1.212 (if Black race)

The estimated GFR is used to classify the renal function in chronic kidney disease as follows:

Table 1.

Stage	Description	GFR (ml/min/1.73 m^2)
1	Normal GFR	> 90
2	Mild decrease in GFR	60 – 89
3	Moderate decrease in GFR	30 – 59
4	Severe decrease in GFR	15 – 29
5	Kidney failure	< 15 or dialysis

(The National Kidney Foundation 2002)

Dosing of some antibiotics, chemotherapy and immunosuppressive agents are often corrected for estimated renal function (Schwartz 2007). Renal function is a major factor affecting the elimination of drugs from the body. Failure to consider reduced renal function with narrow therapeutic to toxic ratio drugs may lead to increased toxicity, especially in elderly women (Grines 2000).

2.4. Drug effect and age

The events following drug administration can be divided into two phases. The pharmacokinetic phase determines the concentration of a given drug at the effect site. The second phase is the pharmacodynamic phase, witch determines the magnitude of the effects produced by the drug at the effect site. The same plasma concentration of a drug may have very different effect depending on patient's age. Warfarin is a good example. Warfarin dosage is not controlled by plasma concentration but the control is taken even a step further and its anticoagulant effect is measured. Even so, the warfarin's risk-benefit ratio depends on patient's age. The risk of major bleeding in elderly people taking oral anticoagulants increases by approximately 50% for every decade over the age of 40 years (Hutten et al. 1999). The age is also a risk factor in upper gastrointestinal tract bleeding in people using NSAID medication (Hernandez-Diaz and Rodriguez 2000).

Opiate medication is the most common modality of severe pain management. Although their effectiveness in pain relief, opiates have many undesirable adverse effects. Opiate adverse effects and poor understanding of pain management in the elderly have resulted in a continuing problem of under-treatment of pain in the elderly (Ferrell et al. 2001). Pain itself is a complex phenomenon with multiple variables affecting how an individual experiences it. Tolerance is a major factor affecting the

pharmacodynamics of opiates. The underlying disease progression and opioid addiction also affects the pharmacodynamics of opioids. Elderly patients seem to develop tolerance to opioids slower and respond to pain treatment differently than young adults (Cutler et al. 1994, Buntin-Mushock et al. 2005).

2.5. Drug metabolism

Drugs are eliminated from the body by metabolism and excretion. Only a small fraction of drugs are eliminated from the body by direct excretion. Lipophilic drugs have to be converted more water-soluble in order to avoid reabsorbtion in the renal tubules or render the drug to be more readily excretable to bile. The metabolism or biotransformation process is catalysed by a number of enzyme-catalysed reactions. Drug metabolism can be divided into two phases. Phase I reactions are oxidation, reduction or hydrolysis reactions where a functional group is added or exposed on the parent drug. Oxidative reactions are the most important phase I reactions. Cytochrome P450 enzymes catalyse most of the oxidation reactions. Phase II constitutes conjugation reactions such as glucuronidation, sulphation and acetylation (Rowland and Tozer 1995).

2.6. CYP enzymes

Cytochrome P450 (CYP) enzymes are a large class of proteins that are expressed in every living organism. CYP enzymes are involved in the metabolism of many different endogenous and exogenous chemical compounds including drugs. They detoxify harmful xenobiotics or sometimes, activate them to reactive species, through biotransformation. The substrates for CYP enzymes vary in chemical character and function e.g. paracrine and endocrine signalling, drugs and mutagens. The transformation reactions catalysed by CYP enzymes can be categorized into a few classes, such as oxidations, dehydrations and reductions. Human CYP superfamily consists of 18 families, 42 subfamilies and in total of 57 different CYP enzymes (Nebert and Russell 2002, Pelkonen et al. 2008). In humans fifteen different CYP enzymes in three families metabolize foreign chemicals including majority of drugs currently in use. Other CYP enzymes are mainly involved in the metabolism of endogenous substrates.

CYP enzymes are the primary enzymes involved in phase I reactions. They are heme-containing proteins that utilize molecular oxygen and the reducing agent. Nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH), donate the electrons via NADPH-450 reductase to the reaction to monooxygenate the substrate. The end result of the reaction is addition of hydroxyl group to the substrate (R) and formation of water. The reaction can be formulated as follows:

$$RH + O_2 + 2H^+ + 2e^- \text{ (from NAD[P]H)} \rightarrow ROH + H_2O$$

Addition of polar hydroxyl group converts the substrate more water soluble thus making it easier to excrete (Brown et al. 2008).

CYP enzyme substrates belong to diverse chemical classes. Depending on molecular features of the CYP substrate such as a molecule's overall structures e.g. size, lipophility, three-dimensional structure, functional groups, steric features and stereochemistry, different types of CYP enzymes bind to different substrates (Brown et al. 2008).

2.6.1. *CYP2D6*

CYP2 substrates are usually low or medium molecular weight molecules with a broad range of polarities. CYP2D6 is capable of metabolizing a large number of drugs even though it accounts only of 1-2% of the total liver CYP content (Shimada et al. 1994). CYP2D6 metabolizes the prodrugs codeine and tramadol to their active forms (Dayer et al. 1988, Poulsen et al. 1996). CYP2D6 is also responsible for O-demethylation of oxycodone to oxymorphone (Lalovic et al. 2004).

CYP2D6 enzyme is the only one among the drug metabolizing CYPs, which is not inducible by other drugs. There is marked genetic variation in CYP2D6 enzyme and it is of great importance for the metabolism of many drugs such as opiate analgesics, antidepressants, neuroleptics, antiarrhythmics, antiemetics and anticancer drugs (Ingelman-Sundberg et al. 2007). Currently more than 120 different CYP2D6 variants have been described (www.cypalleles.ki.se). About 7% of Caucasians have a genotype of two non-functional CYP2D6 alleles that causes poor CYP2D6 enzyme activity and thus are labelled as poor metabolizers (PM) via CYP2D6. 1-2% of Scandinavians have two functional CYP2D6 alleles with extremely high enzyme activity (ultrarapid metabolizers, UM). Distribution of different CYP2D6 genotypes varies between different ethnic groups. PMs are most common in Europe and UMs in North Africa and Oceania. Less than 1% of Chinese population are poor metabolizers and about 25% of Ethiopians are ultrarapid metabolizers via CYP2D6 (Caraco et al. 1999). In Asia CYP2D6 genotyping can explain many cases of nonresponse or adverse drug reactions in patients treated with CYP2D6 substrates (Gasche et al. 2004, Ingelman-Sundberg et al. 2007). The majority of people are classified as extensive metabolizers (EM). People with one non-functional and one allele of decreased activity or two alleles with decreased activity are labelled as intermediate metabolizers (IM). Clinically IM phenotype is not significantly different from PM phenotype. IM genotype is most common in Asia.

2.6.2. *CYP2E1*

CYP2E1 metabolizes many small molecules of anaesthesiologic and toxicologic interest including ethanol, paracetamol, halothane, enflurane and sevoflurane (Fuhr 2000). CYP2E1 is also involved in transforming several endogenous substrates such as acetone, glycerol and different fatty acids. CYP2E1 generates reactive oxygen species (ROS), which can be harmful (Knockaert et al. 2010).

2.6.3. *CYP3A4/5*

CYP3A subfamily accounts for the majority of drug metabolizing enzymes present in adult human tissues. It is probably the most important of all drug-metabolizing enzymes because it has the ability to metabolize many chemically unrelated drugs from different drug classes (Table 2) and CYP3A enzymes are abundant in both the intestinal epithelium and in the liver. It has been estimated that CYP3A is involved in the metabolism of more than half of the therapeutic agents that undergo oxidative metabolism (Wilkinson 2005).

CYP3A family constitutes of four isoenzymes: CYP3A4, CYP3A5, CYP3A7 and CYP3A43 (Stevens et al. 2003, Knockaert et al. 2010). CYP3A4 is the most abundant of the CYP enzymes and it accounts for 30-40% of the total CYP content in human liver. It is also expressed in the intestinal wall (Paine et al. 1997). CYP3A4 and CYP3A7 show an opposite expression pattern during development. CYP3A4 is considered to have little or no impact on fetal CYP3A metabolism while in adults CYP3A4 is the most important CYP3A and CYP3A7 is less significant (Lacroix et al. 1997). The expression of CYP3A5 is independent of developmental stages but it is highly polymorphic. The fetal hepatic CYP3A activity corresponds to CYP3A7 and CYP3A5, while in adults CYP3A activity corresponds mainly to CYP3A4 and CYP3A5 (Stevens et al. 2003). CYP3A43 doesn't have clinical relevance and appears to be a pseudoprotein (Ingelman-Sundberg 2005).

CYP3A4 and CYP3A5 share 84% amino acid sequence identity and thus share most of their substrates, inducers and inhibitors, although some differences in catalytic activities have been found. CYP3A5 genotype doesn't influence the pharmacokinetics or pharmacodynamics of CYP3A substrates alfentanil or midazolam (Kharasch et al. 2007). CYP3A5 gene has several mutations that severely reduce the synthesis of functional CYP3A5 protein, the most common of which is CYP3A5*3. The misplicing causing allele has a frequency of about 88% in Caucasians, 75% in Asians and 35% in Africans. It is detected in reasonable amounts in only 10-30% of liver samples of Caucasians and Asians whereas in Africans it may exceed that of CYP3A4 (Lee et al. 2003). Thus, CYP3A5 polymorphism may be the most important contributor to interindividual differences in CYP3A-dependent drug clearance and in responses to many medicines (Kuehl et al. 2001).

The large number of potential substrates and the inducible or inhibitable character of CYP3A4 make it prone to be involved in drug-drug interactions (DDIs). Several drugs have been removed from the market because of serious adverse effects that followed concomitant administration with other drugs metabolized by CYP3A4 (Wilkinson 2005). Ray et al. reported over two-fold increase of sudden death from cardiac causes in patients using erythromycin (a CYP3A4 substrate) concomitantly with strong CYP3A4 inhibitors e.g. diltiazem and verapamil compared to using only erythromycin (Ray et al. 2004). In recent study, rifampin lowered the exposure to oral and intravenous oxycodone 53% and 86% respectively (Nieminen et al. 2009). CYP3A4 mediates oxycodone N-demethylation to noroxycodone, which is the predominant oxycodone-metabolizing pathway (Lalovic et al. 2004).

Table 2. Common CYP3A substrates, inhibitors and inducers

CYP3A Substrates

Antihypertensive drugs
Diltiazem
Felodipine
Lercanidipine
Nifedipine
Verapamil
Losartan
Propranolol

Corticosteroids
Dexamethasone
Hydrocortisone

Opioids
Alfentanil
Fentanyl
Methadone
Oxycodone

Statins
Atorvastatin
Lovastatin
Simvastatin
(not Pravastatin)

Sedatives and hypnotics
Alprazolam
Midazolam
Triazolam
Buspirone
Zolpidem

Cardiac antirrhythmics
Amiodarone
Dronedarone
Quinidine

Antihistamines
Ebastine
Terfenadine

Steroids
Estradiol
Progesterone
Testosterone

Immunosuppressant agents
Cyclosporine
Tacrolimus

Macrolide antibiotics
Clarithromycin
Erythromycin
Telithromycin

Antipsychotics
Haloperidol
Risperidone

Anti-HIV agents
Neflinavir
Indinavir
Ritonavir
Saquinavir

Cancer medication
Tamoxifen
Taxol

Others
Dextromethorpan
Lidocaine
Ondansetron
Sildenafil

CYP3A Inhibitors

Macrolide antibiotics
Clarithromycin
Erythromycin
Telithromycin
Troleandomycin
(not Azithromycin)

Antihypertensive drugs
Diltiazem
Verapamil

Azole anfungal agents
Itraconazole
Ketoconazole
Voriconazole

Others
Amiodarone
Cyclosporine
Grapefruit juice
Mifepristone

Anti-HIV agents
Delavirdine
Indinavir
Ritonavir
Saquinavir

Antidepressants
Fluoxetine
Fluvoxamine
Nefazodone

CYP3A Inducers

Diabetes medication	Anticonvulsant agents	Cardiac antiarrhythmics
Pioglitazone	Carbamazepine	Amiodarone
(Trogliazone)	Phenobarbital	
	Phenytoin	Sedatives and hypnotics
Rifamycins (antibiotics)		Barbiturates
Rifabutin	Anti-HIV agents	
Rifampicin	Efavirenz	Others
Rifapentine	Nevaripine	St. John's worth

(Cotreau et al. 2005, Wilkinson 2005), (Accessed
http://medicine.iupui.edu/DDIs/ClinicalTable.asp)

2.6.4. *CYP enzymes and the elderly*

There is little information on the effect of age on the CYP enzymes. Opiate analgesic alfentanil is a CYP3A substrate. Studies on age-dependent changes in clearance of alfentanil have resulted in conflicting findings. In a study where alfentanil was given as an intravenous dose for abdominal surgery, Helmers et al found that alfentanil clearance was reduced by approximately 30% in the elderly compared to young patients (Helmers et al. 1984). Lemmens et al observed the age difference only in women (Lemmens et al. 1990) and Scott et al. found no difference in alfentanil clearance between age groups regardless of sex (Scott and Stanski 1987).

Overall many studies show reduced clearance of CYP3A substrates in the elderly. Some of these studies also show that men are more susceptible to the age-related decrements in clearance (Cotreau et al. 2005). Age, however doesn't change the efficiency of hepatic microsomal mono-oxygenases and the changes in clearance of CYP3A substrates with aging are most likely due to reduced liver mass and blood flow (Schmucker et al. 1990, Parkinson et al. 2004).

2.6.5. *Mechanism of CYP inhibition*

Inhibition of CYP enzymes is often classified as reversible, quasi-reversible or irreversible inhibition. The reversible inhibition is the most common type of CYP inhibition. The reversible inhibition can be further divided into competitive, non-competitive, uncompetitive and mixed-type inhibition (Lin and Lu 1998, Pelkonen et al. 2008).

The reversible interaction with CYP enzyme usually occurs as a result of competition at the enzyme reactive site. Binding to the enzyme is weak and bonds are formed and broken easily. In non-competitive inhibition the substrate and the inhibitor bind to different site at the CYP enzyme. Uncompetitive inhibition occurs when the inhibitor binds to the enzyme-substrate complex, but not to the free CYP enzyme. In mixed-

type inhibition there are elements of both competitive and non-competitive inhibition (Pelkonen et al. 2008).

The quasi-reversible inhibition of CYP enzyme is caused by the formation of reactive metabolites. These metabolites form stable complexes with the prosthetic haem of the CYP enzyme, called the metabolic intermediate (MI) complex, so inactivating the CYP enzyme. Clarithromycin for example undergoes CYP-dependent N-demethylation and forms a NADPH-dependent ferrous CYP3A-MI complex. This bond is reversible in vitro but is very stable in vivo and synthesis of new enzymes is the only means of restoring the enzyme activity. Therefore this form of CYP inhibition is called quasi-reversible (Rodrigues et al. 1997, Lin and Lu 1998).

In irreversible mechanism-based, also called suicide inhibition, the CYP substrate is oxidized to reactive intermediates that cause inactivation of the CYP by three different mechanisms. The intermediate can form a covalent binding with an amino acid residue within the active site of the enzyme. The inactivation can occur by arylation or alkylation of the prosthetic haem moiety or by destruction of the haem group (Johnson 2008).

2.7. ABCG2 protein

The breast cancer resistance protein (ABCG2), is an ATP-binding cassette transmembrane transporter, which mediates the extrusion of compunds from the cell. ABCG2 protein is an efflux transporter that is expressed in various normal tissues such as small intestine, colon, liver, kidney, stem cells, placenta, mammary gland, brain and heart, as well as in cancer cells (Hardwick et al. 2007). ABCG2 mediates the cellular efflux of a wide variety of xenobiotics including glucuronide and sulphate metabolites of some compounds such as paracetamol (Suzuki et al. 2003, Zamek-Gliszczynski et al. 2006). *ABCG2* genotype could therefore have an effect on the elimination of the metabolites of paracetamol from the body. Kondo et al. have demonstrated that the *ABCG2* c.421A allele is associated with reduced transport activity of ABCG2, in vitro and in a recent study Keskitalo et al. reported *ABCG2* polymorphism affecting the pharmacokinetics of statins in man (Kondo et al. 2004, Keskitalo et al. 2009).

2.8. Oxycodone

2.8.1. Basic pharmacology

Oxycodone (6-deoxy-7, 8-dihydro-14-hydroxy-3-O-methyl-6-oxomorphine) is a semi-synthetic opiate analgesic, which has been in clinical use since 1916. Although oxycodone has been available for a long time, the worldwide use of oxycodone has been scarce until the orally administered controlled-release forms became available in 1995. The global use of oxycodone has increased several fold during the last decade and it is one the most used strong opioids in Finland and many other countries (Paulozzi 2006, Sullivan et al. 2008, Boudreau et al. 2009, Hamunen et al. 2009, Bell et al. 2009, Bell et al. 2011).

The most commonly used opioid analgesics in clinical practice bind mainly to the μ-receptor and are thus called μ-agonists. The μ-receptor was named using the first Greek letter from morphine, which was the first ligand found to bind to the μ-receptor. Other classical opioid receptors are κ-, named after ketocyclazine and δ-receptor, named after mouse vas deferens tissue in which the receptor was first characterized. Oxycodone is a μ-receptor agonist having $1/5$ of the binding affinity to that of morphine (Lalovic et al. 2006). Opioid receptors are located throughout the nervous system, in somatic and visceral sensory neurons, spinal cord projection and interneurons, midbrain and cortex. Outside of the central nervous system the opioid receptors are found on peripheral processes of sensory neurons. Sympathetic neurons and immune cells have also been found to express opioid receptors (Stein et al. 2003).

2.8.2. *Pharmacokinetics*

Oxycodone can be administered by intravenously, intramuscuscularly, subcutaneously, orally and rectally. The first pass metabolism of orally administered oxycodone is moderate and its bioavailability after oral dosing is 60-87% (Leow et al. 1992, Pöyhiä et al. 1992). The bioavailability of rectally administered oxycodone doesn't differ significantly from that of orally administered (Leow et al. 1995). The time to mean maximal plasma concentration (t_{max}) after oral immediate-release oxycodone dosing is 1-1.5 h. Steady-state oxycodone plasma concentration after oral dosing is achieved in approximately in 24 h (Reder et al. 1996).

Oxycodone, like morphine is hydrophilic compound. About 38-45% oxycodone in plasma is protein bound, mainly to albumin (Leow et al. 1993, Pöyhiä and Seppälä 1994). Oxycodone is extensively metabolized in the liver and to a lesser extent in the intestinal microsomes by oxidative CYP enzymes. Less than 10% of oxycodone is excreted unchanged to urine (Pöyhiä et al. 1992). Renal dysfunction increases the elimination half-life of oxycodone and elimination of oxycodone may be impaired in uremic patients (Kirvelä et al. 1996). In liver the main oxidative pathway is N-demethylation of oxycodone to noroxycodone via CYP3A4/5. The minor oxidative pathway involves O-demethylation of oxycodone to oxymorphone via CYP2D6. These metabolites are further metabolized to noroxymorphone via CYP3A4/5 and CYP2D6. Oxycodone and its oxidative primary metabolites noroxycodone and oxymorphone also undergo reductive metabolism to some extent to form rasemic α- and β- oxycodol, noroxycodol and –oxymorphol (Lalovic et al. 2004, Lalovic et al. 2006).
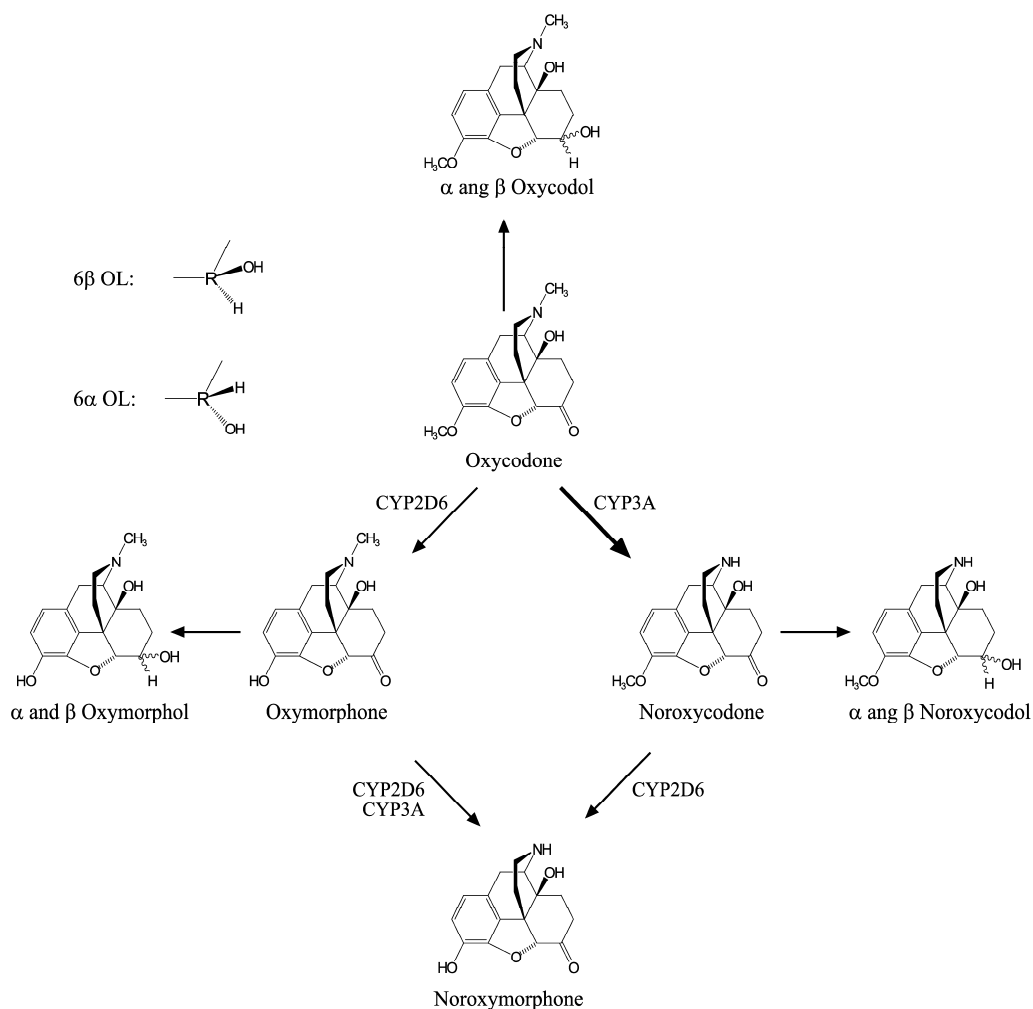
Figure 1. The metabolism of oxycodone. Modified from Lalovic et al. 2006.

2.8.3. *Pharmacodynamics and clinical use*

Oxycodone is a strong opioid used in the treatment of severe pain. Oxycodone closely resembles morphine and its clinical potency in the treatment of pain is often compared to that of morphine. The efficacy of oxycodone is well established in the treatment of postoperative pain and cancer pain (Heiskanen and Kalso 1997, Silvasti et al. 1998, Lenz et al. 2009). Oxycodone has also been proven effective in the treatment of osteoarthritis-related pain, chronic low back pain and lumbar root compression syndrome (Ytterberg et al. 1998, Gammaitoni et al. 2003, Riley et al. 2008).

Oxycodone has also been investigated in the treatment of neuropathic pain. Although oxycodone is not to be used as a first line of treatment against neuropathic pain, there have been promising results for treating post herpetic neuralgia or diabetic neuropathy. Oxycodone is now recommended for treatment of neuropathic pain if tricyclic

antidepressants and gabapentin fail to attain satisfactory pain relief (Finnerup et al. 2005).

Oxycodone, like all μ-receptor agonists, has adverse effects, which are the main obstacle for more extensive use of opioids in pain therapy. The typical opioid adverse effects include depression of breathing, nausea, clouding of consciousness, constipation, development of addiction and tolerance. Oxycodone's adverse effect profile resembles that of morphine. In cancer patients, oxycodone caused less hallucination, nausea and vomiting but somewhat more constipation compared to morphine (Kalso and Vainio 1990, Heiskanen and Kalso 1997). Unlike morphine, oxycodone does not release histamine, which may cause less cardiovascular instability compared to morphine (Rosow et al. 1984, Pöyhiä et al. 2004). Currently there are no studies whether age affects the incidence of adverse effects of oxycodone.

Oxycodone has only $1/5$ of the binding affinity to μ-receptor compared to morphine. Once bound, oxycodone has weaker ability to induce receptor G-protein activation, about $\frac{1}{3}$ of that of morphine (Lalovic et al. 2006). Yet, the analgesic efficacy of the two drugs is almost identical and the equianalgesic doses of oxycodone and morphine vary only a little depending on the study setting and population (Kalso and Vainio 1990, Kalso et al. 1991, Silvasti et al. 1998, Lenz et al. 2009). The discrepancy has been explained by the weak ability of oxycodone to bind to κ-receptors. Also there have been speculations of oxycodone having active metabolites. Oxymorphone and the reduced metabolites of oxymorphone, α- and β- oxymorphol have about 40-fold binding affinity to the μ-receptor compared to parent drug oxycodone but their quantities are so scarce that they have no clinical relevance. Noroxymorphone instead may offer feasible addition to the opiate activity of oxycodone. The exposure to noroxymorphone, the end product of oxidative metabolism pathway of oxycodone, is about half of that of the exposure to oxycodone. Noroxymorphone also exhibits receptor affinity and G-protein activation comparable to that of morphine. However, the clinical effects of oxycodone go in line with plasma $t_{1/2}$ of the parent drug but there is no such correlation with the oxycodone metabolites. In rat studies the brain-to-plasma concentration ratio of oxycodone was 10-fold higher than that of morphine, which may be the underlying cause of the similar effects of oxycodone and morphine despite the differences in their potency of receptor activation (Murphey and Olsen 1994, Lalovic et al. 2006). There are a growing number of studies indicating the existence of an active influx transporter of oxycodone through the blood brain barrier (Boström et al. 2006, Boström et al. 2008). Pyrilamine transporter, a putative organic cation transporter, has been found to, at least partly, mediate the blood-brain barrier transport of oxycodone (Okura et al. 2008).

2.9. Paracetamol

2.9.1. *Basic pharmacology*

Paracetamol (N-acetyl-paraminophenol, acetaminophen) is one of the most widely used nonopioid antipyretic analgesics available without prescription. Paracetamol has been in clinical use since 1887 but wider use started in the 1950s when phanacetin was

abandoned due to its nephrotoxicity (Smith 2009). The popularity of paracetamol is based on its good tolerability and its comparable efficiency as an antipyretic analgesic with nonsteroidal anti-inflammatory drugs (NSAIDs). Paracetamol lacks many of the adverse-effects the classic NSAIDs have. Problems with NSAIDs arise from the risk of gastrointestinal complications (Laporte et al. 1991), their tendency to exacerbate asthma (Settipane et al. 1995), suppressing effect on the renal function (Clive and Stoff 1984) and their effect on blood clothing and postoperative bleeding (Rusy et al. 1995). There is also evidence for NSAIDs inhibiting heterotopic bone formation (Neal et al. 2000) and having disruptive effect on sleep compared to paracetamol (Murphy et al. 1994).

Paracetamol is somewhat unstable molecule in the liquid form and it is poorly soluble in water. Until the turn of the century paracetamol has been commercially available only in orally and rectally administrable form in the United States. In Europe an intravenous propacetamol has been available since the 1990's. Propacetamol is prodrug, which is hydrolysed to paracetamol by plasma esterases in the bloodstream. The use of propacetamol was difficult and it caused irritation at the injection site (Bannwarth et al. 1992, Sinatra et al. 2005). The absence of an intravenous easy-to-administer formulation has detained the use of paracetamol in postoperative pain therapy in adults. As intravenous paracetamol became available in 2002, multimodal analgesia for acute postoperative pain with opioids together with paracetamol has become very popular.

2.9.2. *Pharmacokinetics*

Paracetamol can be administered intravenously, orally and rectally. In surgical patients, the oral bioavailability of paracetamol has been found to vary significantly (Holmer Pettersson et al. 2004, Moller et al. 2005). The absorbtion of rectally administered paracetamol is insufficient compared to oral administration. In order to achieve similar pain relief as with oral route, the rectal dosage must be approximately two-fold (Korpela and Olkkola 1999). Total of 10-30% of plasma paracetamol is protein bound and the proportion doesn't change in overdose (Milligan et al. 1994). Paracetamol is metabolised mainly in the liver by two major hepatic pathways: conjugation with glucuronic acid and conjugation with sulphuric acid to form inactive metabolites. The sulphuric acid conjugation route is rapidly saturable at doses that exceed the common therapeutic doses. A fraction of paracetamol (5-15%) is oxidized by CYP2E1 (and to lesser extent, CYP3A4, CYP2A6, CYP1A2 and CYP2D6) to a reactive intermediate, N-acetyl benzoquinone imine (NAPQI) (Dong et al. 2000). NAPQI, under normal conditions, is rapidly detoxified by reduced glutathione and excreted in the urine after conjugation with cysteine and mercaptouric acid (Mitchell et al. 1974, Miller et al. 1976). Ninety percent of the paracetamol dose is excreted in urine in 24 hours, mainly as glucuronide (60-80%) and sulphate (20-30%) conjugates. Less than 5% of paracetamol is excreted to urine unchanged. Moderate renal failure increases the concentration of glucuronide and sulphate conjugates of paracetamol. However, moderate renal failure does not have a clinically relevant effect on paracetamol concentrations nor does it affect the toxic NAPQI concentrations (Prescott et al. 1989). The pharmacokinetics of paracetamol in the elderly has been

studied but the results have been inconclusive. In most studies, no major differences between young and elderly subjects were reported (Triggs et al. 1975, Divoll et al. 1982, Miners et al. 1988, Wynne et al. 1990, Bannwarth et al. 2001).
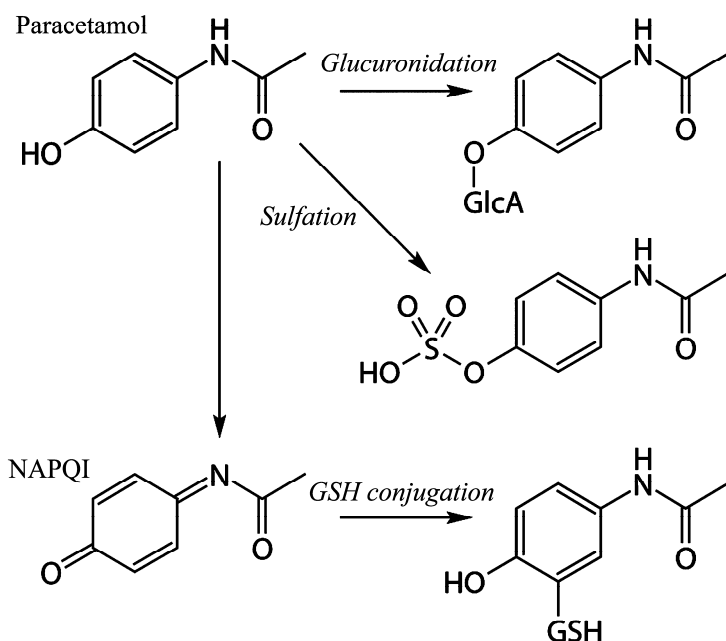
Figure 2. The metabolism of paracetamol.

The safety of paracetamol dosing depends on the availability of electron donors such as glutathione (GSH) and other thiol-containing compounds. In normal conditions GSH supply far exceeds that required to detoxify NAPQI. However, during overdosing, the quantity of this toxic metabolite is increased. The normal therapeutic dose of 10-20 mg/kg has a wide safety margin and significant toxicity generally involves a single dose of over 150 mg/kg (Prescott 1983). Accumulative overdose is more problematic because NAPQI detoxifying reserves diminish over time and slow onset of overdose symptoms, such as nausea, vomiting, pallor and abdominal pain are not specific for paracetamol overdose. Patients with increased risk include elderly, neonates and patients with liver disease (Electronic Medicines Compendium UK). Also alcoholism, chronic malnutrition and concomitant medication with enzyme-inducing drugs such as carbamazepine, phenytoin, phenobarbitone and rifampisin increase the risk of paracetamol overdose (Wallace et al. 2002).

Liver toxicity occurs when NAPQI covalently binds and arylates critical cell proteins which leads to cascade resulting in cell death (Hinson et al. 2004). The process isn't irreversible and it can be prevented, interrupted and reversed even after binding has occurred (James et al. 2003). The toxic process of paracetamol overdose can be prevented with N-acetylcysteine, which replenishes nonprotein sulfhydryls, thus

allowing more substrate for the detoxification of NAPQI (Corcoran and Wong 1986). If untreated, paracetamol overdose leads to massive liver cell necrosis, which manifests in fulminant hepatitis and/or hepatic failure. Paracetamol is the most common drug taken overdose in the United Kingdom accounting for 48% for poisoning admissions to hospital (Thomas et al. 1996). In England and Wales paracetamol overdose resulted in 134 deaths in 2005, from which 33 were over 65 years of age (Office for National Statistics UK. 2007).

2.9.3. *Pharmacodynamics and clinical use*

Paracetamol has been extensively studied and its analgesic efficiency, risks and limitations are well documented. Despite being one of the most used and investigated analgesics to date, the analgesic mechanism of paracetamol is still unclear. Recently it has become evident that the analgesic effects of paracetamol are largely central in origin (Smith 2009).

Paracetamol is well tolerated and adverse effects are rare when dosage is kept below toxic levels. Paracetamol has similar effects with aspirin, which have led many investigations to study the ability of paracetamol to inhibit the cyclooxygenase (COX) enzyme. Paracetamol does not have anti-inflammatory activity and it has no effect on the production of pro-clotting thromboxane. Paracetamol is a weak selective COX-2 inhibitor (Boutaud et al. 2002, Lee et al. 2007). Intrathecal and oral paracetamol has been shown to reverse spinally initiated hyperalgesia in rats. It was shown that the site of systemic drug action was within the neuraxis on mechanisms that mediate the spinal sensitization. This emphasizes the likely central action of paracetamol (Crawley et al. 2008).

Paracetamol penetrates easily to central nervous system (CNS). In the brain and the spinal cord, paracetamol is metabolized by fatty acid amide hydrolase (FAAH) to potent capsaicin receptor agonist N-arachidonoylphenolamine (AM404). AM404 inhibits COX-1 and COX-2 (Högestätt et al. 2005). AM404 acts through serotonergic and opioidergic mechanisms. The serotonergic mechanism is mediated through $5HT_3$-reseptor and the opioidergic mechanism is mediated through μ- and κ-receptors. AM404 is weaker analgesic than paracetamol and actions of paracetamol can only partially be explained through its metabolite AM404 (Ruggieri et al. 2008). The serotonergic system involvement in the analgesic action of paracetamol has been demonstrated in humans and paracetamol been shown to reinforce descending inhibitory pain pathways (Pickering et al. 2006, Pickering et al. 2008). The serotonergic neurons interact with opioid-mediated pain modulatory circuit. AM404 reinforces the endocannabinoid system. The endocannabinoid system has been shown to interact with serotonergic system. Cannabinoid-1-reseptor activity, however indirect, may have a role in antinociceptive actions of paracetamol (Mallet et al. 2008). The activity of paracetamol could occur through a number of mechanisms and the activation of one system may influence the others.
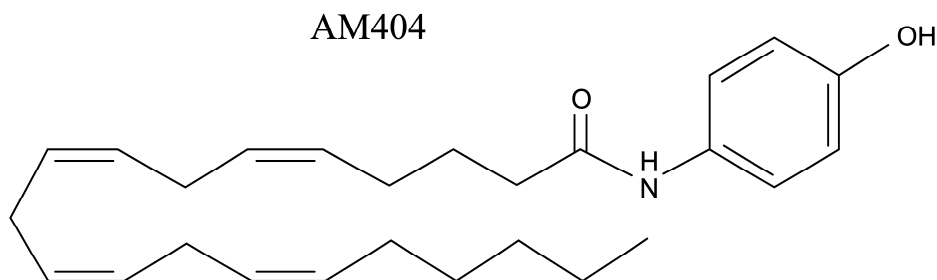
Figure 3. The chemical structure of AM404.

Paracetamol is commercially available in many formulations either alone or in combination with other medications, particularly with opioids. There are a number of studies investigating the potential benefits of combining paracetamol with opioids as part of a multimodal pain treatment. Combining paracetamol with morphine has been found beneficial in a postoperative setting in orthopaedic patients. Paracetamol reduced the morphine consumption significantly. Paracetamol also reduced the pain scores and there were less opioid-related adverse effects (Schug et al. 1998, Hernandez-Palazon et al. 2001). Same type of results were also seen in paediatric day-case surgery patients (Korpela et al. 1999). The meta-analyses, however, are not so convincing. The morphine-sparing effect of combining paracetamol in the pain-therapy has been clearly shown. Whether there are benefits in regard to pain scores or opioid-related adverse effects is still controversial (Curatolo and Sveticic 2002, Elia et al. 2005).

2.10. Clarithromycin

2.10.1. Basic pharmacology

Clarithromycin (6-0-methylerythromycin) is a macrolide antibiotic developed in effort to get more acid stable version of erythromycin in the 1970s. Clarithromycin has been commercially available in the United States and Japan since 1991 (Morimoto et al. 1984). The more acid stable structure makes clarithromycin better tolerated than erythromycin, which has adverse effects such as nausea and stomach ache (Zhou et al. 2008, Zuckerman et al. 2009). Clarithromycin is bacteriostatic inhibiting the translation of bacterial peptides. Clarithromycin has also bactericidal effects on some strains such as Neisseria gonorrhoeae, Haemophilus influezae and Streptococcus pneumoniae (Guay et al. 2001).
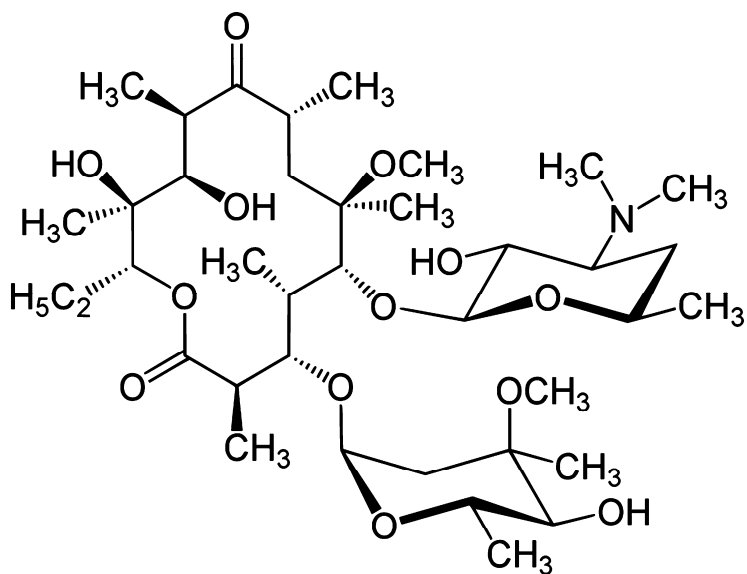
Figure 4. The chemical structure of clarithromycin.

2.10.2. Pharmacokinetics

Clarithromycin is readily absorbed and has a bioavailability of 50%. It is diffused into tissues and phagocytes. Because of high concentration in phagocytes, clarithromycin is actively transported to the site of infection. The tissue-concentration of clarithromycin can be 10-fold to that in plasma. The highest concentrations are found in the lung and in the liver. Clarithromycin undergoes first-pass metabolism to form active 14-hydroxy clarithromycin. The elimination half-life for clarithromycin varies between 3-7 hours depending on dosing. The main routes of elimination of clarithromycin and its metabolites are urinary and biliary excretion. 30-40% of clarithromycin is excreted in urine depending on dose and an additional 10% is excreted in urine as active metabolite (Anonymous 2008).

2.10.3. Drug interactions

Clarithromycin is a potent inhibitor of CYP3A. Inhibition of CYP results from formation of NADPH-dependent ferrous CYP3A-MI complex initiated by CYP-dependent N-demethylation of clarithromycin. Clarithromycin, like erythromycin behaves as quasi-irreversible mechanism-based inhibitors of CYP (Tinel et al. 1989, Rodrigues et al. 1997, Pinto et al. 2005). Clarithromycin has significant interactions with many substrates of CYP3A such as triazolam (Greenblatt et al. 1998), midazolam (Westphal 2000) and trazodone (Farkas et al. 2009). Clarithromycin interactions have caused fatalities. Concomitant administration of clarithromycin with cisapride, pimozide or terfenadine is contraindicated due to increased risk of QT prolongation, ventricular tachycardia, torsades pointes and ventricular fibrillation (Anonymous 2008).

2.10.4. Pharmacodynamics and clinical use

Like erythromycin, clarithromycin is used to treat upper respiratory tract infections, bronchitis, pneumonia, especially atypical pneumonias associated with Chlamydia pneumoniae, uncomplicated skin infections and ulcers. Clarithromycin has also been used to treat Helicobacter pylori, Legionellosis and lyme disease. Clarithromycin has FDA approval for use in children above 6 months and in elderly. Seventy percent of clarithromycin is plasma-bound (Hoffman et al. 2003). Clarithromycin is well tolerated. The most common adverce effects include diarrhoea, nausea, vomiting, abdominal pain, headache and rash (Anonymous 2008).

3. AIMS OF THE STUDY

The number of elderly people is growing rapidly and the rate of increase is expected to accelerate in the upcoming years. The global use of the opioid oxycodone has increased several folds during the past decade, including in the elderly patients, who consume the most of all drugs already now. Likewise the use of multimodal postoperative analgesia including intravenous paracetamol, also in the elderly patients, has become popular. The general aim of this study was to investigate the effect of age on the pharmacokinetics of oral and intravenous oxycodone and intravenous paracetamol in the elderly. Additionally, we assessed the effect of clarithromycin, a known CYP3A inhibitor, on oxycodone pharmacokinetics and pharmacodynamics in young and elderly subjects.

The specific aims were:

1. To study the pharmacokinetics of oral oxycodone in four groups of orthopaedic surgical patients aged 20-40, 60-70, 70-80 and 80-90 years. (Study I)

2. To study the pharmacokinetics of intravenous oxycodone in four groups of orthopaedic surgical patients aged 20-40, 60-70, 70-80 and 80-90 years. (Study II)

3. To study the pharmacokinetics of intravenous paracetamol in four groups of orthopaedic surgical patients aged 20-40, 60-70, 70-80 and 80-90 years. (Study III)

4. To study the effect of clarithromycin on the pharmacokinetics and pharmacodynamics of oral oxycodone in young and elderly subjects. (Study IV)

4. MATERIALS AND METHODS

4.1. Subjects

This summary is based on four studies, which are referred to as Studies I-IV. In addtition some new assays were conducted using the data cathered for the published Studies I-IV. Altogether 101 subjects were recruited in three phases for the four studies. First, 40 patients scheduled to undergo orthopaedic surgery were recruited in Study I for assessment of oral oxycodone pharmacokinetics. Second, 41 orthopaedic surgical patients were recruited for Studies II and III: the same patients received both intravenous oxycodone and intravenous paracetamol. The pharmacokinetic data for each compound is reported separately as Study II and Study III, respectively. Third, 20 healthy volunteer subjects were recruited for Study IV to investigate the effect of clarithromycin on the pharmacokinetics and pharmacodynamics of oxycodone. The demographic details are shown in Table 3.

Ten patients from each age group 20-40 (G 20-40), 60-70 (G 60-70), 70-80 (G 70-80) and 80-90 years of age (G 80-90) were recruited for the first three studies The youngest patients were scheduled for arthroscopic anterior cruciate ligament operation of the knee and the elderly subjects were scheduled for elective knee prosthesis surgery. Patients were approached during a preoperative visit to the hospital approximately two weeks before the scheduled surgery. Oral and written information of the studies were given at that time. Written consent was obtained a day before the scheduled surgery.

Criteria for inclusion (Studies I - III):
1. 20-40 or 60-90 year old person.
2. Scheduled elective knee prosthesis-, arthroscopic knee- or lower limb trauma operation under spinal anaesthesia.
3. Written informed consent from participating subject.

Criteria for exclusion (Studies I - III):
1. A previous history of intolerance to the study drugs or related compounds and additives.
2. Concomitant drug therapy with oxycodone or paracetamol.
3. BMI > 35 kg/m^2.
4. Existing significant hepatic, renal, neurologic, haematologic, endocrine, metabolic or gastrointestinal disease.
5. History of alcoholism, drug abuse, psychologic or other emotional problems that are likely to invalidate informed consent.
6. Donation of blood during and for 6 weeks after completion of the study.
7. Allergy to paracetamol or oxycodone.
8. Blood loss exceeding 1000 ml during surgery.

Patients were judged to have a significant hepatic, renal, neurologic, haematologic, endocrine, metabolic or gastrointestinal disease if they had a clinical diagnosis of any disease of this type. However patients with diabetes mellitus were eligible unless they had significant renal involvement. For study II and III, concomitant use of strong inhibitors or inducers of CYP enzymes was added to the exclusion criteria.

Ten young (18-40 years of age) and ten elderly (over 70 years of age) healthy volunteers were recruited to the study IV. The recruitment of the younger age group was carried out through internet advertisements. All interested young candidates received first an e-mail including detailed information about the study design, the aims of the study, and the study procedures. The elderly subjects were recruited from senior citizen exercise clubs by holding a general presentation about the study at the beginning of one of their weekly exercises after which the subjects interested in the study contacted the investigator directly or by phone.

The volunteers were submitted to physical examination, determination of previous or chronic diseases and comprehensive laboratory testing to prove that they were in good health in regard to age. The young volunteers aged 18-40 years filled in a modified Finnish version of the Abuse Questions (Michna et al. 2004). Laboratory screening included CBC (including haemoglobin, haematocrit, differential WBC, platelet count), SGOT, SGPT, alkaline phosphatase, BUN and creatinine. For women in childbearing age a pregnancy test was performed. Urine was screened for glucose, proteins and drugs with addiction potential. Blood pressure in sitting position was verified to be within normal limits and an ECG was controlled to be within normal limits.

Criteria for exclusion were (Study IV):
1. A previous history of intolerance to the study drugs or to related compounds and additives.
2. Concomitant drug therapy known to cause enzyme induction or inhibition for at least 30 days prior to the study.
3. For the younger age group: Concomitant drug therapy of any kind for at least 14 days prior to the study.
4. For the older age group: Concomitant therapy with opioid analgesic. No other specific limitations were given except drug therapy known to cause enzyme induction or inhibition (see #2) and drug therapy, which was likely to be disturbed by the concomitant administration of clarithromycin or oxycodone. (Table 2)
5. Subjects younger than 18 years and subjects between 40 to 70 years.
6. Bodyweight differing ±15% of the ideal weight for height.
7. Smoking.
8. For the younger age group: Existing or history of asthma, seizures, haematologic, endocrine, significant metabolic, significant cardiovascular, psychiatric or gastrointestinal disease, including gut motility disorders or any other significant disease or drug allergy.

9. For the older age group: American Society of Anesthesiologist's physical status 3, 4 or 5, existing or history of asthma, seizures, haematologic, significant unstable endocrine, metabolic or cardiovascular disease, psychiatric or gastrointestinal disease, including gut motility disorders or any other significant disease such as dementia or drug allergy. Diabetes was a contraindication for the participation in the study.
10. Previous or present alcoholism, drug abuse, psychologic or other emotional problems that were likely to invalidate informed consent, or limit the ability of the subject to comply with the protocol requirements.
11. A positive test result for urine toxicology.
12. A "yes" answer to any one of the Abuse Questions in the younger age group.
13. Pregnancy or nursing.
14. Donation of blood for 4 weeks prior to and during the study.
15. Special diet or life style conditions which would compromise the conditions of the study or interpretation of the results.
16. Participation in any other study involving investigational or marketed drug products concomitantly or within one month prior to the entry into this study.
17. Smoking for one month before the start of the study and during the whole study period.
18. Prolonged QT-syndrome as assessed by family history, previous medical records or ECG.

Table 3. Patient caracteristics

	Patient group	Sex (F/M)	Age (years)	CYP2D6 genotype PM/IM/EM/UM	ASA physical status (I/II/III/IV)	Weight (kg)	Height (cm)	BMI (kg/m²)
Study I	G 20-40	4/6	26.5 ± 4.7	1/0/8/1	6/3/1/0	75 ± 8	172 ± 5	25.3 ± 2.9
	G 60-70	7/3	66.9 ± 3.0	0/0/10/0	1/4/4/1	81 ± 10	168 ± 7	28.8 ± 2.5
	G 70-80	9/1	74.0 ± 2.9	0/2/8/0	0/7/2/1	82 ± 10	164 ± 8	30.4 ± 2.6
	G 80-90	7/3	82.0 ± 2.2	0/0/8/2	0/8/2/0	77 ± 10	165 ± 9	28.3 ± 3.4
Studies II and III	G 20-40	3/8	27.1 ± 4.8	0/1/9/1	9/2/0/0	81 ± 14	177 ± 10	25.7 ± 2.2
	G 60-70	5/5	66.3 ± 2.5	1/0/9/0	0/6/4/0	83 ± 9	169 ± 9	29.0 ± 1.5
	G 70-80	4/6	76.5 ± 2.7	1/0/9/0	0/5/5/0	82 ± 13	169 ± 10	28.9 ± 3.3
	G 80-90	9/1	83.5 ± 2.6	0/0/10/0	0/6/2/2	68 ± 8	160 ± 7	26.3 ± 2.2
Study IV	18-40 years	4/6	22.4 ± 1.6	0/0/9/1	10/0/0/0	73 ± 12	174 ± 10	24.0 ± 2.3
	Over 70 years	3/7	73.5 ± 2.2	2/0/8/0	0/10/0/0	75 ± 12	170 ± 10	25.7 ± 2.3

Values are mean ± SD or number. In Study II, there were 11 patients in group G 20-40 due to dosing error in Study III. In Study II there were seven male patients in group G 20-40.

4.2. Study design

Studies were performed between June 2006 and May 2009 at the Department of Anaesthesiology, Intensive Care, Emergency Care and Pain Medicine at the Turku University Surgical Hospital and the Department of Pharmacology, Drug Development and Therapeutics, University of Turku. All study protocols were approved by the Ethics Committee of the Hospital District of South West Finland and the Finnish Medicines Agency. All studies were also reported to the European EudraCT clinical trials register.

4.2.1. Anaesthesia and treatment of postoperative pain (Studies I-III)

All patients participating in studies I-III were anaesthetized with an appropriate dose of 12.5 – 16.0 mg of intrathecal injection of 5 mg/ml plain bupivacaine. An epidural catheter was placed for postoperative pain management. The epidural solution contained 1.0 mg/ml plain bupivacaine and 5 µg/ml fentanyl. The infusion pump was set to deliver 1–7 ml/h of the solution to the epidural catheter. In case of insufficient pain relief, as determined by subjective visual analogue scale (VAS) score of 4 (minimum 0–maximum 10), patients were set to receive morphine 3 mg intravenously at 15-minute intervals until VAS score was under 4

All patients stayed at the post-anaesthesia care unit (PACU) until the first postoperative morning. For the patients in groups G60-70, G70-80 and G80-90 an oxygen catheter was placed for direct nasal administration of oxygen. In the recovery room and in PACU, the monitoring included ECG-monitoring, blood pressure, peripheral arteriolar oxygen saturation and pain score monitoring. Standard laboratory tests including complete blood count, C-reactive protein, sodium and potassium were taken in the PACU in the first postoperative morning from all patients undergoing knee prosthesis operation and from other patients if clinically indicated. After knee prosthesis surgery the patients were hospitalized for 4–5 days. After anterior cruciate ligament operation the patients were discharged within 24 hours after the operation. During their hospital stay all patients were observed according to the normal routine of our hospital. Further laboratory tests were taken only when clinically indicated.

4.2.2. Administration of the study drug and blood sampling

4.2.2.1. Study I

The study medication, 10 mg of oral oxycodone hydrochloride capsule (Oxynorm 10 mg capsule; Mundipharma, Bard Pharmaceuticals, Cambridge, UK), was swallowed whole by the patient along with 150 ml of water immediately after the operation. Timed venous blood samples were drawn into heparinised tubes before the administration of oxycodone at which time a sample for genomic DNA was taken. Thereafter samples were taken at 15, 30, 45, 60, 90 minutes, 2, 3, 4, 5, 6, 8, 10, 12, 18 and 24 hours.

4.2.2.2. Studies II and III

The study medication (III), 1000 mg of intravenous paracetamol (Perfalgan 10 mg/ml 100 ml solution; Bristol-Myers Squibb, Agen, France), was administered in the recovery room immediately after the surgery. The study drug was administered using an automated infusion device (Braun Infusomat Space Infusor; B. Braun Melsungen AG, Melsungen, Germany) in 15 minutes. At completion of the infusion, the study medication (II), 5 mg of intravenous oxycodone hydrochloride (OxyNorm; Mundipharma, Bard Pharmaceuticals, Cambridge, UK) was administered by bolus injection. The bolus injection was flushed with 5 ml saline solution to ensure correct dosing. Timed venous blood samples were drawn into heparinised tubes before the administration of paracetamol and at 7.5 and 15 minutes during the infusion. After completion of the infusion, samples were drawn at 2.5, 5, 10, 15, 20, 30, 45 and 60 minutes and at 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 18 and 24 hours.

4.2.2.3. Study IV

Study was conducted using a randomized, double blind, balanced, crossover design with two phases in young and elderly subjects. The washout interval between the phases was four weeks. Young and elderly subjects took either clarithromycin (Zeclar 500 mg tablet, Orion Pharma, Espoo, Finland) 500 mg (clarithromycin phase) or placebo (control phase) in a randomized order for five days. On day 4, subjects received a single dose of 10 mg oral oxycodone hydrochloride.

Oral placebo or clarithromycin was self-administered by the subjects on days 1, 2, 3 and 5. Compliance with the dosing schedule was ensured by means of mobile phone short message service. On day 4, the investigators administered the clarithromycin dose at the study facility. The subjects fasted for 8 hours before the oxycodone dosing and were given a standard meal 4 hours after the oxycodone dosing. A light meal was also given 8 hours after the oxycodone dosing.

Blood samples for pharmacokinetic measurements were collected immediately before, and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24 and 48 h after ingestion of oxycodone. The genotyping for CYP2D6 was performed and the plasma concentration of clarithromycin was determined from the samples taken immediately before oxycodone dosing.

4.2.3. Determination of plasma drug concentrations and genotyping

The plasma drug concentration analysis as well as *ABCG2*-genotyping were done in the Department of Clinical Pharmacology, at the University of Helsinki and the *CYP2D6* genotype analysis were done in the Laboratory of Molecular Genetics, HUSLAB, Helsinki University Central Hospital, Helsinki.

4.2.4. Determination of oxycodone, noroxycodone, oxymorphone and noroxymorphone (Studies I, II and IV)

Plasma concentrations of oxycodone, noroxycodone, oxymorphone and noroxymorphone were determined using a validated liquid chromatography tandem

mass spectrometric (LC-MS/MS) method. Following solid-phase extraction, the analytes were separated on a reversed-phase column by gradient elution using on a XBridge C18 (2.1x100mm, 3.5µm I.D.) analytical column protected by a XBridge C18 (2.1x10 mm, 3.5µm I.D.) guard column (Waters Corp., Milford, MASS). The mobile phase consisted of 5 mM ammonium formate (pH 9.4, adjusted with 25 % ammonium hydroxide solution) and methanol. A PE Sciex Api 3000 tandem mass spectrometer (Sciex division of MDS inc., Toronto, ON, Canada) operated in positive ion mode with multiple reaction monitoring. The ion transitions monitored were m/z 316 to m/z 241 for oxycodone, m/z 319 to m/z 244 for d3-oxycodone, m/z 288 to m/z 213 for noroxymorphone, m/z 302 to m/z 227 for noroxycodone and oxymorphone, m/z 305 to m/z 230 for d3-noroxycodone and d3-oxymorphone. The lower limits of quantification (LLQ) were 0.1 ng/ml for oxycodone and oxymorphone and 0.25 ng/ml for noroxycodone and noroxymorphone. The interday coefficients of variation were (CV) < 15% for all analytes at relevant plasma concentrations.

4.2.5. *Determination of paracetamol, paracetamol sulphate and paracetamol glucuronide (Study III)*

Plasma concentrations of paracetamol and its major metabolites, paracetamol glucuronide and paracetamol sulphate, were determined using a minor modification of a high-performance liquid chromatographic method (Vertzoni et al. 2003). We precipitated plasma proteins using methanol, instead of perchloric acid, to avoid a hydrolysis of paracetamol sulphate in the acidic medium. The LLQ were 0.25 mg/L for paracetamol and paracetamol sulphate and 0.5 mg/L for paracetamol glucuronide. The interday CV for paracetamol was 12.8%, 12.5% and 5.1% at 0.398, 2.01 and 10.1 mg/L, respectively (n = 38). The CV for the glucuronide metabolite was 11.5% and 4.5% at 1.76 and 9.48 mg/L (n = 41), respectively. The corresponding values for paracetamol sulphate were 13.6%, 11.6% and 7.4% at 0.342, 1.72 and 8.74 mg/L (n = 38).

4.2.6. *CYP2D6 genotyping (Studies I, II and IV)*

Genotyping for CYP2D6 was performed using a two-step multiplex primer extension method that allows the detection of 11 of the most relevant polymorphic positions, the assessment of whole-gene deletion and duplication and the allele composition of gene duplication (Sistonen et al. 2005).

4.2.7. *ABCG2 genotyping (Study III)*

Genomic DNA was extracted from whole-blood samples using standard methods (QIAamp DNA Blood Mini Kit; Qiagen, Hilden, Germany). The subjects were genotyped for the *ABCG2* c.421C>A single nucleotide polymorphism (p.Gln141Lys; rs2231142) by allelic discrimination with a TaqMan Drug Metabolism Genotyping Assay (assay ID: C__15854163_70; Applied Biosystems, Foster City, CA, USA). (Keskitalo et al. 2009)

4.2.8. Determination of renal function (Studies I-IV)

Renal function was evaluated by estimating the GFR from sex of the subject and his/her serum creatinine by usin abbreviated Modification of Diet in Renal Disease study equation: $GFR = 88.4^{-1} \times 186^{-1.154} \times$ Serum Creatinine$^{-1.154}$ (μmol/l) $\times$ Age$^{-0.203}$ (years) $\times$ (0.742 if female) $\times 88.4^{-1}$ (Levey et al. 2003). The obtained renal function data was not published in Studies I and IV, but since the data was available, it was used in this summary.

4.3. Pharmacokinetic analysis

Pharmacokinetic calculations were performed using the WinNonlin pharmacokinetic program (version 4.1; Pharsight, Mountain View, California). Peak plasma concentration (C_{max}) and the time to maximum concentration (t_{max}) were observed directly from the data. The area under the plasma concentration-time curve with extrapolation to infinity ($AUC_{0-\infty}$) was calculated by the trapezoidal rule. Linear trapezoidal rule was used for successively increasing concentration values, logarithmic trapezoidal rule being used for decreasing values. Individual terminal log-linear phases of plasma concentration-time curves were identified visually, and the elimination rate constant (λ_Z) was determined by regression analysis. The elimination half-life ($t_{1/2}$) was calculated using the following equation: $t_{1/2} = \ln2 / \lambda_Z$. In the Studies I and IV the apparent clearance (CL/F) and apparent volume of distribution during elimination (V_z/F) of oral oxycodone were calculated using standard methods. In Studies II and III standard noncompartmental methods were used to calculate plasma clearance (CL) and steady-state volume of distribution (V_{ss}) of oxycodone and paracetamol. Because a standard dose of 1000 mg of paracetamol was administered to all patients in Study III, standardized $AUC_{0-\infty}$ ($sAUC_{0-\infty}$) for paracetamol and the metabolites was calculated by dividing the $AUC_{0-\infty}$ of paracetamol by paracetamol dose expressed in mg per kg bodyweight. Metabolite-to-parent drug $AUC_{0-\infty}$ ratios (AUC_m/AUC_p) were calculated to describe the activity of various metabolic routes. The ratios of the $AUC_{0-\infty}$ of each substance during clarithromycin to that during placebo phase (AUC_{cl}/AUC_{pl}) were calculated to compare the magnitude of the possible clarithromycin-induced interaction between young and elderly subjects.

4.4. Pharmacodynamic measurements (Study IV)

Behavioural effects of oxycodone were assessed asking the subjects to rate their present state or feeling from the following items: alert / drowsy, good performance / poor performance, no drug effect / strong drug effect, unpleasant feeling / pleasant feeling, no nausea / extreme nausea or vomiting on a 100-mm visual analogue scale (VAS). Central co-ordination of extraocular muscles was assessed using the Maddox wing test (Hannington-Kiff 1970), central processing of sensory information with the digit symbol substitution test (DSST) (Stone 1984). The pupil size was measured with Cogan's pupillometry. Any adverse events reported by the subjects were recorded.

Cold pain threshold and intensity were assessed using the cold pressor test (Jones et al. 1988). The subject immersed his or her left hand into ice water of $0.5 – 2°C$ up to the

wrist for one minute. The latency in seconds from the immersion to the first sensation of pain was defined as the cold pain threshold. During the immersion, the subject reported the intensity of cold pain at 30 s and 60 s using a 11-point numerical rating scale (NRS, 0 = no pain, 100 = maximal pain imaginable). If pain became intolerable, the subject was allowed to withdraw his or her hand from the water, and NRS for pain intensity was recorded as 100.

The area under the effect-time curve for 12 h ($AEUC_{0-12}$) for all pharmacodynamic variables was determined using the linear trapezoidal rule.

4.5.   Safety assessment and precautions

In Studies I-III all patients were monitored in the recovery room by the investigator for the first two hours after the administration of the study medication. After that monitoring continued at the PACU until the first postoperative morning. The triggers for intervention were set to be; heart rate below 40 / min, mean blood pressure below 65 mmHg or systolic blood pressure below 100 mmHg and peripheral arteriolar oxygen saturation below 90%. In Study IV, all subjects were monitored by the investigator and by a study nurse for the first six hours after oxycodone dosing. After that subjects were under the investigators monitoring and care until 12 h post dose at which time the subjects were given a ride home.

4.6.   Statistical analysis

In Studies I-III, eight patients were calculated to be required in each group to demonstrate a 30% difference in the largest and smallest mean values of $AUC_{0-\infty}$ of oxycodone at a level of significance $P < 0.05$ and power of 80%. To prepare for possible dropouts, we decided to recruit the patient to each group. For Study IV, based on earlier studies, ten participants were calculated to be needed to detect a 30% difference in the $AUC_{0-\infty}$ of oxycodone between the clarithromycin and control phases at power of 80% and significance of $P < 0.05$.

In Studies I and II, all pharmacokinetic variables except t_{max} were compared by analysis of variance (ANOVA) or Chi-squared test, as appropriate and a posteriori testing was performed with Tukey's test. The values for t_{max} were compared using the Kruskall-Wallis test followed by the Mann-Whitney U test. For Study IV, the differences in pharmacokinetic and pharmacodynamic variables between clarithromycin and control phases were analysed using Student's t-test for paired samples, except for t_{max}, which was analysed with Wilcoxon signed-ranks test. Two-sample t-test was used to compare the values for AUC_{cl}/AUC_{pl} between young and elderly subjects. Pearson's product-moment correlation coefficient was used to investigate the possible relationship between the pharmacokinetic variables and the estimated GFR. $P < 0.05$ was considered as statistically significant.

Because all 40 patients in Study III had either *ABCG2* c.421CA or c.421CC genotype, the possible effect of *ABCG2* genotype on pharmacokinetic variables was tested using

Student's t-test prior to other statistical analyses. Thereafter, all pharmacokinetic variables except t_{max} were compared using stepwise two-way (age group, sex, age group × sex) analysis of variance. The values for t_{max} were compared using the Kruskal-Wallis test followed by the Mann-Whitney U-test. $P < 0.05$ was considered as statistically significant.

All data were analysed using the statistical program SYSTAT for Windows (version 10.2; Systat Software, Richmond, CA, USA).

5. RESULTS

5.1. Oxycodone

5.1.1. Effect of age on the pharmacokinetics of oral oxycodone (Study I)

The mean exposure (AUC$_{0-\infty}$) to oxycodone was 50-80% greater in groups G 70-80 ($P < 0.01$) and G 80-90 ($P < 0.05$) compared to group G 20-40. The values for CL/F in the two oldest groups were significantly lower ($P < 0.05$) compared to young adults. The mean $t_{1/2}$ of oxycodone was 1.6 hours longer in group G 70-80 ($P < 0.05$) than in the youngest group. The interindividual variation of $t_{1/2}$ in the oldest group was marked and it did not differ in a statititically significant degree from any other group. The individual values for oxycodone AUC and $t_{1/2}$ are shown in Figure 5. The mean C$_{max}$ of oxycodone was similar in all age groups. However, at 12 hours after oxycodone administration, patients over 70 years of age had over twofold higher ($P < 0.05$) mean plasma oxycodone concentration than the youngest group.
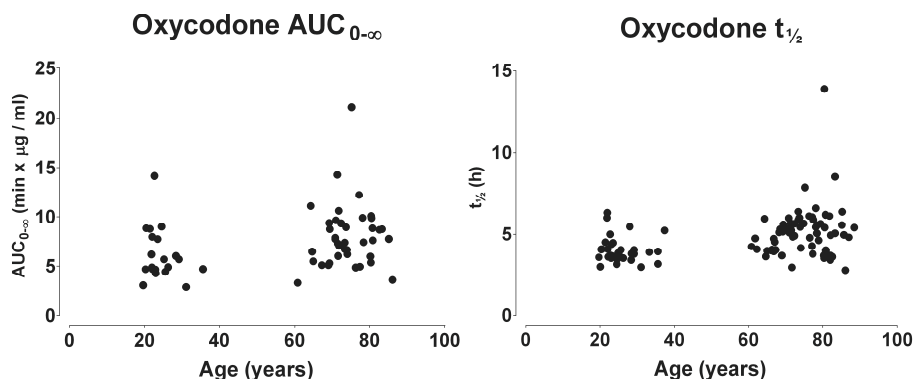

Figure 5. The individual values of oxycodone AUC$_{0-\infty}$ from Studies I and IV and oxycodone $t_{1/2}$ from Studies I, II and IV.

Regarding the metabolites of oxycodone, noroxycodone, oxymorphone and noroxymorphone, the only significant difference in pharmacokinetic parameters was the mean $t_{1/2}$ of noroxycodone, which was 3 hours longer in the two eldest groups compared to young adults ($P < 0.05$). The values for AUC$_m$/AUC$_p$ for both noroxycodone and oxymorphone were similar in all the age groups. There were no significant gender-associated differences in any of the pharmacokinetic parameters.

The effect of renal function on the pharmacokinetics of oral oxycodone was not published in our original publications (Studies I and IV). However, the data was available and assessment was made for this summary. Only four patients in the G 20-40 group in Study I had their serum creatinine value determined prior to the study. Thus for only those four the GFR could be estimated in the G20-40 group. There was a linear correlation between the estimated GFR and the CL/F of oral oxycodone with

the slope of 0.0169 (r = 0.35, P < 0.05). There was also a linear correlation between the estimated GFR and the values of noroxycodone $AUC_{0-\infty}$ with the slope of -0.0436 (r = 0.375, P < 0.01) and $t_{1/2}$ with the slope of -0.0464 (r = 0.40, P < 0.01).

5.1.2. *Effect of age on the pharmacokinetics of intravenous oxycodone (Study II)*

In study II and III, the youngest and oldest age groups differed significantly from each other in respect of gender, ASA physical status, weight and estimated GFR. The study protocol did not include any prior laboratory testing other than those required by the surgical procedure. Only three patients in the G 20-40 group had their serum creatinine value determined prior to the study. Thus for only those three the GFR could be estimated in the G20-40 group. There was an overrepresentation of females in the oldest group compared to other groups. Patients in the oldest group weighed less than patients in the other groups (Table 3).

In group G 80-90, the mean $AUC_{0-\infty}$ of oxycodone was 80% greater (P < 0.01) and the CL 34% lower (P < 0.05) than in the group G20-40. The mean $AUC_{0-\infty}$ was 30-41% greater in group G 80-90 compared to G 60-70 (P < 0.05) and G 70-80 (P < 0.05). In the two oldest groups the mean CL was 28% lower than in group G 20-40 (P < 0.05). The values for V_{ss} tended to be smaller and the values for $t_{1/2}$ longer in the oldest group compared to the youngest group (Figure 5), but there were no statistically significant pharmacokinetic differences between the groups.

Due to the single intravenous dose of oxycodone, the concentrations of metabolites of oxycodone were low. The concentrations of oxymorphone and noroxymorphone were mostly below the LLQ and therefore no pharmacokinetic analysis was possible. The quantity of noroxycodone was high enough to analyse. The values for noroxycodone C_{max} and $AUC_{0-\infty}$ were clearly increased in patients in the oldest group compared with patients in G 20-40 and G 60-70 groups (P < 0.05). The mean values for AUC_m/AUC_p of noroxycodone were similar in all age groups. There were no gender-associated differences in any of the pharmacokinetic parameters.

There was a linear correlation between the estimated GFR and the values of oxycodone CL with the slope of 0.066 (r = 0.505, P < 0.01), V_{ss} with the slope of 0.013 (r = 0.432, P < 0.05) and $AUC_{0-\infty}$ with the slope of -0.058 (r = -0.534, P < 0.01). Linear correlation was also seen between the GFR and values of noroxycodone $AUC_{0-\infty}$ with the slope of -0.065 (r = -0.638, P < 0.01), AUC_m/AUC_p with the slope of -0.0045 (r = -0.482, P < 0.01) and $t_{1/2}$ with the slope of -0.065 (r = -0.458, P < 0.01).

5.1.3. *Effect of clarithromycin on the pharmacokinetics of oxycodone (Study IV)*

Clarithromycin increased the exposure to oxycodone over two-fold. The mean $AUC_{0-\infty}$ of oxycodone during clarithromycin phase was 102% higher (P < 0.01) in young adults and 131% higher (P < 0.01) in the elderly subjects. The values of individual $AUC_{0-\infty}$ during control phase and clarithromycin phase are shown in Figure 6. The ratio of AUC in clarithromycin phase to AUC in placebo phase (AUC_{cl}/AUC_{pl}) was similar in both age groups. The individual changes in the $AUC_{0-\infty}$ of oxycodone and its

metabolites are illustrated in figure 6. Clarithromycin decreased the mean CL/F of oxycodone by 53% in the young (P < 0.01) and 48% in the elderly subjects (P < 0.01). The mean $t_{1/2}$ was prolonged from 4.4 h during placebo phase to 5.8 h during clarithromycin phase in young subjects and from 5.9 to 7.0 h in elderly subjects (P < 0.01) respectively. Compared to placebo, clarithromycin increased the mean C_{max} by 53% in the young (P < 0.01) and 72% in the elderly subjects (P < 0.01).

Clarithromycin inhibited the metabolism of oxycodone and shifted the metabolism pathway away from noroxycodone and towards oxymorphone. Clarithromycin decreased the mean C_{max} of noroxycodone by 42% in the young and 37% in the elderly subjects (P < 0.01). The mean $AUC_{0-\infty}$ of noroxycodone was decreased 54% in the young and 53% in the elderly subjects (P < 0.01) in the clarithromycin phase. The AUC_m/AUC_p for noroxycodone was decreased 71% and 74% in young and elderly (P < 0.01) by clarithromycin, respectively. The values for noroxycodone AUC_{cl}/AUC_{pl} were similar in both age groups. Clarithromycin increased the mean C_{max} of oxymorphone by 1.6- and 1.7-fold (P < 0.05) and the mean $AUC_{0-\infty}$ of oxymorphone by 3.6- and 3.1-fold (P < 0.01) in young and elderly subjects, respectively.

Clarithromycin decreased the C_{max} of noroxymorphone by 52% in the young and 75% in the elderly subjects (P < 0.01). Clarithromycin also decreased the $AUC_{0-\infty}$ of noroxymorphone by 40% (P < 0.01) in young and 62% (P < 0.01) in elderly subjects. The AUC_m/AUC_p for noroxymorphone was 68% (P < 0.01) and 83% (P < 0.05) lower during the clarithromycin phase in young and elderly subjects when compared to the placebo phase. On the basis of the values for AUC_{cl}/AUC_{pl} of oxymorphone and noroxymorphone, the magnitude of the interaction was similar in both age groups.
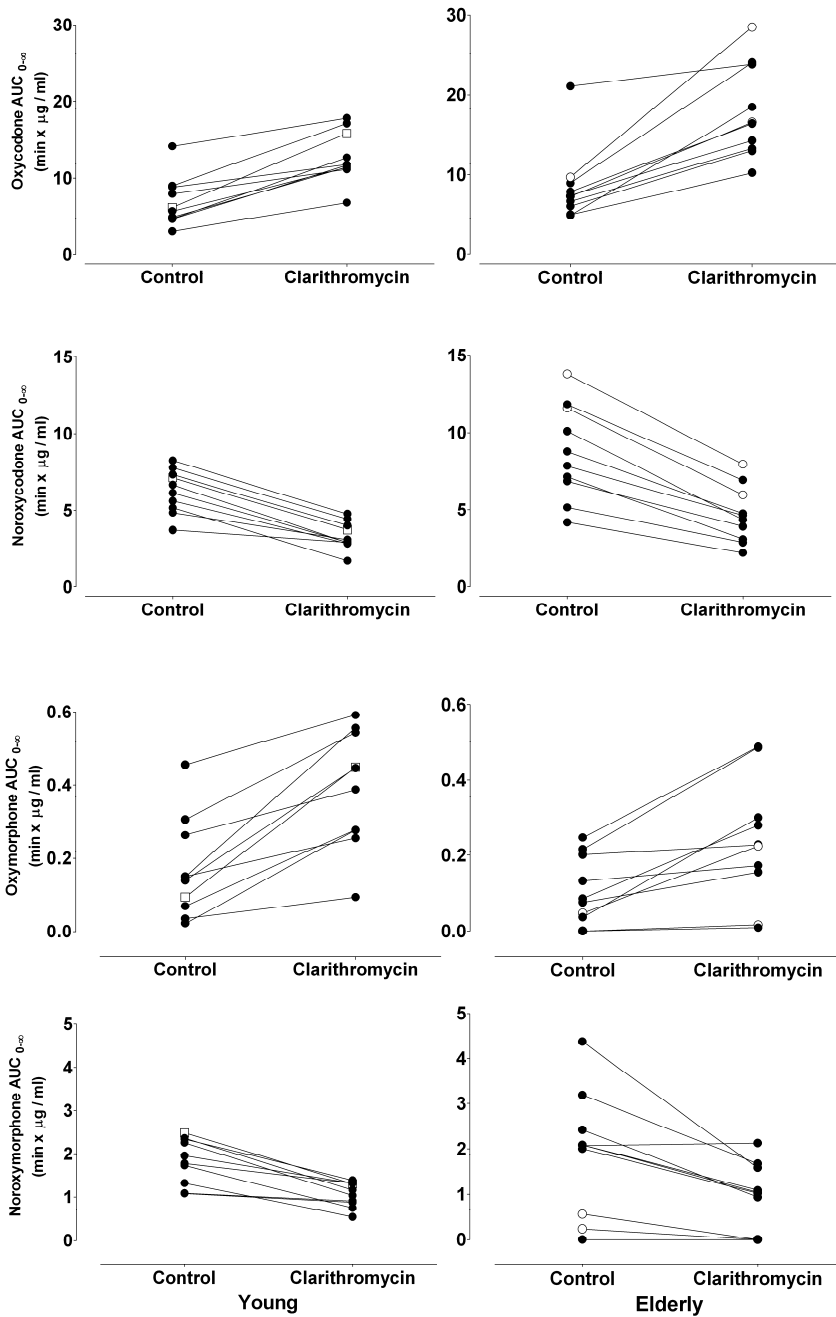
Figure 6. The effect of clarithromycin on the AUC of oral oxycodone and its metabolites. UM via CYP2D6 is depicted with open squares and PMs via CYP2D6 are depicted with open circles.

Three subjects had markedly lower plasma oxymorphone and noroxymorphone concentrations than the rest of the test subjects. The plasma noroxymorphone concentration was below the lower LLQ in one subject during both the placebo and the clarithromycin phase and in one subject during the clarithromycin phase. For two of the subjects all measured oxymorphone concentrations were below the LLQ during the placebo phase. Two out of these three subjects were PMs and one of them was EM via *CYP2D6*.

Plasma clarithromycin concentration varied between 460 and 2930 ng/ml immediately before oxycodone administration on day 4 of the clarithromycin phase of the study. No clarithromycin was detected at the corresponding time point during the placebo phase.

5.1.4.   *Effect of clarithromycin on the pharmacodynamics of oxycodone (Study IV)*

Clarithromycin induced some modest changes in the pharmacologic response to oxycodone. However, the administration of clarithromycin was not associated with an increased oxycodone effect in cold pain threshold, cold pain intensity, self-rated behavioural measurements, pupil size, Maddox wing test or the digit symbol substitution test (DSST) in young or elderly subjects. Two elderly subjects reported nausea 4 hours after oxycodone administration during the clarithromycin phase. For them the individual change in oxycodone $AUC_{0-\infty}$ during clarithromycin was 2.7- and 3.8-fold to that in the placebo phase.

5.1.5.   *Effect of CYP2D6 genotype on the pharmacokinetics of oxycodone (Studies I, II and IV)*

In Studies I, II and IV, there were in total eight PMs or IMs, 88 EMs and five UMs via *CY2D6*. *CYP2D6* genotype affected the concentration of oxymorphone notably, the UMs having higher oxymorphone concentrations. In the Study II the one ultrarapid metabolizer was the only one having detectable amounts of oxymorphone in plasma between 5 minutes to 8 hours after the oxycodone injection and the C_{max} was almost two-fold higher than with the patient with the second highest oxymorphone concentrations. However, no statistical signifant differences were observed.

The PMs and IMs appeared to have higher plasma oxycodone concentrations than the EMs and particularly the UMs irrespective of age (Figure 7), but the difference was not statistically significant. The mean $AUC_{0-\infty}$ of norxoycodone was 90% higher in PMs (P < 0.05) and 40% higher in EMs (P < 0.05) compared to PMs and IMs. The mean noroxymorphone $AUC_{0-\infty}$ was 78% lower in PMs and IMs (P < 0.05) compared to UMs and 71% lower compared to EMs (P < 0.05). Corresponding AUC_m/AUC_p values were higher for noroxycodone in PMs and IMs and lower in UMs. Corresponding AUC_m/AUC_p values were lower for oxymorphone in PMs and IMs and higher in UMs.
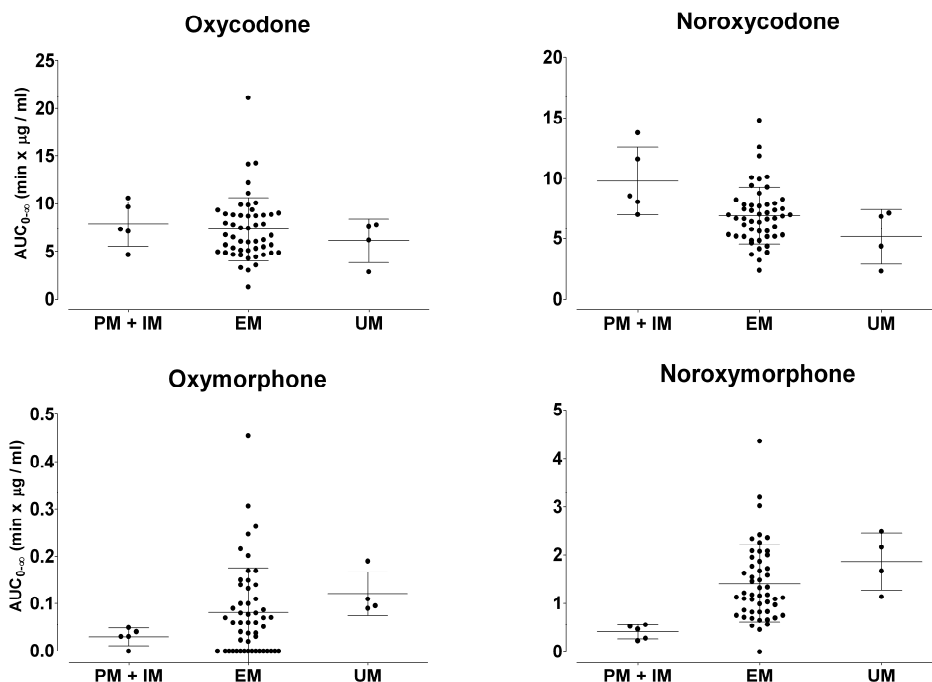
Figure 7. The effect of *CYP2D6* genotype to oral oxycodone metabolism. The mean ± SD values are represented by horizontal line. Data from Studies I and IV.

5.2. Paracetamol

5.2.1. Effect of age on the pharmacokinetics of intravenous paracetamol (Study III)

In the oldest group, the mean value of $AUC_{0-\infty}$ of paracetamol observed after 1000 mg of paracetamol was 54-68% higher (P < 0.01) than in the two youngest groups. The $AUC_{0-\infty}$ was higher in the elderly, when the patients over 70 years of age (G70-80 and G 80-90) were compared to patients under 70 years of age (G 20-40 and G 60-70). Also the standardized $AUC_{0-\infty}$, where the paracetamol dose is expressed in mg / kg bodyweight, was 43% higher (P < 0.01) in the oldest group compared with the youngest group. Patients in group G 80-90 had, on the average 90% higher paracetamol plasma concentrations than young adults 8 hours after the paracetamol infusion. There was also an association with sex. CL showed a statistically significant dependence on age group (P < 0.01), whereas V_z and $t_{1/2}$ with age group, sex and age group × sex interaction. C_{max} was only associated with sex.

The concentrations of paracetamol glucuronide and paracetamol sulphate were significantly higher in the elderly compared to young adults. The mean values for $AUC_{0-\infty}$ of paracetamol glucuronide and paracetamol sulphate were 2.3-fold and 2.7-

fold higher (P < 0.01) in the oldest group compared to the youngest group, respectively.

5.2.2. *Effect of renal function on pharmacokinetics of paracetamol (Study III)*

There was a weak linear correlation between the GFR and $AUC_{0-\infty}$ of paracetamol with the slope of -0.401 (r = -0.435, P < 0.05). The correlations between GFR and paracetamol metabolites, paracetamol glucuronide and paracetamol sulphate were stronger. The plasma concentrations of paracetamol glucuronide and paracetamol sulphate became higher as the renal function got lower. For paracetamol glucuronide, the correlation with GFR had a slope of -4.03 (r = 0.793, P < 0.01) and for paracetamol sulphate, the correlation with GFR had a slope of -1.10 (r = 0.693, P < 0.01).

5.2.3. *Effect of ABCG2 genotype on pharmacokinetics of paracetamol (Study III)*

In Study III, there were 33 patients with the *ABCG2 c.421CC* genotype and 7 patients with the *ABCG2 c.421CA*. None of the patients had the *ABCG2 c.421AA* genotype. The relationship of *ABCG2 c.421C>A* genotype and the $AUC_{0-\infty}$ values of paracetamol and its glucuronide and sulphate metabolites were measured but no statistically significant differences were observed.

5.3. Adverse effects

In Study I, naloxone was given to 13 patients and in study II and III, naloxone was given to eight patients for relief from itching, which was probably caused by the epidural fentanyl. In study II and III, four patients in the oldest group developed transient oxygen desaturation (peripheral arteriolar oxygen saturation < 90%) during the first 35 minutes after the administration of intravenous oxycodone. They were requested to breathe deeply. No other interventions were required. One patient in study II and III belonging to the oldest group, was given intravenous glycopyrrolate because her heart rate fell below 35 beats/minute. Two patients in study II and II experienced nausea, which was treated with intravenous ondansetron. In study IV also two subjects experienced nausea and one of them vomited.

6. DISCUSSION

6.1. Methodological aspects

6.1.1. Pharmacokinetic studies in orthopaedic patients (Studies I-III)

Elderly patients are often excluded from pharmacokinetic studies. This is mainly due to the fact that elderly people have often comorbidities and they are using concomitant medication. This is understandable but on the other hand it would be important to do the studies in the target population.

For pharmacokinetic studies on pain medication in the elderly, the orthopaedic surgical patients are close to the ideal subjects. They are great in numbers and their length of hospital stay makes it possible to complete studies without affecting the discharge date. While in hospital, the environment is standard, the monitoring is easy and if any interventions were to be required, the staff, the medication and the equipment are readily available.

Based on the power analysis of previous studies, eight patients were calculated to be required in each group to demonstrate a 30% difference in the largest and smallest mean values of $AUC_{0-\infty}$. To prepare for possible dropouts, incomplete data collection or other mishaps, ten patients were recruited for each group. Altogether 81 orthopaedic patients were recruited in the studies. The youngest patients were scheduled for arthroscopic surgery of the anterior cruciate ligament of the knee and the elderly patients were scheduled for elective knee prosthesis operation. Although the young and elderly patients were scheduled for different surgical procedures, the procedures were very similar in regard to the length of the operation, blood loss, tourniquet usage time, type of anaesthesia and pain management for the first 24 postoperative hours.

Because all patients were given the same premedication and anaesthetic management, the pharmacokinetic data should be unbiased and likely to be comparable in all groups. For both operations, sufficient pain relief for the first 24 hours was achieved with epidural infusion. In the few cases of insufficient pain relief, patients were given intravenous or intramuscular morphine according to clinical needs. Two patients in study I received one rescue dose of morphine. Morphine should not affect the absorption, distribution, metabolism, excretion or the concentration assays. Patients in the studies were relatively healthy, although the younger groups tended to be healthier than the elderly. The relative healthy status of the elderly patients served the purpose to study the effect of age on oxycodone and paracetamol pharmacokinetics. The pharmacokinetics of oxycodone and paracetamol might be altered even further in frail elderly with significant co-morbidies (Wynne et al. 1990).

Studies I-III were carried out in the immediate postoperative setting. In study I, the study drug was administered orally and in studies II and III, the study drug was administered intravenously. It has been shown that both surgery by itself and the use of opioids may affect the gastrointestinal absorption of drugs (Nimmo et al. 1975, Kennedy and Riji 1998). The timing and the study design made it impossible to

quantify the effect of surgery and perioperatively-administered opioids on the pharmacokinetics of oral and intravenous oxycodone and intravenous paracetamol. Because it is the absorption rather than the distribution or elimination of drugs that is altered in the perioperative period (Nimmo et al. 1975), the results can be of value for clinicians titrating the postoperative maintenance dose of oxycodone or paracetamol. That being said, it cannot be excluded that the pharmacokinetics of oral and intravenous oxycodone and intravenous paracetamol might have been affected by the timing of the study.

In all studies, only a single dose of study medication was administered. Therefore no conclusions can be made regarding the accumulation of oxycodone or paracetamol in the elderly or possible saturation of certain metabolic pathways, e.g. the sulphation pathway of paracetamol, which could increase the formation of the toxic NAPQI metabolite. Also, no conclusions can be made about the possible differences in development of tolerance for oxycodone treatment between young and elderly patients.

6.1.2. *Drug-drug interaction study in healthy young and elderly volunteers (Study IV)*

For the interaction study, 20 individuals, ten young and ten elderly, over 70 years of age, were recruited. A placebo-controlled, balanced, randomized, double-blind, crossover study design in young and elderly subjects was used. In a crossover design each subject serves as his or her own control. The possible changes in the pharmacokinetic and pharmacodynamic variables are calculated within the subject, which minimizes the interindividual variability. Thus, the number of volunteers needed in a crossover study is low. The washout interval between the phases was four weeks to make sure that there would be no carry-over effects. Four weeks was enough so that no clarithromycin was detected in any of the test subjects' plasma during placebo phase of the study. Thus, the likelihood of carry-over effects of clarithromycin is low.

The elderly subjects were recruited from the senior citizen exercise clubs. It could be argued that elderly population in exercise clubs is healthier than the general elderly population. Be that as it may, members of senior citizen exercise clubs are likely to be active, meet the inclusion criteria and be able to give a valid informed consent. Also the likelihood of alcohol or controlled substance abuse is lower in elderly people who exercise compared to general elderly public. There are a large number of individuals present at a given time in a club giving of large exposure for the recruitment information. Still more than half of the elderly volunteers had to be excluded from the study because of existing disease or medication that was included in the exclusion criteria. The most common reason for exclusion from the study with the elderly was statin medication.

The clarithromycin dosing in Study IV was based on the product label information and was aimed to reach steady state concentrations. The dosing 500 mg twice a day is the maximum dose that manufacturer recommends for therapeutic regimen. The dosing

was continued for 35 hours after the oxycodone dosing, so that the concentration of clarithromycin remained at therapeutic level during the whole oxycodone elimination period. None of the test subjects reported any of the possible adverse effects of clarithromycin therapy. The compliance with the pre-treatment was controlled by mobile phone short message service and by determination of clarithromycin plasma concentration in the morning of day 4 of the study. The observed concentrations indicated good compliance.

In our studies the patients and test subjects were not selected according to their *CYP2D6* or *ABCG2* genotypes. The genotyping was done rather to explain the expected variability in the pharmacokinetic variables between the patients/study subjects. All the patients and volunteer subjects in our studies were Scandinavian Caucasians and the number of individuals carrying variant genotypes was low. Therefore the evaluation of the effect of *CYP2D6* genotype on the pharmacokinetics of oxycodone was not possible in a statistically relevant manner.

6.2. Oxycodone

6.2.1. *Effect of age on oxycodone pharmacokinetics*

The pharmacokinetics of oral oxycodone was dependent on the age of the patient. Patients in G 70-80 and G 80-90 had, on the average, 50-80% higher exposure to oral oxycodone than patients aged 20-40, mostly because of decreased clearance in the two oldest groups. Changes in oxycodone clearance were reflected also in $t_{1/2}$, which was increased up to 50% in patients over 70 years of age. At 8-24 hours after the oral oxycodone administration, patients over 70 years of age had over two-fold higher oxycodone plasma concentration compared to young adult patients. In the oral oxycodone study (Study I) the oldest group included two patients, who were ultrarapid metabolizers via CYP2D6. That offers one possible explanation to the finding that the mean $AUC_{0-\infty}$ of oxycodone in the G 70-80 group was higher than in the oldest group.

The pharmacokinetic parameters of oral oxycodone were fairly consistent within the group of young adult patients. The coefficient of variation of $AUC_{0-\infty}$ in these patients was only 9% as compared to values varying from 27 to 37% in the other age groups in Study I. Large interindividual differences in the elderly groups were also observed in the values for t_{max} and $t_{1/2}$. The pharmacokinetic parameters for young patients were in line with those observed in previous studies in young patients or healthy volunteers (Leow et al. 1992, Pöyhiä et al. 1992, Lalovic et al. 2006). The large interindividual variability in the elderly groups is understandable because factors like age-related physiological changes, concurrent diseases, concomitant medication and nutritional factors may influence the pharmacokinetics of drugs (Schwartz 2007, Klotz 2009). Nevertheless, we only included relatively healthy elderly people in our studies.

In Study I, there were three patients using a medication that affects CYP enzymes. One patient in group G 20-40 was using paroxetine medication. Paroxetine is a inhibitor of CYP2D6 (Bertelsen et al. 2003) and the concentrations of oxymorphone and noroxymorphone of that patient remained undetected. The effects of paroxetine on

oxycodone concentrations however, were minimal. One patient in group G 60-70 was on continuous carbamazepine medication. Carbamazepine is a strong inducer of CYP3A (Bertz and Granneman 1997). The elimination of oxycodone in that patient was strongly accelerated. The calculated apparent oxycodone clearance values for her exceeded the next highest clearance value in the study population by a factor of 2.3. She also had the highest metabolite to parent drug ratio for noroxycodone AUC among all the patients in the study. The finding is consistent with the study of Nieminen et al. where rifampin was found to greatly reduce the plasma oxycodone concentrations (Nieminen et al. 2009). One patient in group G 60-70 was using itraconazole medication, which is an effective inhibitor of CYP3A (Olkkola et al. 1994, von Moltke et al. 1996, Saari et al. 2010). The concentrations of the CYP3A-dependent metabolite noroxycodone for her were very low. However, the effects on concentrations of the parent drug oxycodone were small. The individual pharmacokinetic parameters for these three patients were calculated, but they were excluded from further statistical comparisons between the groups.

The pharmacokinetics of intravenous oxycodone was also dependent on the age of patient. Patients aged 60-70, 70-80 and 80-90 years had, on the average 28-34% lower CL of oxycodone than patients aged 20-40 years. Due to changes in the oxycodone CL, patients in the oldest group had up to 80% higher exposure to oxycodone than the patients in the youngest group. The patients over 80 years of age had over two-fold higher oxycodone plasma concentrations at 8-24 hours after oxycodone administration than the young adults. As in Study I with oral oxycodone the pharmacokinetic parameters of intravenous oxycodone were in agreement with previous studies in young patients or healthy volunteers (Pöyhiä et al. 1991, Leow et al. 1992).

6.2.2. *Effect of CYP2D6 genotype on oxycodone pharmacokinetics*

In Studies I, II and IV only a single dose of oxycodone was given and as a result the concentrations of oxymorphone and noroxymorphone were low. In Study II, oxycodone was administered intravenously. Thus, oxycodone was not subjected to first pass metabolism and the concentrations of oxymorphone and to a lesser extent noroxycodone were below the LLQ for most patients the whole study period. Because of low concentrations of oxymorphone and noroxymorphone, no definitive statement can be made about CYP2D6 activity and ageing. Based on the findings in the oral Study I, there appears to be no age-related changes in CYP2D6 activity. There were only three UMs in study I (Table 3). Two of the UMs belonged to the oldest group of patients. The oxymorphone and noroxymorphone concentrations of the UMs far exceeded the corresponding concentrations of any of the other patients regardless of age. In study I, the concentrations of noroxycodone did not change significantly with advancing age and there were no statistically significant differences in the noroxycodone-to-parent drug AUC ratio between the different age groups in Studies I and II. Thus, no age-related changes in the CYP3A activity were observed. During continuous administration of oxycodone the changes in different metabolic pathways, due to genotypic differences or drug-drug interaction, could be more evident.

As stated earlier, all study patients and volunteer subjects were Caucasian. The vast majority of them, 88 out of 101, were EMs through CYP2D6. Eight individuals were PMs or IMs and five individuals were UMs (Table 3). The *CYP2D6* genotype affected the concentration of oxymorphone notably but the difference was not statistically significant. The EMs and especially the UMs produced significantly more oxymorphone than poor or the intermediate metabolizers, which is consistent with an earlier in vitro study (Lalovic et al. 2004). In Study II, the one UM was the only one having detectable amounts of oxymorphone in plasma between 5 minutes to 8 hours after the oxycodone injection and C_{max} was almost two-fold higher than with the patient with the second highest oxymorphone concentrations. Noroxymorphone was also significantly more abundant in the EMs and UMs, a finding that is in good agreement with the fact that noroxymorphone is produced from both noroxycodone and oxymorphone (Lalovic et al. 2004, Lalovic et al. 2006). The PMs and IMs had significantly higher noroxycodone concentrations than the EMs and particularly the UMs and appeared to have higher plasma oxycodone concentrations irrespective of age. Corresponding metabolite-to-parent drug AUC ratios were for noroxycodone higher in PMs and IMs and lower in UMs and for oxymorphone lower in PMs and IMs and higher in UMs.

Three subjects in Study IV had markedly lower plasma oxymorphone and noroxymorphone concentrations than the rest of the test subjects. The plasma noroxymorphone concentration was below the LLQ in one subject during both the placebo and the clarithromycin phase and in one subject during the clarithromycin phase. For two of the subjects all of the measured oxymorphone concentrations were below the LLQ during the placebo phase. Two out of these three subjects were PMs through CYP2D6 and one of them was EM.

6.2.3. *Effect of renal function on oxycodone pharmacokinetics*

None of the patients in Study I and II had renal failure but there were several patients whose estimated GFR suggested mild-to-moderate renal dysfunction. In Study IV, five elderly and one young volunteer had a mild decrease in GFR. Although, on average, only 10% of oxycodone is excreted unchanged in urine (Pöyhiä et al. 1992, Kirvelä et al. 1996), there was a modest linear correlation between the estimated GFR and most parameters describing the pharmacokinetics of oxycodone and noroxycodone. Previous studies have shown that renal failure delays the elimination of oxycodone in humans (Kaiko et al. 1996, Kirvelä et al. 1996). Our findings are consistent with previous studies, although the slope of the regression equation describing the relationship between GFR and $AUC_{0-\infty}$ of oxycodone was only 0.058. According to the regression equation obtained for oxycodone in Study II: $y = -0.058\ x + 11.6$ (r = 0.539, P < 0.01), the deterioration in GFR from 90 ml/min/1.73m^2 body surface area (BSA), to the smallest value observed in our study would increase the exposure to oxycodone by approximately 35%. This is also consistent with the current prescribing information concerning the dosing of oxycodone in renal failure, which recommends a reduction in dose when GFR is below 60 ml/min/1.73m^2 BSA (Purdue Pharma).

6.2.4. *Effect of clarithromycin on oxycodone pharmacokinetics*

In Study IV, we investigated the effect of clarithromycin on the pharmacokinetics and the pharmacodynamics of oral oxycodone in young and elderly subjects. Clarithromycin increased the $AUC_{0-\infty}$ of oxycodone significantly and prolonged its $t_{1/2}$ in both young and elderly subjects. Concomitant use of oxycodone and CYP3A inhibitors has been found to increase the exposure to oral oxycodone in young healthy subjects (Hagelberg et al. 2009, Nieminen et al. 2010, Saari et al. 2010). It can be anticipated that these changes were caused by a simultaneous increase in oral bioavailability and a decrease in clearance of oxycodone. Ageing, however did not influence the interaction between clarithromycin and oxycodone and the relative change in the $AUC_{0-\infty}$ was similar in both age groups. This is consistent with the findings of Schmucker et al. (1990) and Parkinson et al. (2004) who concluded that ageing and gender do not affect the CYP3A content or it's metabolizing activity in human livermicrosomes. In elderly individuals over 65 years of age, the female gender has been suggested to be a more significant determinant of CYP3A activity than age or frailty (Schwartz 2007). In our study (Study IV), there were only three elderly women (Table 3) and due to this, we could not evaluate the gender-related differences in CYP3A inhibition by clarithromycin.

Inhibition of P-glycoprotein by clarithromycin could also be related to our findings in Study IV (Eberl et al. 2007). There are some evidence that oxycodone is a substrate for P-glycoprotein (Bostrom et al. 2005, Hassan et al. 2007). Thus inhibition of P-glycoprotein would increase its bioavailability, concentrations and the penetration through the blood-brain-barrier to the effect site in the brain and subsequently increase its effects. The role of P-glycoprotein in the distribution of oxycodone however, is controversial and the changes in concentrations of metabolites of oxycodone suggest that the pharmacokinetic changes in our study were caused by inhibition of CYP3A.

The concentrations of CYP3A-mediated metabolites of oxycodone, noroxycodone and noroxymorphone were substantially decreased by clarithromycin. Also the metabolism of oxycodone was directed more towards the CYP2D6-mediated route. These findings are in good agreement with the inhibitory effect of clarithromycin on the metabolism of other CYP3A substrates (Rodrigues et al. 1997, Gorski et al. 1998, Pinto et al. 2005).

6.2.5. *Pharmacodynamic considerations*

The age-related differences in the pharmacological response to a single dose of oxycodone in young and elderly subjects have been debatable in previous studies. Kaiko et al. reported differences in perceived drug effect between young and elderly, although their findings had stronger correlation with sex than age (Kaiko et al. 1996). In a recent study, Cherrier et al. reported no differences in neurocognitive or subjective effects after a single dose of oxycodone between middle-aged and elderly subjects (Cherrier et al. 2009). In our study (Study IV), clarithromycin did not affect the pharmacological response to oxycodone in young or elderly subjects. This is probably due to a high interindividual variation and possible gender differences in the behavioural and analgesic responses to oxycodone. Also the log-linear relationship

between plasma drug concentration and effect and the relatively small dose of oxycodone used in our volunteer study could explain the lack of effect by clarithromycin.

In the young and elderly subjects in study IV, the exposure to oral oxycodone was increased more than two-fold by concomitant clarithromycin medication. In the elderly subjects the range was 1.1 – 3.8-fold. The elderly individual with the 3.8-fold increase in the oxycodone AUC was the same subject who experienced nausea and vomited. Also the elderly individual with the third highest increase in oxycodone exposure during clarithromycin phase (2.7-fold) experienced nausea. Although these individual pharmacokinetic changes were not reflected in mean pharmacological responses recorded in this experimental setting, the individual range of responses was notable. Adverse effects are likely to occur within those individuals who are at the extreme ends of the response range.

6.2.6. *Clinical implications of the oxycodone studies*

Although some of the metabolites of oxycodone are pharmacologically active, it is the parent drug that seems to be responsible for the analgesic effects of oxycodone (Heiskanen et al. 1998, Lalovic et al. 2006, Zwisler et al. 2010). Age is an important factor affecting the pharmacokinetics and dose requirements of oxycodone. The interindividual variation in the oxycodone pharmacokinetics in the elderly was notable and the oxycodone dosage used in our studies may not be suitable for all patients. Because oxycodone has potentially serious adverse effects and pharmacokinetics and pharmacodynamics depend to a great extent on the age of the subject, it is important to titrate the analgesic dose individually, particularly in the elderly.

6.3. Paracetamol

6.3.1. *Effect of age on paracetamol pharmacokinetics (Study III)*

The pharmacokinetics of paracetamol was seen to depend on both age and sex. In the oldest group G 80-90, the mean exposure to intravenous paracetamol after a standard dose of 1000 mg was 54-68% higher than in the two younger groups of patients. The exposure to intravenous paracetamol was also higher in the two oldest groups G 70-80 and G 80-90 compared to the two youngest groups G 20-40 and G 60-70. Lower CL and smaller V_z in the oldest group caused the higher concentrations. These changes related to advancing age were also reflected in the higher values for C_{max} and $t_{1/2}$ in the oldest group of patients. Eight hours after its infusion, the patients in G 80-90 had on average almost two-fold paracetamol plasma concentrations compared to young adults.

The oldest group of patients differed from the younger groups in many respects. There was a 9:1 overrepresentation of females in the oldest group (Table 3), which complicated the statistical analysis of age on the pharmacokinetics of paracetamol. When age and sex were taken into account in the stepwise analysis of variance, the primary pharmacokinetic parameters CL and V_z were found to be dependent on both. The CL and V_z were lower in females compared to males and also lower in the elderly

compared to young adults. The oldest group also weighed significantly less than the other groups (Table 3). The dose of 1000 mg was chosen for all patients because that is the common clinical practice. In our study, the highest value of paracetamol $AUC_{0-\infty}$ in the oldest group was 3.8-fold higher than the lowest $AUC_{0-\infty}$ in the youngest group. If the dose had been standardised per bodyweight, the oldest group would still have had on the average 40% higher exposure to paracetamol than the young adults. Also the highest difference in the $AUC_{0-\infty}$ would have been 3.3-fold.

Being such an old and widely used drug, paracetamol has been studied immensely. Older studies however, have given inconclusive information on the pharmacokinetics of paracetamol in the elderly. Methodological aspects might explain some of the discrepancies. Paracetamol is poorly soluble in water and liquid intravenously administrable form of paracetamol is a fairly recent development. Thus, in many studies, paracetamol has been given orally, which has not allowed the determination of clearance and volume of distribution for paracetamol. The pharmacokinetics of oral paracetamol has been studied in the elderly. Trigs et al. and Miners et al. have published studies on the pharmacokinetics of oral paracetamol in young and elderly subjects. They both concluded that there is no difference in paracetamol pharmacokinetics between the young and the elderly. In both studies only male subjects were used and the short sampling period, only 6-8 hours, undermines the results somewhat (Triggs et al. 1975, Miners et al. 1988). Briant et al. reported prolongation of paracetamol $t_{\frac{1}{2}}$ in the elderly as a result of reduction of CL. Their calculations, however, were based on only four samples per patient, which makes reliable assessment of pharmacokinetics debatable (Briant et al. 1976). Divoll et al. studied paracetamol kinetics in four groups of young and elderly men and women. They observed no change in $t_{\frac{1}{2}}$ between the groups. Instead, they reported a lower CL and smaller V_z in the elderly than the young and also in women compared to men (Divoll et al. 1982).

Some of the differences in the studies might be due to different health conditions of the study patients. It has been suggested that frailty and general health conditions are the responsible factors that are related to the changes in pharmacokinetics in advancing age. In a study where hepatic conjugation reactions were investigated in fit young, fit elderly and frail elderly subjects, frailty was reported to have the most profound effect in paracetamol CL. Paracetamol CL was also significantly lower in fit elderly as compared to fit young but when the CL was adjusted to the smaller liver size in the elderly, the clearances were similar in both groups. It was concluded that the decrease in liver volume with age is the major contributor to the decline in CL of paracetamol in healthy elderly subjects (Wynne et al. 1990).

6.3.2. *Effect of renal function on paracetamol pharmacokinetics*

There were not any patients in our study group with manifest renal failure. However, there were patients with mild-to-moderate renal dysfunction implicated by the estimated GFR. In our study, the renal dysfunction had only a minor effect on the pharmacokinetics of paracetamol. The slope of the linear correlation between paracetamol and estimated GFR was -0.401. The finding is consistent with the study

published by Prescott et al. (1989). The correlation values for paracetamol glucuronide and sulphate conjugates with GFR were much stronger. Although the values for $AUC_{0-\infty}$ and C_{max} of paracetamol glucuronide and sulphate conjugates increased with age, the increase can be explained by changes in GFR. The corresponding slopes of the linear correlation between the GFR and $AUC_{0-\infty}$ of the glucuronide and sulphate conjugates were -4.03 and -1.10, respectively. This is also in good agreement with earlier study by Prescott et al. (1989). If the regression equation based on our data were applied to the smallest value observed in our study, exposure to paracetamol would be increased by approximately 40%. It would appear that moderate renal dysfunction does not require dose adjustments in short-term administration of paracetamol. Our finding is consistent with earlier studies (Bannwarth et al. 2001). However, the possible accumulation of the sulphate and glucuronide metabolites of paracetamol in renal dysfunction cannot be ruled out (Martin et al. 1991).

6.3.3. *Effect of ABCG2 genotype on paracetamol pharmacokinetics*

ABCG2 mediates the substrate uptake from the gut lumen by back-transport of variety of absorbed substances including sulphate and glucuronide metabolites of some compounds such as paracetamol (Suzuki et al. 2003, Zamek-Gliszczynski et al. 2006). *ABCG2* genotype may have an effect on the elimination of the metabolites of paracetamol from the body. However, in our study none of the patients had the *c.421AA* genotype associated with the reduced transport activity and only seven patients had the *c.421CA* genotype. Thus, *ABCG2* genotype had no quantifiable effect on paracetamol pharmacokinetics in our study.

6.3.4. *Clinical implications of the paracetamol study*

The current clinical practice is to use a standard dose of 1000 mg paracetamol for all adults irrespective on patient age (EMC UK Perfalgan). Although paracetamol is a reasonably safe drug when used according to dosage recommendations it has a relatively narrow therapeutic index (Sweetman S, 2006). The safety of commonly recommended doses of paracetamol and the liberal use of it has recently been questioned (Kaplowitz 2004). Serum alanine aminotransferase elevations have been reported in healthy adults taking the recommended maximum dose of paracetamol 4 g daily (Watkins et al. 2006). Watkins et al. speculated that fatty liver disease might have had a contribution in their results. A standard clinical dose of 1000 mg of intravenous paracetamol will result on average almost 70% larger exposure to paracetamol in patients aged 80–90 years than in young adults. If the dosage would be bodyweight adjusted, the elderly would still have had 40% larger exposure to paracetamol than the young adults. Thus, the use of standard doses per bodyweight would not protect against paracetamol-induced hepatocellular toxicity as compared to the current practice.

Although paracetamol is mainly metabolized to glucuronide and sulphate metabolites, 5-15% of paracetamol is hydroxylated to NAPQI by cytochrome P450 isoenzymes in the liver and kidney (Manyike et al. 2000, Gelotte et al. 2007). Under normal conditions the small amounts of NAPQI are rapidly detoxified by reduced glutathione

and excreted in the urine after conjugation with cysteine and mercaptouric acid (Mitchell et al. 1974, Miller et al. 1976). However, during overdosing or in vulnerable patients with impaired liver function, the quantity of this toxic metabolite may accumulate and cause liver cell damage (Anonymous 2001).

Age and sex are important factors affecting paracetamol pharmacokinetics. The exposure to paracetamol is increased in advancing age. Female sex is also associated with increased paracetamol concentrations. Paracetamol has proven to be a safe drug when used according to dosage recommendations. However, caution should be exercised when used in very old patients or in patients with prior hepatocellular disease or injury.

6.4. Ethical considerations

So far there has been no systematic information on the pharmacokinetics of oxycodone or intravenous paracetamol in the elderly. Neither has there been systematic information on the effect of clarithromycin on the pharmacokinetics and pharmacodynamics of oxycodone in humans. From a theoretical point of view all studies can cause unforeseen unfavourable effects. Because a change in the rate of metabolism of oxycodone or inhibition of CYP3A4 or 2D6 can lead to an increase of the analgesic / sedative effects of oxycodone and because increase in exposure of paracetamol can have serious, possibly life-threatening effects, it was considered to be justified to do these studies. The study dose of oxycodone was selected to be small enough so that it could be safely administered to the elderly patients and yet large enough, so that the plasma concentrations were quantifiable. The fixed doses of 10 mg of oral oxycodone hydrochloride and 5 mg of intravenous oxycodone hydrochloride were chosen in effort to minimize the risk of dosing errors. The study subjects were monitored diligently, which enabled administering the study drugs safely. The paracetamol dose was the standard dose used in clinical practise. Only single doses of study-medication were used in the studies. All the study patients would have been exposed to the same dosing of both study drugs during their hospital stay regardless of their participation in the studies.

During the test days in the volunteer interaction study (Study IV), the subjects were observed at all times by physicians who were all legitimated specialists in anaesthesia and intensive care. Three psychophysical tests were performed to study whether inhibition of CYP3A4 and 2D6 had an effect on oxycodone-induced analgesia. These tests are non-invasive, widely used, well tolerated and will only cause temporary discomfort to the subjects. The subjects were informed in advance about the temporary pain and unpleasantness they might have experienced during the pain tests.

The cannulation of a peripheral vein causes temporary pain and discomfort to the subject. The venous blood sample for DNA analysis was anonymised with a coded number. The code was known only to the investigators of the study and is stored with the investigator. The genetic information was/is not available to the subject or his/her physician.

One of the long-term adverse effects of opioids is misuse behaviour or addiction, which may occur in some individuals. Although scant data exists on the risk of healthy subjects developing opioid dependence (Simoni-Wastila and Strickler 2004), genetic predisposition, exposure to opioid and environmental factors are known to play a role. In patients with chronic pain, personal or family history of alcohol, substance or drug abuse, and legal problems such as driving under the influence or while intoxicated have been shown to increase the risk of opioid abuse (Michna et al. 2004, Compton and Volkow 2006, Ives et al. 2006). Individuals susceptible to opioid abuse as defined by the above mentioned criteria were not be included in the volunteer interaction study. Subjects were screened for urine toxicology and for risk factors for opioid misuse using the modified Finnish version of the Abuse Questions (Michna et al. 2004) prior to the inclusion. Any subject with a positive urine toxicology test result or a "yes" answer to any of the Abuse Questions were to be excluded. During Study IV, two doses of oral opioids were administered to the subjects on different days. These doses are clinically used in the treatment of postoperative pain. In case of excessive opioid effect, intravenous naloxone was to be used to reverse the opioid effects of oxycodone.

All studies were conducted in facilities where any of study drug related adverse effects could be treated appropriately.

All study protocols were conducted according to the revised Declaration of Helsinki (WMA General Assembly, Washington 2002) and were approved by the Ethics Committee of the Hospital District of Southwest Finland and the Finnish Medicines Agency. The study protocols were registered on the European Union Drug Regulating Authorities Clinical Trials (Eudra CT) clinical trials register.

All subjects received both oral and written information of the studies. They were informed that they could withdraw from the study any time they wanted. All subjects also gave their written informed consent before entering the studies.

Studies I-III were conducted with orthopaedic surgical patients. For them, there were very little additional discomfort or risks involved in participation in our studies. For the volunteers in Study IV, the risks involved in our series of studies, such as opioid exposure, adverse effects of drugs and transient pain were more significant than perhaps with the surgical patients. The theoretical risks, however, were well balanced to the new information given by the present series of studies.

6.5. Limitations of the study

The study design of a sinlge dose pharmacokinetic investigation does not allow making conclusions for continuous dosing, nor does it allow giving recommendations for clinical practise for continuous dosing of the study drugs. Further studies are warranted to investigate the possible accumulation of oxycodone and paracetamol in a continuous dosing in the elderly.

Due to the single dosing of oxycodone, the concentrations of the CYP2D6 dependent metabolite oxymorphone were low. Only five of the subjects in our studies were PMs or IMs and only four were UMs through CYP2D6. During continuous administration of oxycodone the role of CYP2D6 in the production of oxymorphone would have probably been more apparent.

The elderly subjects in our studies were selected to be in fairly good health in an effort to minimize the number of variables affecting the pharmacokinetics of the study drugs. It can be arqued that they do not represent the general elderly population. The pharmacokinetics of oxycodone and paracetamol might be altered even further in patients with comorbidities and concomitant medication.

7. SUMMARY AND CONCLUSIONS

1. Patients aged 70-80 and 80-90 years have 50-80% higher mean exposure to oral oxycodone and over twofold higher plasma oxycodone concentrations at 8-24 hours after the oral oxycodone administration than the young adults (20-40 years of age).

2. Patients aged over 70 years of age are expected to have, on average, 40-80% higher exposure to oxycodone compared to young adults (20-40 years of age) after intravenous administration. Patients over 80 years of age have a twofold oxycodone plasma concentration at 8-24 hours after intravenous oxycodone administration compared to young adults.

3. Age and sex are important factors affecting the pharmacokinetics of paracetamol in the elderly. The administration of 1000 mg of intravenous paracetamol resulted 36-68% higher exposure to paracetamol in patients aged 70-80 and 80-90 years than patients aged 20-40 years. The renal function of the patient somewhat affected the exposure to paracetamol. Plasma concentrations of paracetamol glucuronide and sulphate conjugates are markedly increased as the renal function decreased.

4. Clarithromycin influences the metabolism of oral oxycodone by inhibiting its N-demethylation to noroxycodone via CYP3A, but magnitude of this effect is not, age related. Plasma concentrations of oxycodone are greatly increased in young adults and elderly individuals and the exposure to oral oxycodone is increased by more than 100% during concomitant clarithromycin medication.

8. ACKNOWLEDGEMENTS

These studies were carried out at the Department of Anaesthesiology, Intensive Care, Emergency Care and Pain Medicine and Department of Pharmacology, Drug Development and Therapeutics, University of Turku and Turku University Hospital, during the years 2006-20011. The clinical studies with the orthopaedic surgical patients were conducted at the TYKS Surgical Hospital. I am grateful of the heads of these departments, Professors Klaus Olkkola, Risto Huupponen and Mika Scheinin, docents Kalevi Pihlajamäki and Petri Virolainen and M.D. Jukka Irjala, for the opportunity to work in such excellent facilities. These studies were financially supported by the EVO grants of Turku University Hospital, by the Finnish Society of Anaesthesiologists and by the Clinical Drug Research Graduate School.

I owe my greatest gratitude to my supervisor Professor Klaus Olkkola for tireless enthusiasm for my guidance and studies. His knowledge of the drug research, work ethics and humane attitude has been indispensable throughout the project. I have been truly privileged to work under his supervision.

I want to express my gratitude to my supervisor docent Riku Aantaa, who originated the idea for my research. I could always rely on quick and straightforward answers to whatever problem I had during the project.

Professors Pertti Neuvonen and Mikko Niemi are sincerely thanked for their essential contribution in determination of the drug concentrations and genotyping. Their contributions in preparing the manuscripts were of the utmost importance.

I want to thank Professor Sirpa Hartikainen and docent Leila Niemi-Murola for thorough review of the thesis and constructive comments.

Kristiina Kuusniemi is thanked for all her help and support during the project. Her ability to put things in perspective was essential in overcoming various obstacles during the project.

Docent Nora Hagelberg is thanked for her contribution in Study IV. Her effort enabled a quick publication of the results.

Docent Kalevi Pihlajamäki is thanked for being always willing to help and share his vast knowledge on work for which he received no credit. His influence on the work environment made research much easier.

Chemist Mikko Neuvonen is acknowledged for his expertise in analyzing the study samples. Medical laboratory technologist Elina Kahra and RN Mia Suppanen-Olkkola are thanked for their skilful technical assistance and kindness, which made me, feel at ease in new and somewhat strange environment during the Study IV. Secretaries Kirsti

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