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EFFECTS OF CYTOCHROME P450 ENZYME INHIBITORS AND INDUCERS ON THE METABOLISM OF S-KETAMINE

by

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ABSTRACT

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EFFECTS OF CYTOCHROME P450 ENZYME INHIBITORS AND INDUCERS ON THE METABOLISM OF S-KETAMINE

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The human body eliminates foreign compounds primarily by metabolizing them to hydrophilic forms to facilitate effective excretion through the kidneys. Cytochrome P450 (CYP) enzymes in the liver and intestine contribute to the metabolism of many drugs. Pharmacokinetic drug-drug interactions occur if the activity of CYPs are inhibited or induced by another drug. Prescribing multiple drugs to the improve effectiveness of therapy or to treat coexisting diseases is a common practice in clinical medicine. Polypharmacy predisposes patients to adverse effects because of the profound unpredictability in CYP enzymatic-mediated drug metabolism.

S-ketamine is a phencyclidine derivative which functions as an antagonist of the N-methyl-D-aspartate (NMDA) receptor in the central nervous system. It is a unique anaesthetic producing "dissociative anaesthesia" in high doses and analgesia in low doses. Studies with human liver microsomes suggest that ketamine is metabolized primarily *via* CYP3A4 and CYP2B6 enzymes. In this thesis, in healthy volunteers, randomized and controlled cross-over studies were conducted to investigate the effects of different CYP inducers and inhibitors on the pharmacokinetics and pharmacodynamics of oral and intravenous S-ketamine. The plasma concentrations of ketamine and its metabolite, norketamine, were determined at different timepoints over a 24 hour period. Other pharmacodynamic variables were examined for 12 hours.

Results of these studies showed that the inhibition of the CYP3A4 pathway by clarithromycin or grapefruit juice increased the exposure to oral S-ketamine by 2.6- and 3.0-fold. Unexpectedly, CYP3A4 inhibition by itraconazole caused no significant alterations in the plasma concentrations of oral S-ketamine. CYP3A4 induction by St. John´s wort or rifampicin decreased profoundly the concentrations of oral S-ketamine. However, after rifampicin, there were no significant differences in the plasma concentrations of S-ketamine when it was administered intravenously. This demonstrated that rifampicin inhibited the metabolism of S-ketamine at the intestinal level. When CYP2B6 was inhibited by ticlopidine, there was a 2.4-fold increase in the exposure of S-ketamine. These studies demonstrated that low dose oral S-ketamine is metabolized both *via* CYP3A4 and CYP2B6 pathways. The concomitant use of drugs that affect CYP3A4 or CYP2B6, during oral S-ketamine treatment, may cause clinically significant drug-drug interactions.

Keywords: S-ketamine, drug interaction, pharmacokinetics, cytochrome P450

TIIVISTELMÄ

Marko Peltoniemi

SYTOKROMI P450-ENTSYYMIEN ESTÄJIEN JA TOIMINTAA KIIHDYTTÄVIEN AINEIDEN VAIKUTUS S-KETAMIININ AINEENVAIHDUNTAAN

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Ihmiskeho eliminoi elimistölle vieraita yhdisteitä pääasiassa metaboloimalla ne munuaisten kautta helpommin eritettävään muotoon. Maksan ja suoliston sytokromi P450 (CYP)-entsyymit ovat tärkeässä roolissa lääkeainemetaboliassa. Jos niiden toiminta estyy (inhibitio) tai kiihtyy (induktio) toisen lääkkeen takia, voi tapahtua farmakokineettisiä lääkeaineyhteisvaikutuksia. Modernissa lääketieteessä usean samanaikaisen lääkkeen käyttö on tavanomaista, kun pyritään parantamaan yksittäisen hoidon tehoa tai hoitamaan montaa samanaikaista sairautta. Polyfarmasia kuitenkin altistaa potilaat haittavaikutuksille, koska lääkeainemetabolia voi CYP-entsyymien välityksellä vaihdella arvaamattomasti.

S-ketamiini on fensyklidiinin johdos, jonka vaikutus perustuu keskushermoston N-metyyli-D-aspartaatti (NMDA) -reseptorien salpaukseen. Ketamiini on ainutlaatuinen anestesialääke, koska se toimii dissosiatiivisena anesteettina suurilla annoksilla, mutta kipulääkkeenä pienillä annoksilla. Ihmisen maksasoluilla tehdyissä tutkimuksissa on havaittu, että ketamiini metaboloituu pääasiassa CYP3A4- ja CYP2B6-entsyymien välityksellä. Tässä randomisoidussa, kontrolloidussa ja vaihtovuoroisessa tutkimussarjassa selvitettiin erilaisten CYP-entsyymien toimintaa estävien ja kiihdyttävien lääkeaineiden vaikutusta suun kautta sekä laskimonsisäisesti annostellun S-ketamiinin farmakokinetiikkaan ja farmakodynamiikkaan terveillä vapaaehtoisilla koehenkilöillä. Plasman ketamiinin ja sen metaboliitin norketamiinin pitoisuudet mitattiin 24 tunnin ajan sekä farmakodynaamiset muuttujat 12 tunnin ajan jokaisessa tutkimuksessa.

Tämän tutkimussarjan tulokset osoittivat, että CYP3A-entsyymin toiminnan estäminen klaritromysiinillä tai greippimehulla lisäsivät S-ketamiinialtistusta 2,6- ja 3,0-kertaisiksi. Vastoin odotuksia, CYP3A4-entsyymin estäminen itrakonatsolilla ei kuitenkaan aiheuttanut merkittäviä muutoksia oraalisen S-ketamiinin plasmakonsentraatioihin. Sitä vastoin CYP3A4 entsyymin toimintaa kiihdyttävä mäkikuisma sekä rifampisiini vähensivät selvästi suun kautta annostellun S-ketamiinin pitoisuuksia. Rifampisiini ei kuitenkaan muuttanut merkittävästi laskimonsisäisesti annostellun S-ketamiinin plasmapitoisuuksia. Tämä todisti, että rifampisiini inhiboi S-ketamiinin metaboliaa erityisesti suolistossa. Kun CYP2B6-entsyymin aktiviteettia estettiin tiklopidiinillä, altistus S-ketamiinille nousi 2,4-kertaiseksi. Tutkimukset näyttivät, että matala-annoksinen oraalinen S-ketamiini metaboloituu sekä CYP3A4- että CYP2B6-reittien kautta. Kun oraalisen S-ketamiinihoidon aikana käytetään CYP3A tai CYP2B6 entsyymien aktiviteettiin vaikuttavia lääkkeitä, ovat merkittävät kliiniset lääkeaineyhteisvaikutukset todennäköisiä.

Avainsanat: S-ketamiini, lääkeyhteisvaikutus, farmakokinetiikka, sytokromi P450

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ABBREVIATIONS

ADR	Adverse drug reaction
ANOVA	Analysis of variance
$AUC_{0-\infty}$	Area under plasma concentration-time curve extrapolated to infinity
AUC_{0-t}	Area under plasma concentration-time curve from zero to t hours
$AUEC_{0-t}$	Area under effect-time curve from zero to t hours
AUC_m/AUC_p	Metabolite to parent drug area under plasma concentration-time curve ratio
BMI	Body mass index
BCRP	Breast cancer resistance protein
CI	Confidence interval
CL	Plasma clearance
C_{max}	Peak plasma concentration
CPT	Cold pain threshold
C_{trough}	Trough concentration of a drug
CYP	Cytochrome P450
CV	Coefficient of variation
DDI	Drug-drug interaction
DSST	Digit symbol substitution test
EDTA	Ethylenediaminetetra-acetic acid
EMA	European Medicines Agency
F	Oral bioavailability of drug
FDA	Food and Drug Administration agency
GABA	Gamma-aminobutyric acid
GFJ	Grapefruit juice
GMR	Geometric mean ratio
HPLC	High performance liquid chromatography
K_i	Inhibition constant
k_{el}	Elimination rate constant
mRNA	Messenger ribonucleic acid
NMDA	N-methyl-D-aspartate
OAT	Organic anion transporters
OATP	Organic anion transporting polypeptide
OCT	Organic cation transport
P	Probability
PCP	Phencyclidine
P-gp	P-glycoprotein
r	Correlation coefficient
SD	Standard deviation
$t_{½}$	Elimination half-life
t_{max}	Time when C_{max} occurs
V_{ss}	Steady-state volume of distribution
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-V. The original publications have been reproduced with the permission of the copyright holders.

I Hagelberg NM, Peltoniemi MA, Saari TI, Kurkinen KJ, Laine K, Neuvonen PJ, Olkkola KT. Clarithromycin, a potent inhibitor of CYP3A, greatly increases exposure to oral S-ketamine. Eur J Pain 2010; 14: 625-629.

II Peltoniemi MA, Saari TI, Hagelberg NM, Laine K, Neuvonen PJ, Olkkola KT. St. John´s wort greatly decreases the plasma concentrations of oral S-ketamine. Fundam Clin Pharmacol 2012; 26: 743-750.

III Peltoniemi MA, Saari TI, Hagelberg NM, Reponen P, Turpeinen M, Laine K, Neuvonen PJ, Olkkola KT. Exposure to oral S-ketamine is unaffected by itraconazole but greatly increased by ticlopidine. Clin Pharmacol Ther 2011; 90:296-302.

IV Peltoniemi MA, Saari TI, Hagelberg NM, Laine K, Kurkinen KJ, Neuvonen PJ, Olkkola KT. Rifampicin has a profound effect on the pharmacokinetics of oral S-ketamine and less on intravenous S-ketamine. Basic Clin Pharmacol Toxicol 2012; 111:325–332.

V Peltoniemi MA, Saari TI, Hagelberg NM, Laine K, Neuvonen PJ, Olkkola KT. S-ketamine concentrations are greatly increased by grapefruit juice. Eur J Clin Pharmacol 2012; 68:979-986.

1. INTRODUCTION

Drugs are used in combination to improve the effectiveness of therapy or to treat coexisting diseases making multiple drug therapy common in current clinical practice (Bucsa et al. 2012). However, some combinations may alter the exposure to drug or drugs with consequential undertreatment causing adverse effects. Pharmacokinetic drug-drug or drug-herb interactions can occur when one drug or herb affects the absorption, distribution, metabolism or excretion of another drug (Magro et al. 2012). These drug-drug interactions (DDIs) occur during drug metabolism. Cytochrome P450 (CYP) enzymes, in the liver and intestine, play a major role in the metabolism of drugs and other foreign compounds (Dresser et al. 2000). Parent drug and its metabolites concentration levels are dynamic. These levels are influenced by specific CYP modulating agents (Pelkonen et al. 2008). In addition, drug transporters in various tissues can modulate the absorption, excretion and extent of drug entry into liver and target organs (DeGorter et al. 2012).

PCP was first synthesized in the 1950s, but the demand of a less hallucinogenic anaesthetic and analgesic led to invention of ketamine, which is a PCP derivative and antagonizes the NMDA receptors (Domino 2010). A racemic mixture of ketamine was integrated into clinical use in 1970 as a well tolerated but potent sedative agent, which produces analgesia in low doses and anaesthesia in high doses (Sinner and Graf 2008). The S-enantiomer of ketamine (S-ketamine) is 2-4 times more potent anaesthetic and analgetic than the R-enantiomer (Arendt-Nielsen et al. 1996; Oye et al. 1992). Furthermore, S-ketamine has less psychotomimetic adverse effects yielding a practical compound for clinical use compared to the racemate (White et al. 1985). Currently, there is a renewed interest in low dose applications of S-ketamine for various acute and chronic pain conditions (Bell et al. 2003; Bell et al. 2005; Bell 2009).

Ketamine is N-demethylated by CYP3A4 and CYP2B6 enzymes into its major metabolite, norketamine (Hijazi and Boulieu 2002; Yanagihara et al. 2001). Oral usage of ketamine limits the bioavailability of ketamine to approximately 17-24% in humans (Clements et al. 1982; Chong et al. 2009). Because of its oxidative and extensive first-pass metabolism, ketamine is prone to pharmacokinetic drug-drug interactions. There are no previous systematic *in vivo* DDI studies of ketamine, but low concentrations of ketamine and high ketamine metabolite concentrations appear after concomitant diazepam, secobarbital or barbiturate use (Lo and Cumming 1975; Idvall et al. 1983; Koppel et al. 1990). Because the long term use of low dose S-ketamine is increasing among chronic pain patients, it is important to examine the effects of different CYP inducers and inhibitors on the pharmacokinetics of S-ketamine.

2. REVIEW OF THE LITERATURE

2.1. Drug metabolism

Xenobiotics are foreign compounds that include drugs, dietary supplements, and food additives. Some drugs are administered intravascularly but many compounds are able to enter systemic blood stream *via* extravascular tissues. Drugs can be administered by oral, intramuscular, subcutaneous, intradermal, buccal, sublingual, pulmonary (inhalation) or rectal route. The oral route of administration is preferred because it is the most natural and convenient. Orally administered drugs are exposed to enteric metabolism before entering the liver, which is the principal site of metabolism (Krishna and Klotz 1994). The absorption of xenobiotics from the intestinal lumen to portal circulation can be altered by different efflux and uptake transporter proteins, polypeptides and enzymes located in the intestinal enterocyte cells (Glaeser et al. 2007; Nishimuta et al. 2007; Hanley et al. 2011).

Elimination of xenobiotics occurs by metabolism and excretion. Few lipophilic drugs may be directly excreted to the bile or urine. In general, convertion of compounds to hydrophilic forms facilitates effective excretion (Rowland and Tozer 2011). Metabolism transpires in the liver but it can also occur in many other tissues such as in the lungs, blood, brain, kidneys and especially in the gastrointestinal wall. These metabolic reactions include functionalization (*e.g.* oxidation, hydrolysis, reduction) and conjugation. In functionalization, a functional group is added or an existing part is modified whereas in conjugation, an endogenous molecule such as methyl group, sulphate, glucuronic acid, or glutathione is attached to an eligable functional group making the molecule more hydrophilic (Testa and Kramer 2008). Usually these biotransformations occur simultaneously by several competing pathways, although functionalization is often the initial step. Alternatively, some drugs are directly conjugated. Metabolites, after functionalization, may be further metabolized. Elimination of metabolites is therefore a complex process.

Usually metabolites are pharmacologically less active than their parent drug (Sinner and Graf 2008). Some drugs are prodrugs such as codeine (Tirkkonen and Laine 2004), tramadol (Poulsen et al. 1996) and tamoxifen (Wu et al. 2012). Prodrugs are converted to active forms by metabolic processes. Codeine is demethylated to its active form, morphine, tramadol to *trans*-O-desmethyltramadol and tamoxifen to its active metabolite, endoxifen by CYP2D6 (Tirkkonen and Laine 2004; Wu et al. 2012).

2.2. Cytochrome P450 (CYP) enzyme family

Cytochrome P450 enzymes are haemoproteins coded by 57 different genes. These proteins are very diverse at the individual level due to pharmacogenetic variance. This ancestral cytochrome exists in bacteria, plants, fungi, animals and man (Nelson et al. 1996). This enzyme group received its name in 1962, because a spectral peak of its chromophore occurred at 450 nm (Omura and Sato 1962). CYP enzymes have a pivotal role in the metabolism of xenobiotics and the biosynthesis of endogenous compounds (*e.g.* cholesterol, bile acid, steroids, vitamin D_3 and retinoic acid) (Nebert and Russell 2002).

CYP enzymes exist in almost all human tissues. The systemic metabolic clearance of drugs transpires in the endoplasmic reticulum of hepatocytes and in intestinal wall enterocytes. The proximal small intestine acts as a major site for presystemic drug metabolism (Paine et al. 2006). It is estimated that CYPs can be involved in over 50-75% of all drug metabolic reactions (Nebert and Russell 2002; Guengerich 2008). Many gene polymorphisms associate with individual CYP enzymatic functions. Yet, only ten human CYP isoforms catalyze the paramount CYP-mediated reactions involved with oxidation and reduction. In the oxidation reaction, a hydroxyl (OH^-) group is formulated from the parent drug and water is generated as by-product. At the molecular level, oxidation is the loss of electrons whereas reduction is the gain of electrons. CYPs main function is to be a monooxygenase and cleave oxygen molecule from the substrate and add one oxygen atom into the drug (D) as follows:

$$O_2 + DH + 2e^- + 2H^+ \rightarrow DOH + H_2O$$

or

$$O_2 + D + 2e^- + 2H^+ \rightarrow DO + H_2O$$

Usually xenobiotics are converted to more water soluble alcohols, phenols, aldehydes and ketones. There are other enzymes than CYPs (*e.g.* flavin-containing monooxygenases and oxidoreductases) that interact with CYPs. Their action is limited because they react with specific compounds contrary to CYPs, which have a vast amount of substrates (Testa and Kramer 2007).

CYP enzymes are arranged into 18 families and 42 subfamilies. To be in the same family (Arabic numeral), enzymes must have over 40% similarity in their amino acid sequence, whereas over 55% identity justifies classification into the same subfamily designated by a letter from A through E, *e.g.* CYP3A (Nebert and Russell 2002). In humans, CYP1 to 3 are the main families that metabolize xenobiotics. Pharmacogenetic heterogeneity exists in various CYPs, which leads to a variance in the drug response because of genetic allelic variants. Most drugs modified by the CYP system are metabolized by two or more CYP

forms. CYP3A4, CYP2D6, CYP2C19, CYP2C9 and CYP1A2 are responsible for the majority of drug metabolism in humans (Wienkers and Heath 2005).

2.2.1. CYP3 gene family

CYP3A function is the sum activity of the family of CYP3A genes. The CYP3 family contains one subfamily (CYP3A) and is the most important CYP subfamily because they are capable of metabolizing 50% of all therapeutic drugs (Wilkinson 2005; Ingelman-Sundberg et al. 2007). CYP3A is the most common P450 enzyme in the human liver and intestine (Figure 1). There are only four members of CYP3A in humans: CYP3A4, CYP3A5, CYP3A7 and CYP3A43 (Gibson et al. 2002). Traditionally, CYP3A7 was considered to exist only in fetal liver but recent reports shows that it exists in 10-20% of all adult livers. The clinical role on CYP3A7 in drug metabolism is still unclear (Koch et al. 2002; Sim et al. 2005; Pelkonen et al. 2008). Most probably CYP3A43 protein has no clinically significant role in drug metabolism, because its expression level in the liver is very low (Westlind et al. 2001).

CYP3A4

CYP3A4 is the major metabolic enzyme in small intestine and liver. In the gut, it acts with several ingested drugs before they enter the liver thus contributes significantly to first-pass metabolism (Paine et al. 2006). It is the best studied CYP3A enzyme in humans and have remarkable interindividual variability in its expression due to many allelic variants (Lown et al. 1994; Shimada et al. 1994). CYP3A4 transcription is activated during the first week after birth with a concomitant decrease in the expression of fetal CYP3A7 (Lacroix et al. 1997). Kinetic interactions among CYP3A4 and its substrates is complex. The active site of CYP3A4 permits one or multiple molecules to be in contact within this large pocket simultaneously in relatively unselective way making the prediction of CYP3A4-mediated drug-drug interactions problematic (Ekins et al. 2003; Pelkonen et al. 2008). Several clinically important drugs, such as midazolam, alfentanil, simvastatin, atorvastatin, quinidine, cyclosporine A, erythromycin, nifedipine and felodipine are substrates of CYP3A4 (Pelkonen et al. 2008). In addition, studies with human liver microsomes reveal the important role of CYP3A4 in the metabolism of ketamine (Hijazi and Boulieu 2002). The most well-known inhibitors of CYP3A4 include all azole antifungals (Venkatakrishnan et al. 2000), clarithromycin (Yeates et al. 1996), erythromycin (Olkkola et al. 1993), diltiazem, (Zhang et al. 2009) and GFJ (Pelkonen et al. 2008). Some drugs can increase CYP3A activity through transcriptional activation such as St. John´s wort, the antibiotic, rifampicin, steroid dexamethasone. Antiepileptic drugs, phenytoin and carbamazepine, are important inducers of CYP3A4. They can enhance the metabolism of many drugs and reduce their therapeutic efficacy (Liu et al. 2007).

CYP3A5

The role of CYP3A5 in overall drug metabolism is unclear. It is expressed rarely. It can be found in 10 - 20% of all human livers and in the adrenal gland, prostate and kidney, CYP3A5 quantities are unknown (Koch et al. 2002; Westlind-Johnsson et al. 2003; Daly 2006). High levels of metabolically active CYP3A5 enzymes are found in approximately 30% of Caucasians (Europeans), 30% of Japanese, 40% of Chinese and more than 50% of African Americans due to a polymorphic distribution of the CYP3A5*1 allele. Furthermore, the frequency of enhanced 3A5 activity among Finnish people is variable due to common homozygous CYP3A5*1 allele (Hilli et al. 2007). Polymorphisms may explain interindividual variability in the clearance of drugs inactivated by CYP3A (Kuehl et al. 2001). A clear association exists between the CYP3A5 genotype and exposure of the immunosuppressive agent tacrolimus in several studies (Barry and Levine 2010).

2.2.2. CYP2 gene family

CYP2 family is the largest and most diverse P450 enzyme group, in humans, containing 5 families and 13 subfamilies. CYP2D6 is involved in the metabolism of more than 75 drugs. Furthermore, CYP2C8, CYP2C9, CYP2C18 and CYP2C19 collaborate to metabolize over half of all prescribed drugs (Nebert and Russell 2002). The important role that CYP2B6 plays in drug elimination was elucidated only a few years ago (Turpeinen et al. 2006). CYP genes have a polymorphic nature including CYP2A6, CYP2B6, CYP2C9, CYP2C19 and CYP2D6 (Ingelman-Sundberg et al. 2007).

CYP2D6

CYP2D6 is the most studied CYP enzyme. It plays an important role in drug metabolism and has a wide variability in its enzymatic activity among individuals due to genetic polymorphism (Pelkonen et al. 2008). Currently, there are 78 different functional polymorphic CYP2D6 alleles (www.cypalleles.ki.se). Approximately 20-25% of drugs in clinical use are metabolized by CYP2D6 (Evans and Relling 1999; Ingelman-Sundberg 2005; Eichelbaum et al. 2006). Different CYP2D6 alleles have ultra-rapid, normal, decreased, and even null enzyme activity predisposing populations to drastic alterations in metabolism and in drug concentrations at individual levels (Ingelman-Sundberg et al. 2007). Poor metabolizers (5-10% of all caucasians) are mainly found in Europe and ultrarapid metabolizers are population's indigineous to North Africa and Oceania. Asian people are typically intermediate metabolizers *via* CYP2D6 (Ingelman-Sundberg 2005). However, ethnically tailored drug therapies are difficult to achieve because of population admixture and the fact that there are only few region-specific haplotypes (Sistonen et al. 2007). Typical CYP2D6 substrates are beta-blockers (*e.g.*

metoprolol, carvedilol, propranolol and timolol), tricyclic antidepressants (*e.g.* amitriptyline, nortriptyline, clomipramine and imipramine), antipsychotic agents (*e.g.* fluoxetine, haloperidol and paroxetine), opioids (*e.g.* codeine and dextromethorphan) and some antiarrhythmic agents (*e.g.* flecainide, mexiletine and propafenone). CYP2D6 genotype-based dosage recommendations have been suggested for substrates such as warfarin, proton pump inhibitors and some antidepressants (Kirchheiner et al. 2001; Eichelbaum et al. 2006). Well-known inhibitors of CYP2D6 include fluoxetine, haloperidol and paroxetine (Wilkinson 2005).

CYP2B6

CYP2B6 is mainly expressed in the liver and represents 1-10% (Figure 1) of the total hepatic CYP content (Turpeinen et al. 2006). However, total human liver microsomal CYP2B6 content can vary up to 100-fold (Gervot et al. 1999; Lamba et al. 2003; Zanger et al. 2007). It can contribute to a broad range of drug metabolism and procarcinogen activation reactions (Code et al. 1997). Due to its inherent polymorphisms, there is considerable interindividual variability in the hepatic levels of mRNA of CYP2B6 and its corresponding protein (Yamano et al. 1989; Shimada et al. 1994). In fact, CYP2B6 is one of the most polymorphic CYP genes, in humans, having over 100 defined single nucleotide polymorphisms and 28 different alleles (Zanger et al. 2007). CYP2B6 gene is regulated apparently by pregnane X and constitutive androstane receptors, which can be induced by several drugs such as phenobarbital and rifampicin (Goodwin et al. 2001; Wang and Negishi 2003; Turpeinen et al. 2006). Well-known substrates of CYP2B6 are bupropion, propofol and ketamine (Turpeinen et al. 2006). Ticlopidine, clopidogrel and thiotepa are known inhibitors of CYP2B6 (Rae et al. 2002; Richter et al. 2004; Turpeinen et al. 2006). Especially, ticlopidine is a relatively selective CYP2B6 inhibitor *in vitro* and *in vivo* (Turpeinen et al. 2005).

2.2.3. CYP1 gene family

CYP1A2 is the only member of the CYP1 family to be expressed in the liver (Figure 1). Two other members of the CYP1 gene family are CYP1B1 and CYP1A1, which are expressed in many other human tissues. They can effectively metabolize polycyclic aromatic hydrocarbons (PAHs) rather than clinical drugs (Nebert and Russell 2002; Ding and Kaminsky 2003). CYP1 family members are connected to chemical carcinogenesis. High CYP1B1 expression is found in some specific tumors (Boobis et al. 1994; Murray et al. 1997). In addition, CYP1B1 associates with congenital glaucoma (Vasiliou and Gonzalez 2008).

CYP1A2

CYP1A2 metabolizes about 10-20 different drugs in the liver. Consistent with other CYP1 family members, it is expressed also in the lungs (Wei et al. 2002). There is obvious interindividual alteration in CYP1A2 activity, although no clear polymorphisms are known (Ingelman-Sundberg et al. 2007). Caffeine and melatonin are probes used to study individual CYP1A2 phenotypes (Faber et al. 2005). Other CYP1A2 substrates are theophylline, clozapine and tizanidine (Laine et al. 1999; Pelkonen et al. 2008). Activity of CYP1A2 can be inhibited by fluvoxamine, ciprofloxacin and some hormone replacement therapy drugs, such as estradiol and levonorgestrel (Laine et al. 1999; Pelkonen et al. 2008).

2.2.4. Orphan proteins

At present, genomic sequences are categorized quicker than the functionality of these genes can be determined. One-fourth of the human CYP proteins are not characterized in regards to their cellular or clinical function and they are called "orphans" (Guengerich et al. 2010). Some orphans are capable of oxidations of carcinogens, retinoic acid and fatty acids, but the clinical significance of these reactions are not clear (Capdevila et al. 1996).

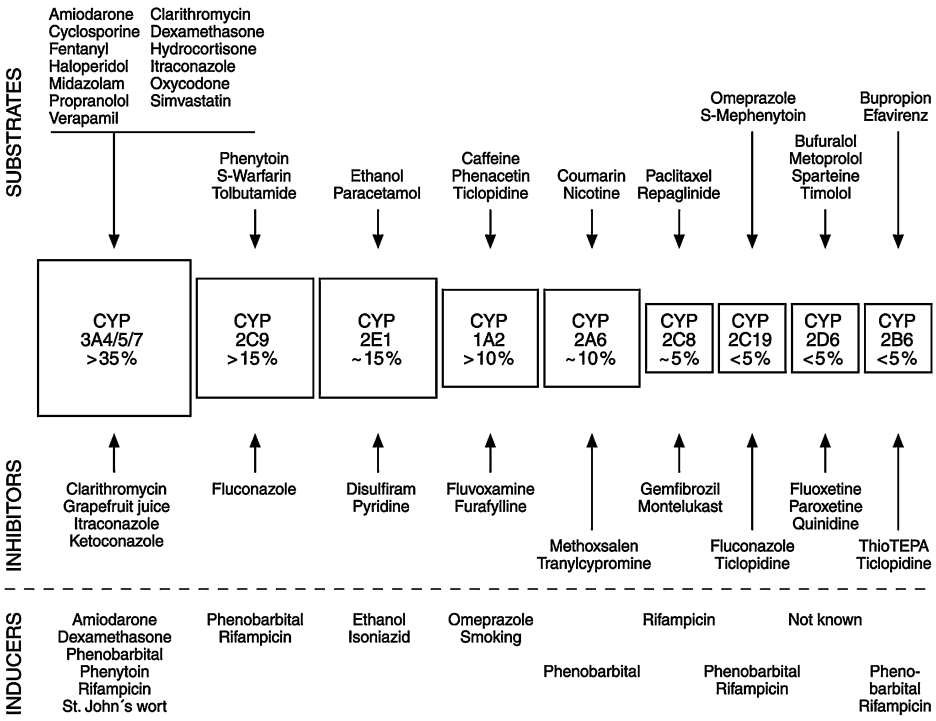

Figure 1. Relative abundance of different forms of cytochrome P450 enzymes in the human liver and some examples of substrates, inhibitors and inducers. Modified from Pelkonen et al. 2008.

2.3. Mechanism of CYP inhibition

The inhibition of CYP substrate metabolism occurs during substrate binding specifically during the binding of molecular oxygen to the ferrous (Fe^{2+}) atom in the substrate. The transfer of activated oxygen from the heme iron in the substrate is positioned at a vulnerable phase in the inhibition cascade (Hollenberg 2002). This inhibition of metabolism of one drug by another drug may cause considerable elevations in the exposure of one or both drugs which puts the patient at risk to serious adverse effects. However, CYP inhibitors may cause a decrease in the concentrations of the active metabolite when interacting with prodrugs, such as clopidogrel, causing an inactivation of the drug (Norgard et al. 2009). The onset of inhibition usually occurs relatively fast compared to induction. Generally, CYP inhibition is divided into reversible, quasi-irreversible and irreversible interactions. The nature of the substrate binding to the iron of the heme is a complex mechanism (Lin and Lu 1998).

Irreversible inhibition (mechanism-based inhibition) is relatively rare. Clinically important drugs that can inhibit CYP irreversibly include the antibacterial agent clarithromycin (CYP3A4), the sex steroid, gestodene (CYP3A4) and the antidepressant, paroxetine (CYP2D6) (Bertelsen et al. 2003; Zhou et al. 2005). Oxygenated, reactive intermediates form complexes with CYP or adhere with covalent binding to the CYP protein or heme. Consequently, the native catalytically active enzyme becomes permanently inactive. Irreversible inhibition is long lasting because new catalytically active enzymes must be *de novo* synthesized. Quasi-irreversible inhibition is reversible *in vitro*, but *in vivo,* the inhibitor-CYP complex is stable enough to be irreversibly inhibited (Lin and Lu 1998).

When inhibition is reversible, the inhibitor binds to the CYP enzyme only after competition with the substrate and weak bonds form and do not permanently inhibit the enzyme's activity (Lin and Lu 1998; Hollenberg 2002; Pelkonen et al. 2008). Reversible inhibition can be further divided into competitive, non-competitive, uncompetitive, and mixed-type inhibition (Pelkonen et al. 2008). In competitive inhibition (the most common type), the substrate and the inhibitor competes with equal chance for the same position on the active site of CYP. Inhibition can be overcome by raising the concentration of the substrate. In the non-competitive mode of inhibition, the binding site of the inhibitor and substrate is different from each other and the inhibitor has no direct effect on substrate binding. However, the formed inhibitor-substrate-enzyme complex is dysfunctional. In uncompetitive inhibition, the inhibitor binds to the enzyme-substrate complex, instead of the free enzyme alone, making this whole complex inert. Uncompetitive inhibition is seldom seen in drug metabolism. In

the case of mixed-type inhibition, components of both competitive and noncompetetive inhibitions occur (Lin and Lu 1998; Hollenberg 2002).

2.4. Mechanism of CYP induction

The human body has the ability to reduce environmental xenobiotic pressure by accelerating the function of specific drug-metabolizing enzymes. Increased CYP enzyme synthesis is mediated by ligand-activated transcription factors and receptors such as the aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR) and constitutive androstane receptor (Pelkonen et al. 2008). In addition to enhanced transcription of the CYP gene, the concentrations of intracellular CYPs may be elevated by a decreased rate of protein degradation (Hollenberg 2002). In some cases, non-receptor-mediated induction processes may be involved. Many receptors that mediate CYP enzyme transcription are presently unknown (Zhu 2010). Maximal induction is a gradual process and these levels may be reached after a few days to two weeks. Typical, inducible CYP enzymes include CYP1A1, 1A2, 2A6, 2C9, 2C19, 2E1 and 3A4. In humans, the antimicrobial agent, rifampicin, is a potent enzyme inducers (Kanebratt et al. 2008).

2.5. Drug-drug and food-drug interactions

Five-to-twenty percent of serious ADRs needing hospital care are caused by DDIs (Kongkaew et al. 2008; Astrand 2009). ADRs which cause hospital admissions lead to a prolonged length of stay and almost a two-fold increased risk of death occurs (Classen et al. 1997; Astrand 2009). Because CYP enzyme inhibitors usually cause an increased drug effect, reversible inhibitors may cause the majority (62%) of potent *in vivo* DDIs (Isoherranen et al. 2009). Similarly, CYP inhibitors, with prodrugs, may reduce the concentrations of active metabolites and impair clinical drug effects. By contrast, CYP inducers can cause subtherapeutic levels of drug plasma concentrations. In DDIs, drugs having a narrow therapeutic window may cause more serious ADRs.

CYP-mediated interactions in the liver, intestine and other tissues have a crucial role in the pharmacokinetics of DDIs, but other mechanisms are also involved. In addition, any inhibition or induction affecting drug absorption, distribution and elimination may alter the concentrations of substrate drug. For example, GFJ inactivates intestinal CYP3A4 metabolism and augments the oral bioavailability of felodipine and nifedipine (Bailey et al. 1991; Dresser et al. 2002). Intestinal influx (into the cell) and efflux (out of the cell) transporter proteins, such as P-gp and OATP, are increasingly being recognized as important determinants of drug movement from the gut lumen to portal circulation. Thus, xenobiotics affecting the function of transporter proteins may

alter the oral bioavailability of many drugs *e.g.* cyclosporine, digoxin, erythromycin and many chemotherapeutic agents (Kim 2000).

2.6. Prediction and investigation of drug-drug interactions

Many pharmacodynamic DDIs can be predicted based on a drug's pharmacodynamic profile and clinical pharmacokinetic interaction studies with humans provide validation. The interest in animal models mimicing *in vivo* interactions, based on *in vitro* data, is increasing. Recently, quantitative prediction of DDIs using K_i values derived from *in vitro* experiments was investigated (Ito et al. 1998; Tachibana et al. 2009). Considerable uncertainty exists when trying to determine interaction mechanisms for a specific inhibitor/inducer/CYP combination (Lutz and Isoherranen 2012). When predicting complicated DDIs involving, for example, multiple inhibitors, many simplifications must be made (Lutz and Isoherranen 2012). Extrapolation of *in vitro* data to clinical practice is problematic. Using *in vitro* studies, inhibitor effects on CYP metabolism can be totally different from that *in vivo*. Furthermore, extensive first pass metabolism, active metabolites and the role of transport proteins *in vivo* make the prediction of DDIs more complex (Bachmann 2006). Therefore, clinical DDI-studies are warranted to determine the accurate effects of interactions among two or more drugs.

Before marketing, authorization approval by the EMA and FDA of the new drugs must be tested for their potential to cause DDIs (EMA 2010). Additional post-marketing evaluation and safety studies may be required. In the last decade, increased knowledge in the areas of enzyme induction has increased, but even more studies are needed to fully understand the clinically relevant DDIs affecting drug transport system. In humans, due to notable differences among species, pharmacokinetic interaction studies should generally be performed. As stated before, the characterization of the major enzymes involved in the metabolism of specific drugs is practical to initiate *in vitro* studies using human liver microsomes, hepatocytes, or other cells expressing the investigated enzymes and transporters (EMA 2010).

Clinical studies are composed of multiple investigations where individuals are exposed to both experimental CYP drugs and validated CYP inhibitors and inducers such as rifampicin, ticlopidine, itraconazole and clarithromycin. As with CYPs, the evaluation of the role of the active transporter proteins are examined similiarly beginning with *in vitro* studies with and without inhibitors which proceed to *in vivo* studies. Presently, clinically relevant transporters including: P-gp, OATP1B1, OATP1B3, OCT2, OCT1, OAT3 and BCRP (EMA 2010, Grandvuinet et al. 2012). *In vitro*, transporter and metabolism

using investigational drugs should be performed over a physiological concentration range (EMA 2010).

2.7. S-ketamine

2.7.1. History and basic pharmacology

Ketamine [2-(2-chlorophenyl)-2-(methylamino)-cyclohexanone] is a phencyclidine derivative (Figure 2). Ketamine was first synthesized in 1962 by Calvin Stevens. Ketamine was introduced into clinical use as a well tolerated and potent sedative agent in 1970 and was used during war-time as an anaesthetic drug (Sinner and Graf 2008). Ketamine is a unique drug because it has hypnotic, analgesic and amnesic effects and it produces "dissociative anaesthesia" in high doses and analgesia in low doses. The ketamine molecule is a chiral structure consisting of two optical enantiomers, S(+)-ketamine and R(-)-ketamine. The different anesthetic potencies of the two enantiomers were known in 1970, but it was not until 1992 when the FDA required that optical isomers of drugs must be studied separately. This led to a renewed interest in S-ketamine. In Finland, S-ketamine gained marketing authorization in 2000 under the brand name Ketanest-S (Pfizer). Ketamine is lipid-soluble and its dissociation constant is near physiologic pH, which makes it easy to administer *via* various routes. It rapidly crosses the blood-brain barrier.

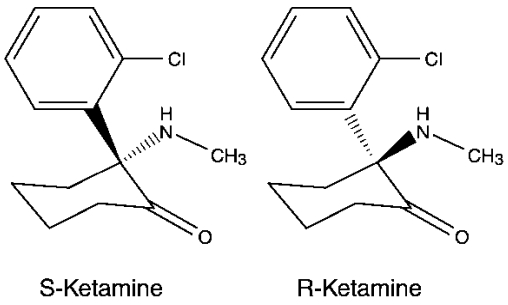

Figure 2. Optical isomers of ketamine.

2.7.2. Pharmacokinetics

Bioavailability of ketamine after oral dose is 17-24% and after intranasal dose 25-50%. Following intramuscular dose, the bioavailability of ketamine is approximately 93% (Grant et al. 1981; Clements et al. 1982; Yanagihara et al. 2003; Sinner and Graf 2008; Chong et al. 2009). After entering systemic circulation, ketamine is rapidly distributed into the brain and other well perfused tissues. After oral administration, peak plasma concentrations are reached in 30 minutes (Grant et al. 1981; Yanagihara et al. 2003; Chong et al. 2009). Both isomers are characterized by a short α half-life (2-4 minutes) and longer β half-life (8-16 minutes). The α half-life reflects the rate of decline of

plasma concentrations of a drug due to its redistribution from the central to the peripheral compartment. β half-life reflects the elimination of a drug *via* metabolism (Teboul and Chouinard 1990). The "dissociative anaesthetic" state is terminated due to redistribution, followed by hepatic and renal elimination with a half-life of 2-3 hours. Plasma protein binding of ketamine is 12% (Hijazi et al. 2003; Sinner and Graf 2008). There are no stereoselective differences in the N-demethylase activities of ketamine enantiomers in human liver microsomes (Sinner and Graf 2008; White et al. 1985). R-ketamine is not produced after administration of S-ketamine, in human volunteers, *i.e.* interconversion does not exist (Ihmsen et al. 2001). However, R-ketamine inhibits the metabolism of S-ketamine *in vitro* and *in vivo*. The clearance of S-ketamine is delayed in the racemate compared to the pure S-isomer (Kharasch et al. 1992; Geisslinger et al. 1993; Ihmsen et al. 2001). The underlying mechanism for the reduction of the elimination of S-ketamine in the racemate is not clear.

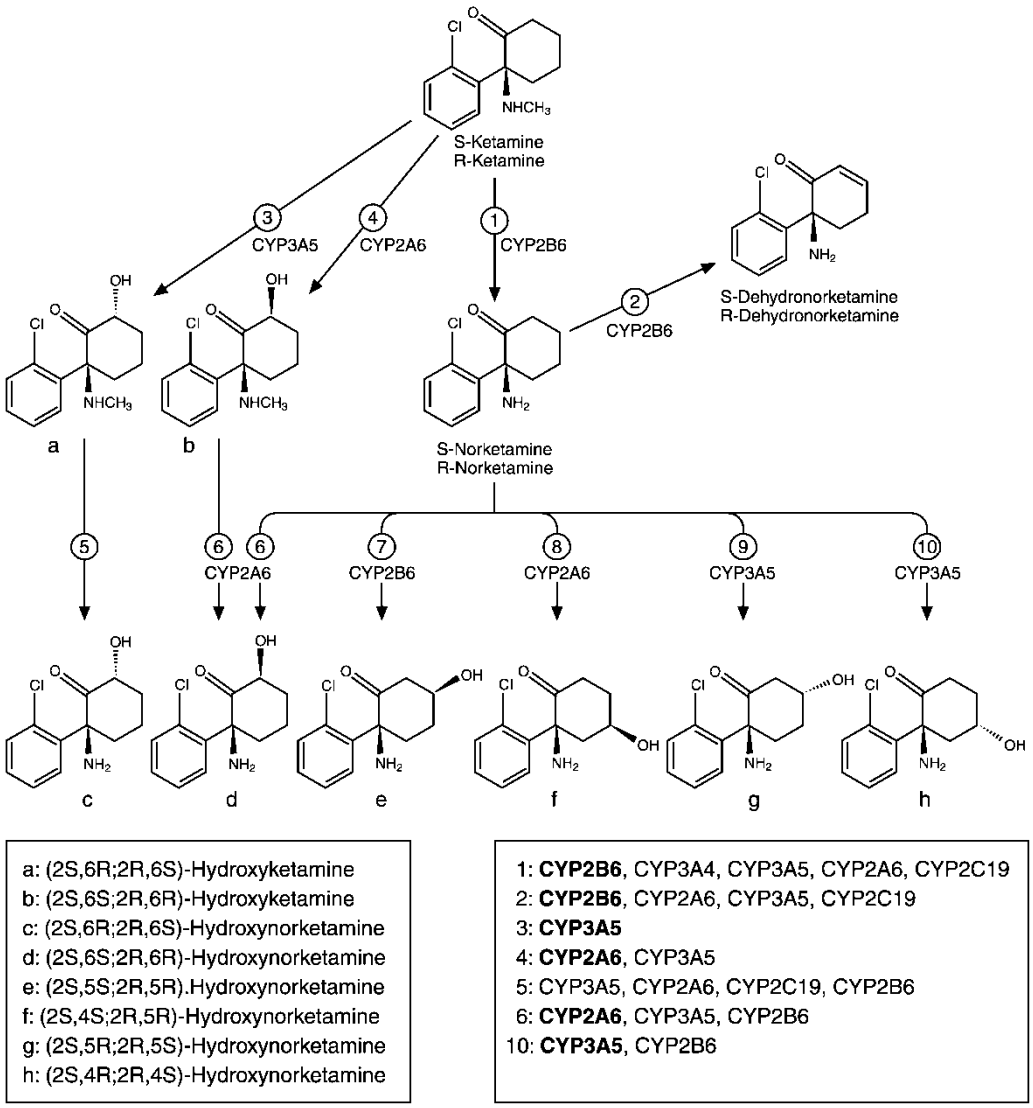

Figure 3. The metabolism of ketamine and norketamine enantiomers. Modified from Williams et al. 2004.

2.7.3. Metabolism

In vitro, ketamine (Figure 3) is extensively N-demethylated by CYP3A4 and to a lesser extent by CYP2B6 and CYP2C9 isoforms into S-norketamine, R-norketamine, 4-hydroxy-ketamine and 6-hydroxy-ketamine (Woolf and Adams 1987; Hijazi and Boulieu 2002; Yanagihara et al. 2003). Norketamine (Figure 3) is considered to be the major metabolite, in humans, being also a substrate for further metabolism to 5-hydroxy-norketamine (Woolf and Adams 1987). Norketamine and other metabolites are excreted renally and only minimal

quantities of the parent ketamine is excreted unmodified (Geisslinger et al. 1993).

Ketamine N-demethylation, *in vitro*, is biphasic and CYP3A4 and CYP2B6 mediates this process (Hijazi and Boulieu 2002). In experimental models with human liver microsomes, the action of CYP2B6 increases as ketamine concentrations decreases (Yanagihara et al. 2001; Hijazi and Boulieu 2002). The inhibition of CYP3A with different concentrations of ketoconazole inhibits the N-demethylation pathway of ketamine by 40-65% (Yanagihara et al. 2001; Hijazi and Boulieu 2002). In addition, orphenadrine, an inhibitor of CYP2B6, decreases norketamine formation by 20-67% (Yanagihara et al. 2001; Hijazi and Boulieu 2002). Also medetomidine, which inhibits the activity of a broad number of CYP enzymes, is capable to inhibit the metabolism of ketamine (Kharasch et al. 1992). Phenacetin, a CYP1A2 inhibitor, and coumarin, a CYP2A6 inhibitor, produces minor inhibition of norketamine formation (Hijazi and Boulieu 2002). *In vitro* studies demonstrate that both CYP3A and CYP2B6 have a role in the metabolization of ketamine (Yanagihara et al. 2001; Hijazi and Boulieu 2002). Still, systematic *in vivo* studies are lacking. Chronic barbiturate users have lower than expected plasma ketamine levels due to enzymatic induction in the liver (Koppel et al. 1990).

2.7.4. Pharmacodynamics

S-ketamine is 2-4 times a more potent anaesthetic and analgetic than the R-isomer (White et al. 1980; Oye et al. 1992; Arendt-Nielsen et al. 1996). Both enantiomers have different affinities to the receptors, which are also optically active. Ketamine, in contrast to many other anaesthetics, has no affinity for $GABA_A$ receptors in the human brain at subanaesthetic doses (Salmi et al. 2005). The $GABA_A$ receptor is the most abundant inhibitory neurotransmitter receptor in the human central nervous system (Garcia et al. 2010). The S-isomer has a higher affinity for the NMDA receptor and lower doses are required compared to racemic ketamine. In addition, fewer adverse effects in central nervous system (CNS) occur with S-ketamine compared to racemate (White et al. 1985). Ketamine acts on a variety of receptors, *e.g.* NMDA, muscarinic, nicotinic, dopaminergic, serotonergic and different voltage-gated receptors (Scheller et al. 1996; Sinner and Graf 2008). Part of the analgesic effect of ketamine in animals are derived from the agonism of opioid receptors (Smith et al. 1987; Finck et al. 1988; Hustveit et al. 1995). However, naloxone, a potent opioid antagonist, had no effects on the action of ketamine in humans (Mikkelsen et al. 1999).

Noncompetitive antagonism of NDMA receptors by ketamine produce a primary anaesthetic and analgesic effect. NMDA receptors are located both in

the brain and the spinal cord. They are triggered by excitatory amino acids such as glutamate and glycine. The activation of the NMDA receptors leads to influx of Ca^{2+}, which activates intracellular nitric oxide synthase and formation of nitric oxide (Sinner and Graf 2008). Nitric oxide (NO) plays a crucial role in the nociception and neurotoxicity (Fan et al. 2012). Ketamine blocks the NMDA receptor and reduces the channel mean open time and frequency and prevents Ca^{2+}-influx (Orser et al. 1997). Central sensitization is considered to be the main factor for the development and maintenance of chronic pain (Pelissier et al. 2003). When spinal nociceptive neurons are exposed to repetitive C-fiber stimulation, they increase their spontaneous activity and receptive fields. This wind-up phenomen is related to the enhanced activity of NMDA receptors and blockade of receptors by ketamine may prevent the pain to become chronic (Bennett 2000).

Norketamine binds to the PCP site on the NMDA-receptor complex and acts as a noncompetitive antagonist. However, the affinity of norketamine to receptor is much lower than that of ketamine (Ebert et al. 1997). In animal models, S-norketamine has antinociceptive properties with the potency of one-fifth to one-third of ketamine (Ebert et al. 1997; Holtman et al. 2008).

2.7.5. Clinical use

Because ketamine acts *via* several different receptors, the potential applicable clinical spectrum is wide. An intravenous dose producing general anaesthesia is 1-2 mg/kg for racemic ketamine and 0.5-1 mg/kg for S-ketamine (Sinner and Graf 2008). However, low doses of ketamine are sufficient for analgesic, anti-hyperalgesic or opioid sparing effects (Schmid et al. 1999). For analgesia, typical intravenous doses of S-ketamine range from 0.125 mg/kg to 0.25 mg/kg (Sinner and Graf 2008).

Anaesthesia and analgosedation

Ketamine is mainly used as an anaesthetic by intravenous injection, intravenous infusion or intramuscular injection. It is a rapidly acting general anaesthetic which produces “dissociative anesthesia.” The patient falls into a trance-like state and their eyes may remain open. Psychotomimetic adverse effects in CNS, such as vivid dreams, hallucinations and changes in perception can be blunted by co-application of sedatives or hypnotics (*e.g.* propofol or benzodiazepines) (Sinner and Graf 2008). Ketamine is an appropriate anaesthetic for haemodynamically unstable patients because it preserves cardiac output *via* central sympathetic stimulation and inhibition of neuronal catecholamine uptake (Sinner and Graf 2008). A major advantage in using ketamine as a field anaesthetic is that it preserves protective pharyngeal and

laryngeal reflexes without depressing respiration (Green and Krauss 2004). Besides being an analgosedative, ketamine has favorable effects on cardiovascular and pulmonary parameters, making it an attractive option for the maintenance of sedation in intensive care units (Miller et al. 2011). Furthermore, in contrast to opioids, ketamine has no negative impact on gut motility (Freye et al. 1998).

Pain therapy

Low dose ketamine can be advantageous in diverse clinical settings related to pain. In an extensive review (Bell et al. 2006), low dose ketamine was concluded to reduce postoperative pain, curtailing postoperative morphine requirements and postoperative nausea and vomiting. Analgesia may persist throughout the recovery period by endogenous modulation of nociceptive stimuli. A recent meta-analysis shows the poor efficacy for the preemptive effects of intravenous ketamine in the setting of acute postoperative pain (Ong et al. 2005). Besides perioperative pain, ketamine is frequently utilized for analgesia in chronic pain, *e.g.* central pain, complex regional pain syndromes, fibromyalgia, orofacial pain, postherpetic neuralgia and in combination with opioids in cancer related pain (Eide et al. 1994; Mathisen et al. 1995; Sorensen et al. 1997; Mercadante et al. 2000; Bell et al. 2003; Sigtermans et al. 2009b). Long-term use of ketamine for chronic pain is limited because of safety concerns.

Other indications, adverse effects and abuse

Recent clinical studies show the rapid-acting antidepressant effects of ketamine. This mechanism may act through the modulation of the glutamate system by NMDA antagonism in patients suffering from treatment-resistant depression (Murrough 2012). A single low dose of ketamine may last up to ten days (Larkin and Beautrais 2011). In addition, ketamine is an effective and safe choice as an anesthetic agent during electroconvulsive therapy (ECT) in the treatment of therapy-resistant depression. Ketamine has synergistic antidepressant effects with ECT (Kranaster et al. 2011). Theoretically, ketamine may reduce bronchoconstriction during severely exacerbated asthma, but the clinical relevance is unclear (Lau and Zed 2001). The expression of NMDA receptors increases in prolonged epilepticus. Some case reports claim that ketamine is efficacious at reducing the amplitude and duration of seizures (Kranaster et al. 2011; Fernandez and Claassen 2012).

Ketamine produces hemodynamically stable anaesthesia by increasing blood pressure and heart rate *via* sympathetic activation. Ketamine also preserves

respiratory activity and protects airway reflexes making lethal overdose unlikely. The most problematic adverse effects are psychotomimetic reactions (www.medicines.org.uk/emc/medicine) varying from slight nightmares and blurred vision to delirium, psychosis and paranoia (Table 1). However, sedative medications are effective in preventing and managing these symptoms. Other inconvenient, but clinically minor adverse effects are hypersalivation, which can be treated by concomitant anticholinergic administration (Brown et al. 2008). Chronic consumption of ketamine is linked to severe and persistent urinary diseases (Winstock et al. 2012).

Ketamine is defined as a class C drug in the classification of controlled drugs in the United Kingdom, where class A represents the most dangerous drugs. As a party drug, ketamine can produce euphoria and dream-like hallucinations in a dose dependent manner (Winstock and Mitcheson 2012).

Table 1. Adverse effects of ketamine

Adverse events	Frequency	Undesirable Effects
Psychiatric disorders	Common	Hallucination, Abnormal dreams, Nightmare, Confusion, Agitation
	Rare	Delirium, Flashback
Nervous system disorders	Common	Nystagmus, Hypertonia, Tonic clonic movements
Eye disorders	Common	Diplopia
Cardiac disorders	Common	Blood pressure increased, Heart rate increased
Respiratory disorders	Common	Respiratory rate increased
	Uncommon	Laryngospasm
Gastrointestinal disorders	Common	Nausea, Vomiting
	Uncommon	Salivary hypersecretion
Skin	Common	Erythema
Renal and urinary disorders	Rare	Cystitis
Immune system disorders	Rare	Anaphylatic reaction

2.7.6. Pharmacogenetics of ketamine

Pharmacologic effects of drugs depend on the individual genotype and phenotype affecting the quality and quantity of isoenzymes (*e.g.* cytochrome P450, glucuronyltransferases, esterases and reductases) involved in the drug metabolism and transport. As previously stated, ketamine is metabolized by polymorphically expressed CYP2B6 and CYP2C9 enzymes. Although there is

no clinical pharmacogenetic evidence, this metabolic variance may have an impact on the exposure to ketamine at the individual level.

2.7.7. Drug-drug interactions of S-ketamine

Based on the studies with human liver microsomes, the oxidation of ketamine is mediated mainly by CYP3A4, CYP2B6 and CYP2C19 enzymes. Orphenadrine (CYP2B6 inhibitor) inhibited ketamine N-demethylase activities by 20-67% and sulfaphenazole (CYP2C9 inhibitor) by 57-62% in human liver microsomes (Yanagihara et al. 2001; Hijazi and Boulieu 2002). Ketoconazole, a CYP3A inhibitor, reduced the N-demethylation activity of ketamine by 40-65% *in vitro* (Yanagihara et al. 2001; Hijazi and Boulieu 2002). Inhibition depends on the concentrations of the specific inhibitor.

There are limited *in vivo* reports about DDIs with ketamine and known CYP inhibitors and inducers in humans. Patients taking long-term barbiturates have higher concentrations of hydroxylated metabolites than patients without previous CYP enzyme induction (Idvall et al. 1983). Similarly, low plasma levels of ketamine exist even after long-term use of barbiturates (Koppel et al. 1990). In addition, the CYP2B inhibitor, secobarbital, increased the plasma half-lives of ketamine in humans (Lo and Cumming 1975). In animal models, the metabolism of bupivacaine was inhibited by ketamine (Gantenbein et al. 1997). When ketamine was administered to rats, a slight decrease in the clearance of flecainide and ethosuximide was observed (Loch et al. 1995).

2.8. CYP inhibitors

2.8.1. Clarithromycin

Clarithromycin is a semisynthetic macrolide antibiotic, which exhibits broad-spectrum antibacterial activity against gram-positive, gram-negative, and atypical respiratory tract and skin pathogens (Guay et al. 2001). It is primarily used in the treatment of respiratory tract infections and Helicobacter pylori-associated gastric and duodenal ulcers (Sturgill and Rapp 1992). Clarithromycin is better tolerated than its precursor erythromycin, which has multiple gastrointestinal adverse effects.

Clarithromycin has an oral bioavailability of 50% with a $t_{1/2}$ of 4 to 5 hours. The 14-(R)-hydroxylation and N-demethylation of clarithromycin to 14-(R)-hydroxy-clarithromycin is mediated by CYP3A enzymes (Rodrigues et al. 1997). In humans, clarithromycin is a potent inhibitor of hepatic and intestinal CYP3A (Gorski et al. 1998). The inhibition of intestinal CYP3A activity by clarithromycin is reversible and caused by the formation of metabolic intermediate (MI) complex (Pinto et al. 2005). Numerous clinical human studies revealed the inhibitory effects on CYP3A by clarithromycin. The

typical substrates of CYP3A, which interact with clarithromycin are cyclosporine, midazolam, omeprazole and tacrolimus (Wolter et al. 1994; Gustavson et al. 1995; Sadaba et al. 1998; Pinto et al. 2005).

2.8.2. Ticlopidine

Ticlopidine is a thienopyridine derivate that inhibits platelet aggregation. It is widely used for the prevention of atherothrombotic events in cardiovascular, cerebrovascular and peripheral vascular disease (Kam and Nethery 2003). Ticlopidine is a prodrug, which is metabolized in the liver by CYP1A subfamily (Savi et al. 1994). The clinical effect of the inhibition of platelet aggregation starts 24-48 hours after administration. Maximal inhibition is achieved after 3-5 days. The binding of ticlopidine to $P2Y_{12}$ receptors is irreversible. The inhibitory effects on platelet function are terminated by *de novo* synthesis of platelets after drug withdrawal (Weber et al. 2001). Diarrhea, nausea, vomiting and skin rash are common adverse effects of ticlopidine (Quinn and Fitzgerald 1999).

Ticlopidine is rapidly absorbed from the intestine with an oral bioavailability of 80%. It has nonlinear pharmacokinetics and its clearance decreases with repeated dosing (Kam and Nethery 2003; Richter et al. 2004; Turpeinen et al. 2004). The $t_{1/2}$ of ticlopidine is about 12.6 hours after a single oral dose but increases to 4-5 days after repeated dosing. It is largely bound to plasma proteins and extensively metabolized in the liver. *In vitro*, ticlopidine is a very potent and relatively selective CYP2B6 inhibitor reducing bupropion hydroxylation in human liver microsomes (Richter et al. 2004, Turpeinen et al. 2004). Moreover, it has some inhibitory action on human CYP2C19 enzyme, which is related to phenytoin toxicity in clinical studies (Donahue et al. 1997; Ha-Duong et al. 2001; Turpeinen et al. 2004). The potent inhibitory effect of ticlopidine on CYP2B6 has been demonstrated in a human study where ticlopidine clearly inhibited the CYP2B6-catalyzed bupropion hydroxylation (Turpeinen et al. 2005).

2.8.3. Itraconazole

Itraconazole is a triazole antimycotic used for the prophylaxis and treatment for fungal infections (Boogaerts and Maertens 2001; De Beule and Van Gestel 2001; Sabatelli et al. 2006). All azole antifungals, including itraconazole, are traditionally considered as an extremely interactive class of drugs causing DDIs. Itraconazole blocks the substrate-binding site of fungal CYP enzyme which inhibits the synthesis of ergosterol, a vital component of the fungal cell membrane (Marichal and Vanden Bossche 1995). Itraconazole is well tolerated and can be administered as capsules, oral solutions or intravenous injections.

Itraconazole is a weak base, lipophilic and water insoluble (Poirier and Cheymol 1998). It is tightly bound to blood cells and plasma proteins (99%). Therefore, plasma concentrations of itraconazole are relatively high compared to some other body fluids, *e.g.* cerebrospinal fluid. Itraconazole has a large distribution volume (round 11 L/kg). It accumulates in tissues such as lungs, kidney, liver, skin, nails and in the female genital tract (Grant and Clissold 1989). Itraconazole has over 30 different metabolites because of numerous hepatic biotransformation pathways. Itraconazole and its metabolites act as substrates and potent competitive inhibitors of CYP3A4 in human liver microsomes (Isoherranen et al. 2004; Niwa et al. 2005a; Niwa et al. 2005b). Examples of drugs that are metabolized by human CYP3A4 enzymes and whose elimination is inhibited by itraconazole include cyclosporine, midazolam, diazepam and simvastatin (Back and Tjia 1991; von Moltke et al. 1996; Tran et al. 2002). Inhibitory effects of itraconazole on CYP3A4 was demonstrated in multiple clinical trials, for example with midazolam, quinidine and lovastatin (Olkkola et al. 1994; Kaukonen et al. 1997; Kivisto et al. 1998). Because the toxicological profile of itraconazole is well documented, it is recommended as a standard compound when investigating potential DDIs *in vivo via* CYP3A4 (EMA 2010).

Itraconazole inhibits P-gp function *in vitro* (Wang et al. 2002a). In line with *in vivo* findings, coadministration of itraconazole reduces the clearance of poorly metabolized P-gp substrates such as digoxin, celiprolol and fexofenadine in humans (Jalava et al. 1997; Lilja et al. 2003; Shimizu et al. 2006). *In vitro,* itraconazole inhibits breast cancer resistance protein (BCRP), which is another efflux transporter (Gupta et al. 2007). Many CYP3A4 inhibitors inhibit P-gp (Katragadda et al. 2005).

2.8.4. Grapefruit juice

GFJ is a mixture of several hundred phytochemicals, which can induce food-drug interactions. Furanocoumarins (bergamottin, 6′,7′-dihydroxybergamottin, bergapten), flavonoids (naringin, naringenin) and quercetin are major contributors to the CYP3A4-mediated DDIs *in vitro* (Ho et al. 2001; Hanley et al. 2011). In humans, the administration of naringin and quercetin had no effect on the pharmacokinetics of some CYP3A substrates (Bailey et al. 1993a; Bailey et al. 1993b; Rashid et al. 1993). Clinical pharmacokinetic studies propose, that furanocoumarins, which are mechanism-based inhibitors of enteric CYP3A, play an important role in the food-drug interactions of GFJ *in vivo* (Ducharme et al. 1995; Paine et al. 2008). When furanocoumarins were removed from GFJ, no alterations were detected in the AUCs of substrates, such as felodipine and cyclosporine, compared to the control beverage (Paine et al. 2008). It is notable, that the inhibitory effects by normal consumption of

GFJ occurs mainly in the intestinal level while hepatic enzymes remain unaffected (Ducharme et al. 1995). In addition to intestinal CYP3A4 inhibition, GFJ inhibits the OATP, P-gp and reduces the hydrolysis of drugs by inhibiting specific esterase and sulfotransferase activities (Hardin et al. 1988). Behind GFJ induced CYP3A4 inhibition occurs through the irreversible loss of enteric CYP3A protein. Recovery of enzyme activity requires resynthesis of this isozyme. The half-life for this action is 23 hours. Up to three days may be necessary for enzymatic activity to return back to normal levels after GFJ ingestion (Lown et al. 1997; Greenblatt et al. 2003). In contrast, the GFJ-mediated OATP inbitition is short-lived (2-4 hours) and it can occur with a single dose of GFJ (Bailey 2010). Pharmakenetics are needed to map the net effects of inhibition *versus* induction. Currently, evidence for clinically significant GFJ-drug interactions exists only for CYP3A and OATPs (Hanley et al. 2011).

There are about 40 different drugs that interact with GFJ. Pharmacokinetic extremes are simvastatin (a 16-fold increase in exposure) and celiprolol (with nearly 90% reduction in exposure) in concomitant use with GFJ (Lilja et al. 1998; Lilja et al. 2003). In other words, the most drastic exposure alterations are reported among CYP3A4 substrates with low oral bioavailability (Kantola et al. 1998; Kivisto et al. 1999).

2.9. CYP inducers

2.9.1. St. John´s wort

St. John´s wort (*Hypericum Perforatum*) is a wild plant with medicinal properties. It is a herbal remedy for the self-treatment of mild-to-moderate depressive symptoms, anxiety and sleep disorders (Linde et al. 1996). It has a clear inhibitory effect on the neuronal uptake of many central nervous system transmitters such as serotonin, noradrenaline, dopamine, GABA and L-glutamate (Muller 2003). St. John´s wort contains variable quantities of phytochemicals, such as pseudohypericin, quercetin, chlorogenic acid, flavonols and especially hyperforin, which has been identified as the possible active component in the treatment of depression (Nahrstedt and Butterweck 1997; Obach 2000).

The extent of DDI is related to the hyperforin content of the St. John´s wort preparation (Mueller et al. 2006; Mueller et al. 2009). Hyperforin can activate the pregnane X receptor and induce the production of CYP3A4 *in vitro* (Moore et al. 2000). St. John´s wort inhibits CYP2D6, CYP2C9, CYP3A4 and CYP1A2 enzymes in experimental *in vitro* models (Obach 2000). In humans, St. John´s wort induces the expression of CYP3A4 and P-gp in the intestine and accelerate the function of CYP3A4 in the liver (Durr et al. 2000). The $t_{1/2}$

of hyperforin is nine hours in healthy volunteers (Biber et al. 1998). St. John´s wort products are mainly well tolerated and only a very low frequency of adverse effects occur (Muller 2003).

Clinically, St. John´s wort reduces the plasma concentrations of many CYP3A substrates such as simvastatin (Sugimoto et al. 2001), omeprazole (Wang et al. 2004) and oral contraceptives (Hall et al. 2003). Furthermore, St. John´s wort decreases cyclosporine concentrations and causes acute cellular heart transplant rejection (Ruschitzka et al. 2000). Coadministration of St. John´s wort with the P-gp substrate, digoxin, decreased the plasma concentration of digoxin (Johne et al. 1999). The plasma concentration of CYP3A4 and CYP2C19 substrate voriconazole decreased extensively with the concomitant use of St. John´s wort (Rengelshausen et al. 2005).

2.9.2. Rifampicin

Rifampicin is a wide spectrum semisynthetic antibiotic, which plays a key role in the treatment of tuberculosis. It can also be used in the combination with other antibacterial agents in various infections (van Ingen et al. 2011). After oral administration the absorption of rifampicin is very rapid and complete. The serum concentrations peak in two hours after a single 600 mg dose of rifampicin (Acocella 1978). On repeated administration, the clearance of rifampicin increases with consequent reduction in the $t_{1/2}$ due to autoinduction of the metabolism (Loos et al. 1987). Rifampicin is well tolerated compared to other drugs currently used against tuberculosis (van Ingen et al. 2011).

In vivo, rifampicin induces the CYP3A4 enzyme in the liver and small intestine by activating pregnane X receptor (PXR), which is a critical regulator of CYP3A gene expression (LeCluyse 2001; Kanebratt et al. 2008). Full induction is reached after one week after initiation of treatment. The induction resolves in about two weeks after discontinuing rifampicin (Niemi et al. 2003). Also P-gp is strongly induced by rifampicin. That makes orally administered CYP3A4 and P-gp substrates ineffective during rifampicin treatment. In addition, rifampicin accelerates CYP3A4 formation in intestinal enterocytes in healthy volunteers (Kolars et al. 1992); Greiner et al. 1999). The plasma concentration of midazolam (Backman et al. 1996; Villikka et al. 1997), triazolam (Villikka et al. 1997) and simvastatin (Kyrklund et al. 2000) are reduced after rifampicin treatment. Furthermore, rifampicin decreases the exposure to digoxin by inducing P-gp (Greiner et al. 1999). CYP2C9 substrates, warfarin and sulfonylureas interact with rifampicin, which cause reduced plasma consentrations of these substrates (Niemi et al. 2003). However, rifampicin is only a weak inducer of CYP1A2 as shown in interaction studies with caffeine and tizanidine (Backman et al. 2006).

3. AIMS OF THE STUDY

The use of ketamine as an adjuvant in the multimodal management of chronic pain is increasing worldwide. Ketamine is an intravenous anaesthetic and analgesic agent for surgical operations. Currently, there is a need to develop various oral formulations of this drug. *In vitro* studies show a major role for CYP3A4 and CYP2B6 in the metabolism of ketamine but systematic human DDI-studies are lacking.

The specific aims of these five studies were:

1. To investigate the effects of the inhibition of CYP3A by the antibiotic clarithromycin, the antifungal agent itraconazole and grapefruit juice on the pharmacokinetics of low dose oral S-ketamine (Study I, III and V)

2. To determine if the induction of CYP3A4 by the herbal remedy St John´s wort and antibiotic rifampicin modulates the pharmacokinetics of low dose oral and intravenous S-ketamine (Study II and IV)

3. To assess the impact of inhibition of CYP2B6 by the antithrombotic agent ticlopidine on the pharmacokinetics of low dose oral S-ketamine (Study III)

4. MATERIALS AND METHODS

4.1. Subjects

Forty-one healthy male and female volunteers (21 male, 20 female), who were not active smokers, participated in five separate studies. Thirteen of the 41 subjects participated in two different studies and one person participated in three different studies. The number of subjects and demographic data is shown in Table 2. All subjects gave their written informed consent. Before entering the study volunteers were classified as healthy by medical history, clinical examination and laboratory tests including complete blood count, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, and for women a pregnancy test. Urine was screened for glucose, proteins and some addictive drugs. Also a 12-lead electrocardiogram was obtained. The susceptibility of participants to develop ketamine addiction was estimated to be low as evaluated by the Finnish modified version of the Abuse Questions (Michna et al. 2004). The volunteers were not allowed to drink additional GFJ (or other grapefruit containing products) or take any drugs known to cause CYP-enzyme inhibition or induction for four weeks before the study.

Exlusion criteria in all five studies were:

1. A previous history of intolerance to the study drugs or to related compounds.
2. Concomitant drug therapy of any kind for at least 14 days prior to the study.
3. Subjects younger than 18 years and older than 40 years.
4. Existing or history of asthma, seizures, hematological, endocrine, metabolic, cardiovascular, QT prolongation (in Study I, clarithromycin), psychiatric or gastrointestinal disease, including gut motility disorders or any other significant disease or drug allergy.
5. Previous or present alcoholism, drug abuse, psychological or other emotional problems that are likely to invalidate informed consent, or limit the ability of the subject to comply with the protocol requirements.
6. A positive test result for urine toxicology.
7. A “yes” answer to any one of the Abuse Questions.
8. Pregnancy or nursing.
9. Donation of blood for 4 weeks prior and during the study.
10. Special diet or life style conditions which would compromise the conditions of the study or interpretation of the results.
11. Participation in any other studies involving investigational or marketed drug products concomitantly or within one month prior to the entry into the study.
12. Smoking for one month before the start of the study or during the whole study period.

Table 2. Demographic data of the subjects in studies I – V. Values are given as mean (range).

Study	No of subjects	Age (yr)	BMI (kg/m^2)
I	10	23 (19-27)	23 (20-25)
II	11	25 (20-35)	24 (20-27)
III	11	23 (20-35)	22 (19-26)
IV	12	22 (20-27)	21 (19-26)
V	12	22 (20-27)	21 (20-26)

4.2. Study design

All studies were conducted using a randomized, placebo controlled, balanced, cross-over design. Pretreatments in Studies I - IV were double blinded, whereas the GFJ or water treatments in Study V were open-label. In placebo controlled studies a hospital pharmacist, not involved in the study, packed the study drugs and placebos in identical plastic containers according to a randomization list. The number of drug capsules/tablets or placebo capsules per day, during the pretreatment phases were equal (Table 3). For example, in Study III ticlopidine was ingested two times daily at 8:00 a.m. and 8:00 p.m., whereas during the itraconazole phase, one itraconazole capsule was given at 8:00 a.m. and one placebo capsule administrated at 8:00 p.m. Studies I, II and V consisted of two phases and Study III three phases. In Study IV, a four-session, paired design was used. A four-week wash-out period between the phases was used in Studies II - V. In Study I, the wash-out period was two weeks. The clinical parts of these studies were performed between June 2008 and March 2010 in the Departments of Pharmacology, Drug Development and Therapeutics, University of Turku and Anaesthesiology, Intensive Care, Emergency Care and Pain Medicine, University of Turku and Turku University Hospital.

S-ketamine and pretreatment protocols

In these studies, we used Ketanest-S® (Table 4), which is made for parenteral use but also can be used by the oral route for pain patients (Soto et al. 2012). Orally administered S-ketamine syrup was prepared at the Pharmacy of the Hospital District of Southwest Finland. S-ketamine syrup was composed of Ketanest-S 5 mg/ml (Pfizer, Sandwich, UK) 10 ml, Diluend glycyrrh. DF-93 20 grams, Aqua sterilisata 10 grams, Syrupus saccari DF-04 ad 50 ml yielding a S-ketamine concentration of 1 mg/ml in the syrup. Intravenously administered S-ketamine was Ketanest-S 5 mg/ml (Pfizer, Sandwich, UK). The syrup was stored according to the directions of the local Pharmacy at +8 to +15 degrees to ensure pharmacological stability of S-ketamine.

Pretreatment drugs or placebo (or GFJ/water in Study V) were self-administered at home according to the dosing schedule. Compliance was followed with mobile phone text messages to ensure the right timing of pre-medication. On the study day, the pretreatment drugs were administered by the investigators in the study facilities. The dosing schedules of pretreatment drugs and S-ketamine are summarized in Table 2. The dose of oral S-ketamine was 0.2 mg/kg in Studies I, III and V (with CYP inhibitors) and 0.3 mg/kg in Study II and in oral part of Study IV (with CYP inducers). Intravenous S-ketamine dose in Study IV was 0.1 mg/kg given as an injection in two minutes. Ticlopidine and itraconazole concentrations in Study III and clarithromycin concentrations in Study I were measured in the morning of the study day (*i.e.* day of S-ketamine administration) to ensure the use of medication. The volunteers fasted overnight before the study day and continued fasting until standardized meals were served 4 and 8 hours after S-ketamine administration. Ingestion of alcohol, coffee, tea, or cola drinks was not allowed during pretreatment periods and test days. On study days, subjects stayed in the study facilities from 6:30 a.m. to 8:30 p.m.

In Study I (clarithromycin), II (St. John´s wort) and III (ticlopidine, itraconazole), the last pretreatment was taken at 8:00 a.m. and S-ketamine was administered at 9:00 a.m. In Study IV, rifampicin or placebo capsules were taken at 8:00 p.m. (the sixth dose was administered in the study evening) and S-ketamine was ingested or administered intravenously at 8 a.m., 12 hours after the fifth dose of rifampicin or placebo. In Study V, GFJ (Valio Ltd, Helsinki, Finland) or water was taken at 7:00 a.m., 1:00 p.m., and 7:00 p.m. The ingestion of GFJ or water was continued during the study day until 7:00 p.m. In addition, on the study day, S-ketamine was given at 8:00 a.m. with an additional dose of either 150 ml of GFJ or water depending on the treatment phase.

Table 3. Design of Studies I – V

Study	Pretreatment				Ketamine dosing
	Phase	Drug	Dose	Duration	
I	Phase 1	Placebo	1 caps. x 2	4 days	S-ketamine
	Phase 2	Clarithromycin	500 mg x 2	4 days	0.2 mg/kg *p.o.*
II	Phase 1	Placebo	1 caps. x 3	14 days	S-ketamine
	Phase 2	St. John´s wort	300 mg x 3	14 days	0.3 mg/kg *p.o.*
III	Phase 1	Placebo	1 caps. x 2	6 days	S-ketamine
	Phase 2	Itraconazole	200 mg x 1	6 days	0.2 mg/kg *p.o.*
	Phase 3	Ticlopidine	250 mg x 2	6 days	
IV	Intravenous part				
	Phase 1	Placebo	1 caps. x 1	6 days	S-ketamine
	Phase 2	Rifampicin	600 mg x 1	6 days	0.1 mg/kg *i.v.*
	Oral part				
	Phase 1	Placebo	1 caps. x 1	6 days	S-ketamine
	Phase 2	Rifampicin	600 mg x 1	6 days	0.3 mg/kg *p.o.*
V	Phase 1	Water	200 ml x 3	5 days	S-ketamine
	Phase 2	Grapefruit juice	200 ml x 3	5 days	0.2 mg/kg *p.o.*

Table 4. Drugs used in Studies I – V

Drug	Trade name, dosage	Manufacturer	Place of manufacture
S-ketamine	Ketanest-S 5 mg/ml	Pfizer	Sandwich, UK
Clarithromycin	Zeclar 500 mg	OrionPharma	Espoo, Finland
St. John´s wort	Jarsin 300 mg	Klosterfrau, Lichtwer Pharma	Hamburg, Germany
Itraconazole	Sporanox 200 mg	Janssen-Cilag	Latina, Italy
Ticlopidine	Ticlid 250 mg	Roche	Nutley, New Jersey
Rifampicin	Rimapen 600 mg	Orion	Espoo, Finland
Grapefruit juice	Greippi täysmehu	Valio	Helsinki, Finland

4.3. Blood sampling

During the morning of each study day, a forearm vein was cannulated using a 18-gauge intravenous catheter and timed venous blood samples were drawn into 10 ml EDTA containing tubes immediately before and 20 minutes (min), 40 min, 1 hour (h), 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h, 12 h and 24 h after ingestion of S-ketamine. An additional blood sample was drawn 10 min after intravenous S-ketamine administration in Study IV. In Study I (clarithromycin) and Study III (ticlodipine and itraconazole), a blood sample was drawn before administration of any drugs in the morning for the determination of trough concentrations of the above interacting drugs. After the last blood sample of the study day, all the subjects reported feeling well and were transported home. Subjects returned to the study facility the following morning to give a blood sample at 24 h after S-ketamine administration. Plasma was separated within 30 min and stored at -70ºC until analysis.

4.4. Determination of plasma drug concentrations

Determination of ketamine and norketamine

The doses of S-enantiomer of ketamine were administered, but the plasma concentrations of ketamine and norketamine were analyzed. There is no interconversion between the enantiomers of ketamine (Ihmsen et al. 2001). In consequence, the actual enantiomers we assayed were S-ketamine and S-norketamine. The determination method for ketamine used in our study was not enantioselective (Feng et al. 1995; Ihmsen et al. 2001).

Ketamine and norketamine were extracted from plasma and their concentrations were quantified using an API 2000 liquid chromatography-tandem mass spectrometry system (Sciex Division of MDS, Toronto, Ontario, Canada) with ketamine-D4 and norketamine-D4 as internal standards (Feng et al. 1995). The lower limit of quantification was 0.025 ng/ml for both ketamine and norketamine. The interday CVs were less than 13% for both ketamine and norketamine at the relevant plasma concentrations in all five studies.

Clarithromycin

Plasma concentrations of clarithromycin were determined by a Q Trap liquid chromatography–tandem mass spectrometry system (Sciex Division of MDS Inc.) as previously described (Laakso et al. 1990; van Rooyen et al. 2002). The lower limit of quantification was 10 ng/ml. The CVs were 1.3%, 2.4%, and 1.2% at 30 ng/ml, 300 ng/ml and 3000 ng/ml, respectively.

Itraconazole and OH-itraconazole

Itraconazole and its metabolite OH-itraconazole were extracted from timed plasma samples taken just before and up to 12 h after ketamine administration. Their plasma concentrations were quantified using R51012 as the internal standard and carrying out high-performance liquid chromatography with ultraviolet detection as described earlier (Compas et al. 1996; Gubbins et al. 1998). The lower limit of quantification was 10 ng/ml for both itraconazole and OH-itraconazole and their CVs were < 10% at the relevant concentrations.

Ticlopidine

The plasma samples for ticlopidine concentrations were protein precipitated with a three-fold volume of acetonitrile using Sirocco protein precipitation plates (Waters, Milford, MA). Chromatographic separation was carried out with the Waters Alliance 2695 HPLC system (Waters) using a WatersXBridge C18 analytical column (2.1 mm x 50 mm, particle size 3.5 μm) with a Phenomenex C18 2.0 mm x 4.0 mm precolumn (Phenomenex, Torrance, CA). The eluents were 0.1% acetic acid (A) and methanol (B). A linear gradient elution with profile 10% - 98% - 98% B in 0 - 1.0 - 3.0 min was employed, followed by 4 min of column equilibration. The flow rate was 0.4 ml/min, and the column oven temperature was 30ºC. The data were acquired using a Waters Quattro Micro triple quadrupole mass spectrometer equipped with a Z-spray electrospray source, using multiple reaction monitoring mode detection. The positive ionization mode was used. The fragmentation reactions monitored were from 264 *m/z* to 154 *m/z* for ticlopidine and from 322 *m/z* to 212 *m/z* for clopidogrel, which was used as a reference. The back-calculated accuracy and precision over the quantification range were 86-107% and 1-15%, respectively.

4.5. Pharmacokinetic measurements

Pharmacokinetic variables of ketamine and norketamine were determined using the WinNonlin pharmacokinetic program (version 4.1; Pharsight, Mountain View, California). The $AUC_{0-\infty}$ of S-ketamine was the primary outcome variable in the study, and all other pharmacokinetic and all pharmacodynamic parameters were secondary variables.

The C_{max} and corresponding times (t_{max}) of ketamine and norketamine were observed directly from the data. The k_{el} and the $t_{1/2}$ were calculated by means of standard noncompartmental methods. The $t_{1/2}$ was calculated using the following equation $t_{1/2} = \ln 2/k_{el}$. The areas under the ketamine and norketamine concentration-time curves (AUC) were estimated by means of the trapezoidal rule with extrapolation to infinity ($AUC_{0-\infty}$) and with last quantifiable ketamine

concentration value (AUC_{0-t}). The linear trapezoidal rule was used for successively increasing concentration values and the logarithmic trapezoidal rule for decreasing concentration values. The AUC_m/AUC_p ratio was calculated for comparison of the relative abundance of norketamine during each phase. The F of S-ketamine was calculated as follows: $F = (AUC_{0-\infty,\ oral} \bullet Dose_{intravenous}) / (AUC_{0-\infty,\ intravenous} \bullet Dose_{oral})$.

4.6. Pharmacodynamic measurements

The subjective pharmacological effects of S-ketamine were evaluated up to 12 hours at the time of blood sampling immediately before S-ketamine administration and 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h and 12 h after ingestion or intravenous dose of S-ketamine. The volunteers were trained to perform all the pharmacodynamic tests before attending the first study day experiment. The area under the self reported pharmacodynamic effect-time curve from zero to t hours ($AUEC_{0-12}$) was determined using the trapezoidal rule for each pharmacodynamic variable.

Subjective effect

Psychomotor effects of S-ketamine were evaluated with VAS for the following seven items: alert/drowsy, very good performance/very poor performance, no drug effect/very strong drug effect, relaxed/anxious, unpleasant feeling/pleasant feeling, no nausea/very strong nausea, calm/restless (Bond and Lader 1974). In this test, the subject drawed a vertical line across a horizontal 100-mm-long scale with opposite psychological states at its ends. The forms were collected away after each timepoint so that the volunteers were not able to see the previous scales they had already filled. Any adverse events spontaneneously reported by the volunteers were recorded. In Study IV (intravenous S-ketamine), the subjects were recommended to write down all the abnormal sensations that could not be ruled out by VAS-forms during the study day.

Digit symbol substitution test

The level of psychomotor and cognitive capacity of the volunteers was determined by using the DSST by Stone (Stone 1984). In the DSST, the subjects had 3 minutes to substitute digits (1-9) with corresponding simple symbols determined in the upper part of the paper. The DSST form consists of 300 digits in randomized order and the number of correct symbols was recorded. We used ten different DSST forms at each time point to prevent the memorizing of the code. The volunteers trained to do DSST on the study mornings before first recorded DSST.

Maddox Wing test

The Maddox Wing test measures the effect of S-ketamine on the coordination of the extraocular muscles (Hannington-Kiff 1970). When looking through the Maddox wing apparatus, a person's vision is divided by oblique and vertical wings so that the right eye views an arrow while the left eye views a horizontal numbered scale. The difference in extraocular muscle tone results in an image in which the arrow moves along the numeric scale. The number, which the arrow stopped to point at, was reported by the subjects and the result was given in dioptres.

Cold pressor test

A cold pressor test was used to assess cold pain sensitivity (Garcia de Jalon et al. 1985). The subject immersed his/her left hand into ice-water of 0-2ºC for 1 min. The latency from the immersion occurred if the hand to the first sensation of pain (cold pain threshold, CPT) was determined with a stop-watch in seconds. The subjects rated cold pain intensity and unpleasantness at 30 s and 60 s (CPI30, CPI60, CPU30, CPU60) after immersion on a numerical rating scale (NRS; 0 = no pain or unpleasantness, 100 = the maximal imaginable pain or unpleasantness). If cold pain became intolerable before the end of the test, the volunteer was instructed to discontinue the immersion. If CPT was not reached in 1 min, it was recorded as a maximum value of 60 s.

4.7. Statistical analysis

A pre-study power analysis was made to determine the number of volunteers needed. It was calculated that ten subjects would be required in order to demonstrate a 30% difference in the area under S-ketamine AUC with a statistical power of 80% and a type I error of 5% in each study. Enrolling 12 subjects allowed a 20% dropout rate without affecting the statistical power.

All data were analyzed using the statistical program SYSTAT for Windows (version 10.2; Systat Software, Richmond, California). Before statistical analysis, data were evaluated for normality of distribution using Shapiro-Wilk´s W-test. All non-normally distributed data were log-transformed for analysis but reported as non-transformed results to correct for non-normality of the distribution. Differences in pharmacokinetic and pharmacodynamic variables between phases (two-phase studies, Studies I, II, IV and V) were analysed using paired Student´s t-test except for t_{max}, which was analysed with Wilcoxon signed-ranks test in all five studies. The relative changes compared to the placebo phase were calculated individually for each subject. The means and ranges of these values were reported. In the three-phase study (Study III),

the ANOVA for repeated measures was used to compare pharmacokinetic and pharmacodynamic variables between different phases. A posteriori testing was performed using the Tukey´s test. These tests were used to investigate the contributions of treatments, gender, phases and sequences to overall variance. Differences between phases were regarded significant if $P < 0.05$. We also calculated the GMRs for pharmacokinetic results with 90% CI to determine the biological equivalence (bioequivalence) of drugs and placebo in all studies. The lack of interaction was concluded if the 90% CI of GMRs for ketamine $AUC_{0-\infty}$ was in the range of 0.8 to 1.25 (EMA 2010).

The Pearson product-moment correlation coefficient was used to detect a possible association of plasma concentrations of S-ketamine with pharmacological effects. The results were expressed as mean ± SD or median (range) whenever appropriate. P values < 0.05 were considered as statistically significant.

4.8. Ethical aspects

All studies were conducted according to the guidelines in the revised Declaration of Helsinki (2008). All study protocols were approved by the ethics committee of the Hospital District of Southwest Finland and by the Finnish National Agency for Medicines. In addition, the studies were registered to the EudraCT (European Union Drug Regulating Authorities Clinical Trials) under a specific code number. Before the screening visit, all subjects received written information about the study by email. After that, during preliminary medical examination, additional oral explanation of the study protocol and pharmacodynamic tests was given. Finally, the volunteers gave their written informed consent before entering the studies and they were told that they were allowed to withdraw from the study at any time.

All the doses of pretreatment drugs were similar to those used in the clinical practice. The oral doses of S-ketamine were equal to doses used in the treatment of chronic pain (Bell 2009). The dose was low enough to be safely administered to the healthy volunteers.

5. RESULTS

The plasma concentrations (mean values ± SD) of ketamine (combined values from studies I - V in the placebo phases) after different oral and intravenous doses of S-ketamine are shown in Figure 4. Mean changes in the pharmacokinetic parameters of ketamine and norketamine during treatment periods are shown in Figures 5 and 6. The subjective pharmacodynamic effects of S-ketamine during the placebo and the treatment phases are shown in Figure 7.

5.1. Plasma concentrations of ketamine

Plasma concentrations of ketamine were low during the placebo phases. When the oral dose of S-ketamine was 0.2 mg/kg, the mean C_{max} of ketamine varied from 5.3 to 10.6 ng/ml. When the ingested dose was increased to 0.3 mg/kg, the mean C_{max} of ketamine ranged from 12.4 to 16.2 ng/ml in the control phases. Intravenous S-ketamine produced a mean C_{max} of 32.5 ng/ml (Figure 4).

5.2. Effect of clarithromycin on oral S-ketamine (I)

The dose of oral S-ketamine was 0.2 mg/kg. Clarithromycin increased the mean $AUC_{0-\infty}$ of oral ketamine by 263% ($P < 0.001$) and the mean C_{max} by 355% ($P < 0.001$) compared to the control. The mean C_{max} of ketamine was 5.3 ± 2.7 ng/ml and 16.2 ± 5.8 ng/ml in the placebo and treatment phases, respectively. The mean metabolite-to-parent ratio (AUC_m/AUC_p) was decreased by 54% after clarithromycin treatment compared to placebo. During the clarithromycin phase, the GMRs for $AUC_{0-\infty}$ and C_{max} for ketamine were outside the bioequivalence acceptance limits. The mean $t_{1/2}$ of ketamine was prolonged from 4.2 to 6.5 h after clarithromycin ($P = 0.001$). The median t_{max} of ketamine was 0.9 h and 0.5 h in the control and clarithromycin phases, respectively.

The mean $AUC_{0-\infty}$ of norketamine did not differ between the phases but the mean C_{max} of norketamine was increased by 35% by clarithromycin ($P = 0.009$). The mean C_{max} of norketamine was 38.4 ± 7.8 ng/ml in the control phase and it was increased by 35% by clarithromycin ($P = 0.09$). The mean $t_{1/2}$ of norketamine was prolonged from 5.2 h to 7.0 h by clarithromycin but the difference was not significant.

Plasma concentrations of clarithromycin, immediately before S-ketamine administration on the study day, ranged from 494 to 2310 ng/ml. The change in the mean $AUC_{0-\infty}$ of ketamine between the placebo and clarithromycin phases did not correlate with plasma concentrations of clarithromycin.

Pharmacodynamic effects of clarithromycin

There were no statistically significant differences in the mean $AUEC_{0-\infty}$ values of any pharmacodynamic test variables between control and clarithromycin phases. The maximum subjective drug effect was reached at 1 h after ketamine ingestion. There were no correlations between the plasma concentrations of ketamine and pain scores in the analgesic tests.

5.3. Effect of St. John´s wort on oral S-ketamine (II)

Pretreatment with St. John´s wort decreased the mean $AUC_{0-\infty}$ of orally administered ketamine by 58% ($P < 0.001$) and the mean C_{max} by 66% ($P < 0.001$) compared to placebo. The decrease in the $AUC_{0-\infty}$ of ketamine was observed in all 12 subjects. The mean $t_{1/2}$ of ketamine diminished by 36% ($P = 0.001$) by St. John´s wort. The time to achieve t_{max} did not differ significantly between the phases (0.8 h vs. 0.7 h).

St. John's wort decreased the mean $AUC_{0-\infty}$ of norketamine by 18%. The mean C_{max} on norketamine decreased 23% after St. John´s wort treatment. The corresponding AUC_m/AUC_p ratio was increased by 2.2-fold.

Pharmacodynamic effects of St. John´s wort

No statistically significant differences were observed in the mean $AUEC_{0-\infty}$ values of behavioral or analgesic tests between the phases. A statistically significant linear correlation between the self-reported drug effect and C_{max} of ketamine was detected ($r = 0.55$; $P < 0.01$).

5.4. Effect of ticlopidine and itraconazole on oral S-ketamine (III)

Pharmacokinetic effects of ticlopidine

The CYP2B6 inhibitor, ticlopidine, elevated the mean $AUC_{0-\infty}$ of ketamine by 318% ($P < 0.001$). The exposure to ketamine increased in all subjects and the greatest individual elevation was 10.4-fold. Ticlopidine increased the mean C_{max} of ketamine by 231% but the effect was variable. The C_{max} of ketamine increased in eight subjects by 126-570% but decreased in three subjects by 16-47% after ticlopidine treatment. The mean $t_{1/2}$ of ketamine was unaffected but median t_{max} was slightly prolonged by ticlopidine. The formation and the mean $t_{1/2}$ of norketamine were practically unaffected by ticlopidine. Only insignificant decreases in the mean C_{max} of norketamine was observed after ticlopidine. The mean AUC_m/AUC_p decreased by 49% after ticlopidine treatment ($P < 0.001$).

Pharmacokinetic effects of itraconazole

Itraconazole did not change the mean $AUC_{0-\infty}$ of ketamine. Furthermore, the mean C_{max}, $t_{1/2}$ and t_{max} did not differ significantly between the placebo and itraconazole phases. The mean $AUC_{0-\infty}$ and C_{max} of norketamine were slightly decreased by itraconazole, but the changes were not significant. The mean AUC_m/AUC_p was significantly decreased during the itraconazole phase as compared to the placebo phase ($P = 0.006$). There were no linear correlations between the individual $AUC_{0-\infty}$ values of ketamine and the C_{trough} values of itraconazole ($R = 0.45$; $P = 0.17$) or metabolite OH-itraconazole ($R = 0.28$; $P = 0.41$). The mean C_{trough} values for itraconazole and OH-itraconazole just before the S-ketamine ingestion in the study morning were 117 ng/l (range 18-223 ng/l) and 184 ng/l (range 45-352 ng/l), respectively.

Pharmacodynamic effects of ticlopidine and itraconazole

During the first hours after S-ketamine ingestion, all the subjects reported poor performance and slight drowsiness in all three phases. The $AUEC_{0-\infty}$ values for drowsiness and performance were the only pharmacodynamic parameters that were higher in the ticlopidine ($P = 0.024$ and 0.025) and itraconazole ($P = 0.036$ and 0.027) phases than in the placebo phase, even if itraconazole produced no differences in the exposure to ketamine between the phases. There was a linear correlation between the plasma ketamine concentration and all of the mean $AUEC_{0-\infty}$ values ($P < 0.001$).

5.5. Effect of rifampicin on oral and intravenous S-ketamine (IV)

Oral S-ketamine, pharmacokinetics

Rifampicin decreased the mean $AUC_{0-\infty}$ and the mean C_{max} of oral ketamine by 86% and 81% ($P < 0.001$), respectively. The mean C_{max} was 12.4 ± 5.9 ng/ml with placebo and 1.9 ± 0.9 ng/ml with rifampicin treatment. The mean oral bioavailability of ketamine decreased from 11% to 2% ($P < 0.001$) by rifampicin. Rifampicin shortened the mean $t_{1/2}$ of ketamine by 28% from 6.0 h to 4.2 h ($P = 0.005$). The median t_{max} was slightly shortened from 0.7 h to 0.3 h but the difference was statistically insignificant ($P = 0.17$). Rifampicin decreased the mean $AUC_{0-\infty}$ of norketamine by 74% ($P < 0.001$) and the mean C_{max} of norketamine by 44% ($P < 0.001$) compared to placebo. The mean AUC_m/AUC_p was increased by 147% ($P < 0.001$).

Intravenous S-ketamine, pharmacokinetics

When S-ketamine was administered intravenously, rifampicin treatment decreased the mean $AUC_{0-\infty}$ of ketamine by 14% (P = 0.005). The mean C_{max} of ketamine remained unchanged. Rifampicin increased the mean plasma CL by 19% (P = 0.003) and decreased its $t_{1/2}$ from 5.9 h to 5.1 h (P = 0.012). The mean V_{ss} and t_{max} of ketamine remained unchanged between the phases. In all subjects, the mean $AUC_{0-\infty}$ of norketamine decreased by 72% (P < 0.001) and the mean C_{max} by 46% (P < 0.001). The mean AUC_m/AUC_p was 66% lower during the rifampicin phase compared placebo (P < 0.001). The mean $t_{1/2}$ of norketamine shortened from 7.8 h to 5.6 h by rifampicin (P < 0.001). The time to achieve t_{max} did not differ between the phases.

Pharmacodynamic effects of rifampicin

There was a significant linear correlation between all the behavioral effects and plasma ketamine concentrations (P < 0.05). No statistically significant differences were detected between any of the studied pharmacodynamic variables ($AUEC_{0-12}$) between the phases. Reduced ketamine concentrations by rifampicin did not modify the analgesic effects of intravenous or oral S-ketamine compared to placebo.

5.6. Effect of grapefruit juice on oral S-ketamine (V)

GFJ increased the mean $AUC_{0-\infty}$ of ketamine by 320% (P < 0.001) and C_{max} by 228% (P < 0.001). The mean $t_{1/2}$ of ketamine was slightly prolonged from 4.9 h to 5.7 h (P < 0.05) and t_{max} from 0.7 h to 1.0 h (P < 0.05) by GFJ. The mean C_{max} of norketamine remained unchanged after GFJ. The mean $AUC_{0-\infty}$ of norketamine was increased by 27% (P < 0.05) and the mean $t_{1/2}$ from 5.9 h to 6.5 h (P = 0.04) by GFJ. The mean AUC_m/AUC_p was decreased by 57% (P < 0.001).

Pharmacodynamic effects of grapefruit juice

Two subjects reported the feeling of drunkenness after ketamine ingestion. GFJ appeared to enhance the perceived subjective drug effect during the first 5 h after ketamine ingestion, but no statistically significant differences were observed between the phases (P = 0.16). Relaxation rates were subjectively assessed and were significantly decreased and the performance in the DSST increased after GFJ treatment compared to control (P < 0.05). However, a significant linear correlation was detected between the plasma ketamine concentrations and the self reported drowsiness, performance, drug effect and calmness (P < 0.05). The maximum pain relieving effect of oral S-ketamine on

the CPT scores were observed 1-2 h after S-ketamine ingestion. A linear correlation was detected between the plasma ketamine concentrations and CPT ($r = 0.79$; $P < 0.001$), maximum pain at 60 s ($r = 0.62$; $P = 0.003$), and pain unpleasantness at 60 s ($r = 0.71$; $P < 0.001$). However, no statistically significant differences were detected between the phases.

5.7. Adverse effects

When S-ketamine was administered perorally, there were no significant observed or reported adverse effects at any time during the study. Only slight drowsiness and poor performance were reported. All psychomimetic incidents (e.g. hallucinations, nightmares) were absent. One subject was treated with tropisetron for nausea and vomiting during one test evening.

As expected, intravenous S-ketamine (0.1 mg/kg) caused more profound adverse effects than oral S-ketamine. Six of eleven subjects from both pretreatment phases reported transient visual disturbances, altered perception of light or sound within one hour after dosing. One day after intravenous S-ketamine in the Study IV, one of the subjects complained about nausea, vomiting and diarrhoeae and was not willing to continue the study. The symptoms were interpreted to be caused by a common gastroenteritis.

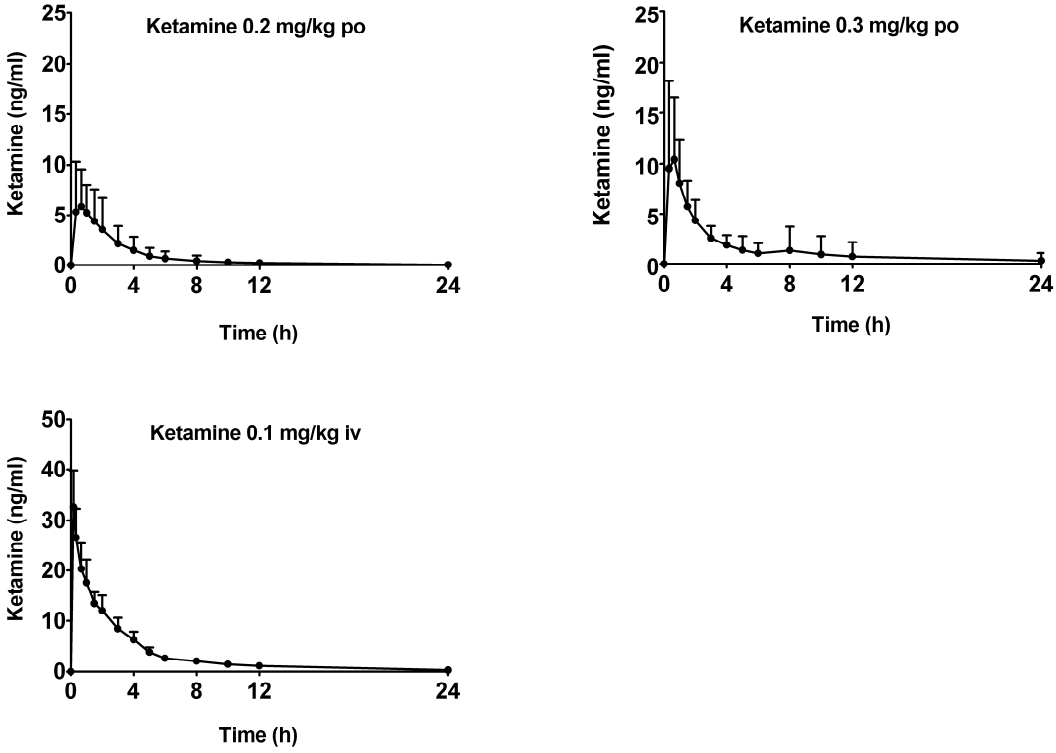

Figure 4. Combined (Study I - V) mean plasma (±SD) concentrations of ketamine after oral 0.2 mg/kg, oral 0.3 mg/kg and intravenous 0.1 mg/kg dose of S-ketamine under placebo phases in the Studies I - V.

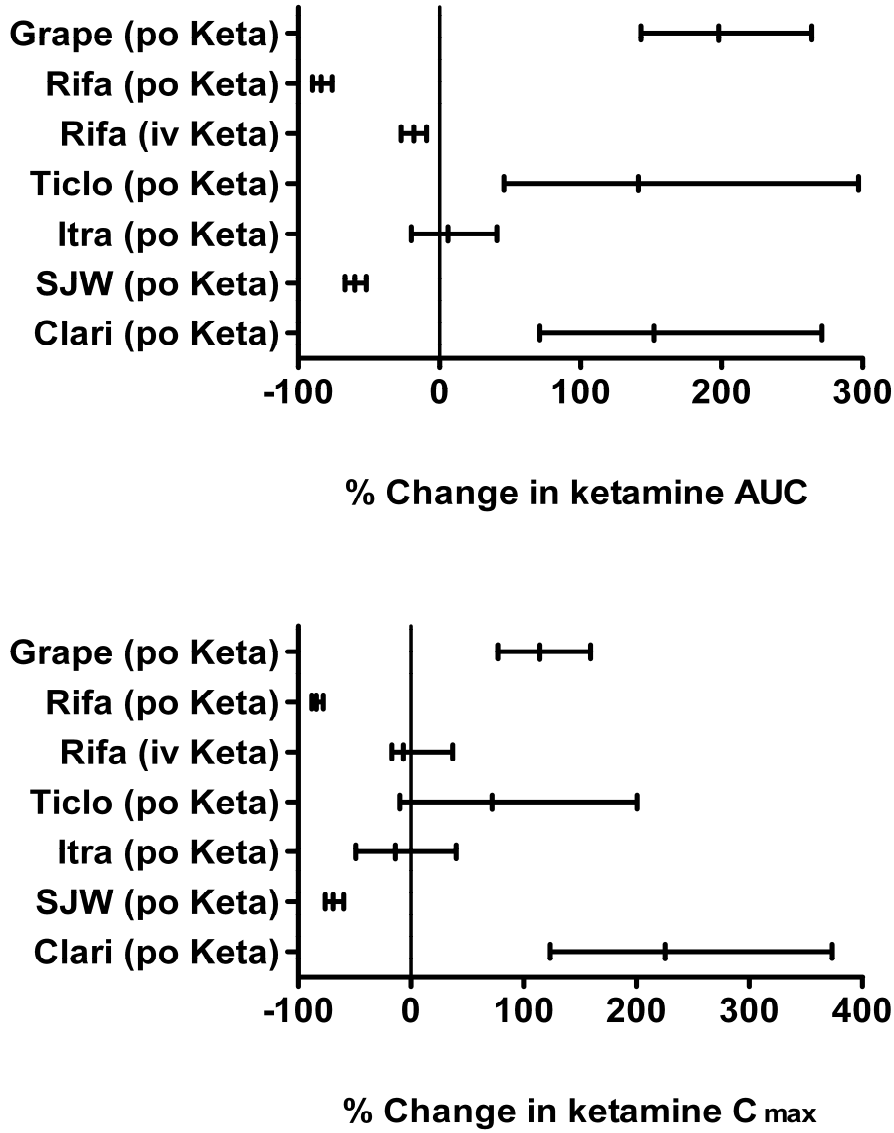

Figure 5. Percent changes in the AUC and C_{max} of ketamine with 95% confidence intervals, after pretreatment with GFJ (Grape), rifampicin (Rifa), ticlopidine (Ticlo), itraconazole (Itra), St. John´s wort (SJW), and clarithromycin (Clari).

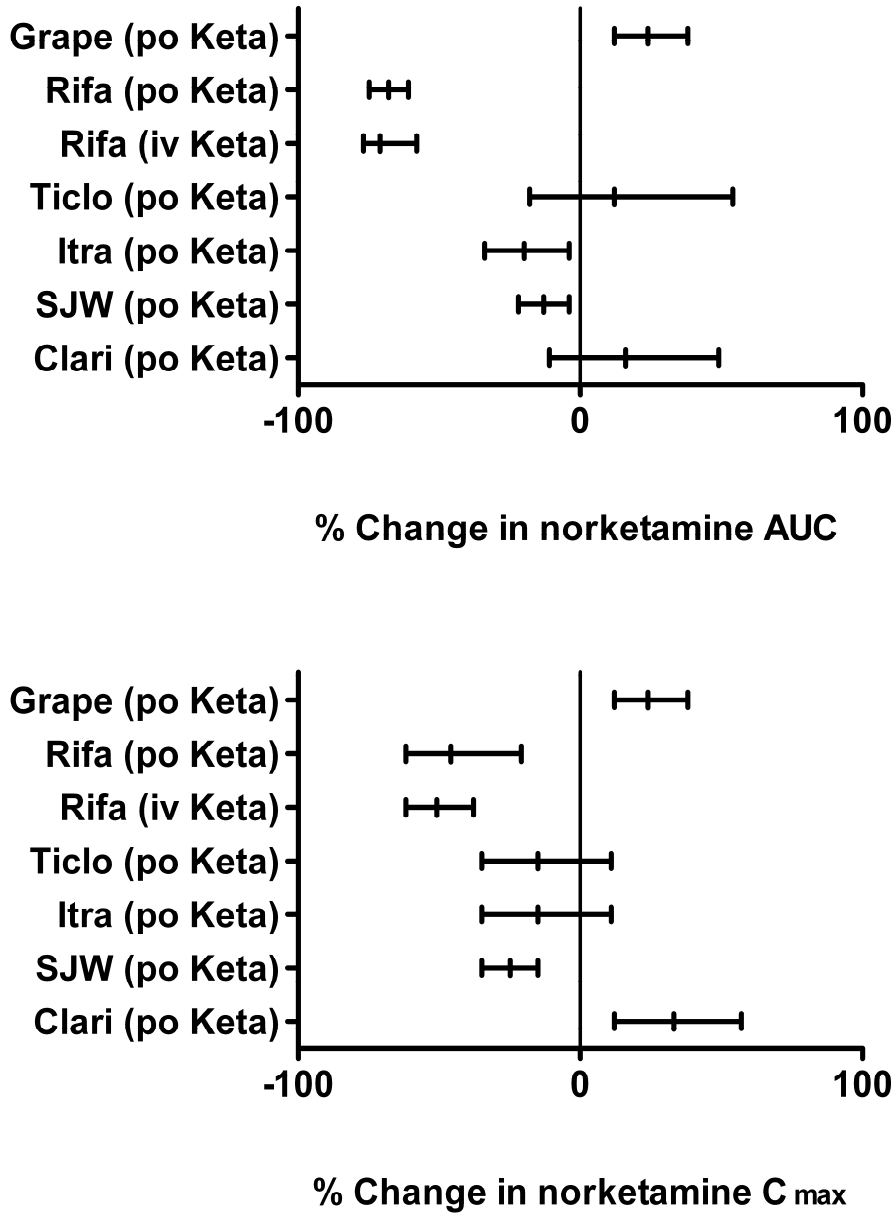

Figure 6. Percent changes in the AUC and C_{max} of norketamine with 95% confidence intervals, after pretreatment with GFJ (Grape), rifampicin (Rifa), ticlopidine (Ticlo), itraconazole (Itra), St. John´s wort (SJW), and clarithromycin (Clari).

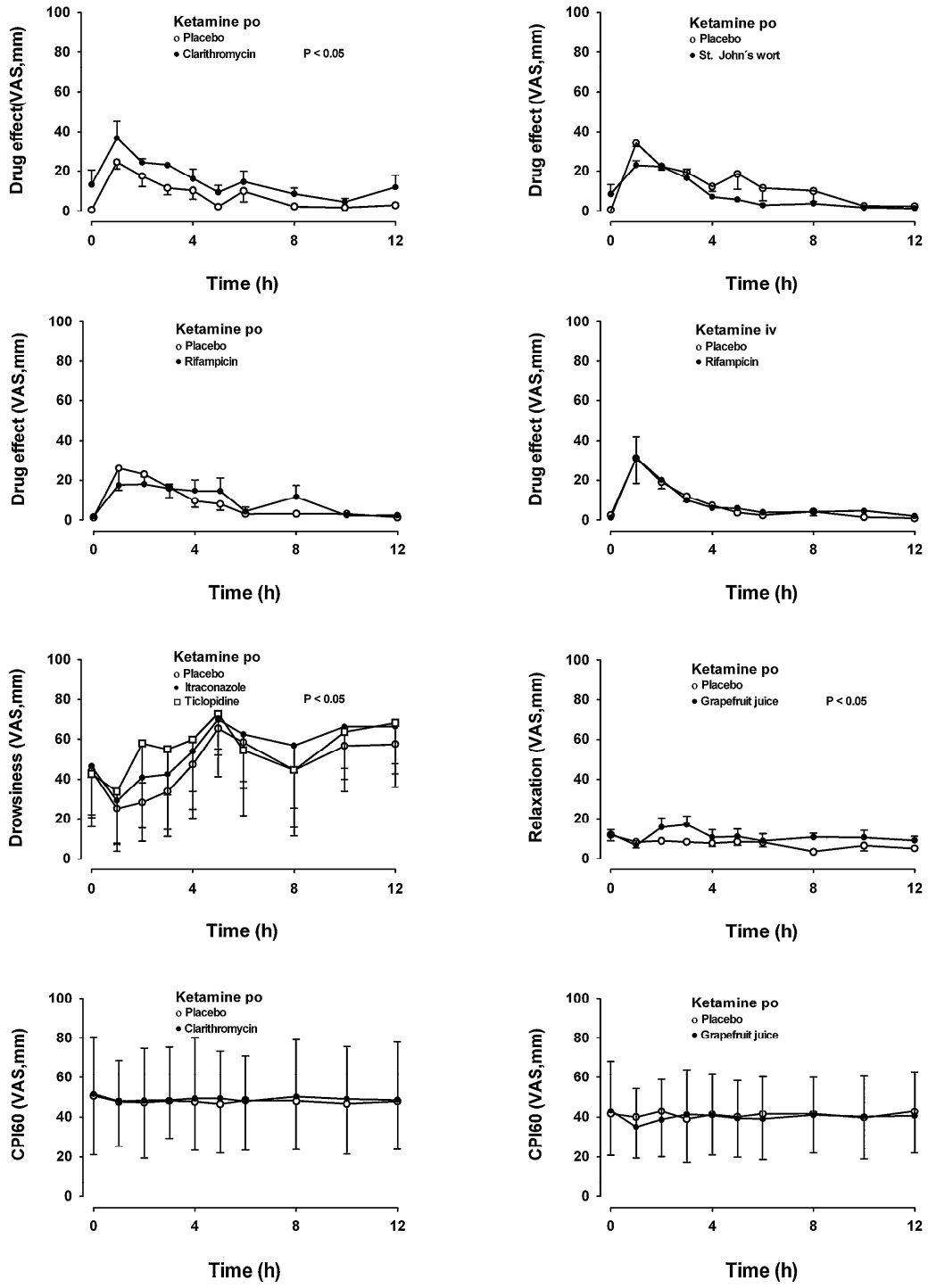

Figure 7. The mean (± SD) of some subjective pharmacodynamic effects of S-ketamine during the placebo and treatment phases.

6. DISCUSSION

6.1. Methodology

The principal purpose to conduct these studies was to investigate the pharmacokinetic DDIs of oral and intravenous S-ketamine with different CYP enzyme inducers and inhibitors in healthy volunteers. Because of the small size in the study groups, the interindividual variance was minimized by choosing a balanced, cross-over study design. All the subjects served as their own controls, received the same number of pretreatment drugs and participated in an equal number of study phases. Studies I – IV were conducted in a double-blinded, placebo-controlled manner in order to enable, reliable pharmacokinetic measurements. In the Study V, where pretreatment agents were liquid (GFJ or water), a liquid placebo was used which mimicked GFJ but open-label study design was employed.

According to power analysis calculations, the sample size of 10 volunteers was estimated to be sufficient to show a 30% difference in the $AUC_{0-\infty}$ of ketamine at a power of 80% at significance level of 0.05 between the phases. However, 10 volunteers were enrolled only in Study I. Enrolling 12 subjects to the rest of the studies allowed for 2 dropouts. Altogether 41 different volunteers were enrolled in the five studies. Half of the subjects were female. The menstrual cycle has considered to have minimal effects on drug elimination (Kharasch et al. 1997). There can be small sex-related differences in the drug-metabolizing enzymes, especially when comparing men to pregnant women (Kharasch et al. 1997; Soldin and Mattison 2009). In addition, intake of oral contraceptives inhibits CYP2C19 activity (Laine et al. 2000). However, we considered these issues clinically insignificant because women in our study were nonpregnant and were not taking hormonal contraceptives. It was allowed for the same subject to take part in more than one study if all the inclusion criteria were met.

To objectively ensure compliance with the pretreatments in the studies I and III, the concentrations of clarithromycin, ticlopidine and itraconazole were measured. Adherence with the pretreatment schedule was also assured by using mobile phone text messages in all studies. The dose of the pretreatment drugs was based on the Summary of Product Characteristics. Usually, the use the highest clinical dose is recommended to achieve steady state conditions (EMA 2010). However, for safety reasons (potential QT prolongation), the daily dose of clarithromycin was not the maximum clinical dose but typical dose used in previous DDIs (Liukas et al. 2011; Shin et al. 2011). Clinically, the duration of clarithromycin treatment is short. However, DDIs occur already after three days clarithromycin exposure (Lilja et al. 2007). Four days of clarithromycin exposure was considered to be sufficient in this study. Rifampicin and

itraconazole can be used for long periods in the treatment of tuberculosis and fungal infections. However, rifampicin has profound autoinductive properties reducing its own oral bioavailability (Loos et al. 1985). A pretreatment time of six days with rifampicin and itraconazole was not long enough to achieve steady state concentrations (Loos et al. 1985). This duration was selected to avoid adverse effects. However, similar doses and duration of itraconazole and rifampicin have profound effect on CYP3A4-mediated metabolism of different drugs (Kaukonen et al. 1997; Backman et al. 1998). The ingestion of GFJ in study V was started four days before the test day and continued until the end of the study day to ensure the inhibition of the CYP3A-mediated effect and maintain possible short term intestinal OATP inhibition, as shown in previous studies (Bailey 2010; Nieminen et al. 2010a). The administration of St. John´s wort was started two weeks before S-ketamine to ensure steady-state concentrations and to select the length of treatment, which is also used in many clinical studies (Mai et al. 2004; Rengelshausen et al. 2005). Six-day duration of ticlopidine treatment is sufficient to inhibit CYP2B6 *in vivo* and is well tolerated (Turpeinen et al. 2005).

The dose of S-ketamine was kept low enough (0.2 mg/kg with inhibitors and 0.3 mg/kg with inducers) to ensure the safety of the participants. Similarly, when S-ketamine was administered intravenously and high peak concentrations were predicted, the dose of S-ketamine was kept low (0.1 mg/kg). In the management of acute clinical pain (CPT in our studies), higher doses may be needed at least when ketamine is administered orally. Usually, intravenous doses of 0.125-0.25 mg/kg are used when treating acute clinical pain (Sinner and Graf 2008). Oral doses of 0.5 mg/kg of racemic ketamine improve analgesia during chronic pain (Bell 2009; Bell et al. 2012). Because S-ketamine is a more potent analgetic than its racemate, its recommended starting dose in chronic pain is 0.25 mg/kg (Blonk et al. 2010). At the moment, there are no other formulations on the market than intravenous S-ketamine, but stable oral, sublingual and intranasal compounds are under development (Chong et al. 2009; Huge et al. 2010). Low dose S-ketamine (intravenous formulation administered orally) is in clinical use when treating different chronic pain conditions at pain clinics.

All $AUC_{0-\infty}$ values of ketamine were equal to the corresponding AUC_{0-24} values indicating that 24 h blood sampling time was appropriate in all five studies. Wash-out periods were 2 weeks in Study I and 4 weeks in Studies II - V, which are sufficient to minimize the possible carry-over effects of the studied inhibitors and inducers.

Considerable inter-individual variation was observed in the mean $AUC_{0-\infty}$ and mean C_{max} of ketamine both n control and treatment phases in all studies. This was to some extent caused by genetic polymorphisms in the activity of CYP3A4 and CYP2B6, which play a major role in the metabolism of S-ketamine (Yanagihara et al. 2001; Hijazi and Boulieu 2002; Noppers et al. 2011). Because S-ketamine is metabolized at least *via* three different routes (CYP3A4, CYP2B6 and CYP2C9) and a cross-over study design was used, we considered it unnecessary to genotype the volunteers for CYP3A4, CYP2B6 and CYP2C9 polymorphisms.

Pharmacodynamic effects of S-ketamine were evaluated with methods used earlier in pharmacokinetic studies with opioids (Grach et al. 2004; Grönlund et al. 2010; Nieminen et al. 2010a; Nieminen et al. 2010c). These methods included VAS-scores, Maddox wing test, digit symbol substitution test and cold pressor test in all studies. However, VAS-scores were not specific to S-ketamine which has unique effect on the central nervous system *via* antagonism of NMDA receptors. The most sensitive VAS-score was the subjective drug effect, which peaked at the 1 h timepoint after ingestion of S-ketamine. An intravenous bolus of S-ketamine produced an almost immediate subjective drug effect, as expected. We used the cold pressor test as the pain model in all studies, because it resemples clinical tonic pain (Jones et al. 1988). In addition, the cold pressor test was non-invasive and easy to repeat under study days. However, pharmacodynamic differences between phases remained minor in all studies. This may reflect the small dose of S-ketamine, small sample size or a log-linear relationship between drug concentration and effect.

6.2. Pharmacokinetic considerations

6.2.1. Effect of CYP3A inhibition

Pharmacokinetics of oral S-ketamine was profoundly affected by CYP3A inhibitors clarithromycin and GFJ. Unexpectedly, the CYP3A4 inhibitor itraconazole failed to affect the pharmacokinetics of oral S-ketamine.

Inhibition of CYP3A-mediated metabolism by clarithromycin produced 2.6 times higher mean $AUC_{0-\infty}$ and a 3.6 times higher mean C_{max} of ketamine compared to control phase. The interactions of another CYP3A inhibitor, GFJ with ketamine was in line with that of clarithromycin. GFJ increased the mean $AUC_{0-\infty}$ of ketamine by 3.0-fold and C_{max} by 2.1-fold compared to placebo. These findings confirm the important role of CYP3A in the elimination of oral ketamine.

Previously, concomitant use of clarithromycin inhibits the metabolism of several CYP3A substrates, such as oxycodone, midazolam and triazolam

(Greenblatt et al. 1998; Westphal 2000; Liukas et al. 2011). Clarithromycin is a strong CYP3A4 inhibitor but has no effect on CYP2B6-mediated metabolism, which made it appropriate pretreatment drug in this study (Walsky et al. 2006). Clarithromycin produced significant changes in the relative formation of norketamine as judged from 54% decrease in the mean AUC_m/AUC_p ratio. The mean $t_{1/2}$ of ketamine was only slightly prolonged. These results suggest that the inhibitory effects of clarithromycin take place mainly during the first-pass phase in the metabolism of ketamine, as detected in previous studies with midazolam and clarithromycin (Gorski et al. 1998; Pinto et al. 2005).

GFJ prolonged the mean $t_{1/2}$ of ketamine from 4.9 to 5.7 h but the mean C_{max} of norketamine remained unchanged between the phases. GFJ is considered to be a rather selective CYP3A4 inhibitor at the intestinal level. The overall effect of GFJ on the elimination of ketamine was similar compared to clarithromycin. The decrease in the AUC_m/AUC_p ratio was equal suggesting the important role of CYP3A4 rather than OATP or other transporter proteins. However, GFJ is a complex mixture of phytochemicals making the definitive interpretation of the interaction mechanism difficult. Clinically significant GFJ-drug interactions have been reported only for CYP3A and OATPs (Bailey et al. 1989; Kantola et al. 1998; Hanley et al. 2011). In fact, also clarithromycin can inhibit the uptake of pitavastatin from human hepatocytes *via* OATP (Hirano et al. 2006). However, the major role of the inhibition of CYP3A, in our study, was more likely, because there is no evidence to support ketamine being an OATP substrate.

Both clarithromycin and GFJ were able to increase the plasma concentration of S-ketamine, which is a substrate of CYP3A, *in vivo*. The findings were in line with previous DDI works with clarithromycin and GFJ (Ozdemir et al. 1998; Nieminen et al. 2010b; Liukas et al. 2011). Unexpectedly, a well documented CYP3A4 inhibitor, itraconazole, had no significant interactions with S-ketamine. Only a slight decrease in the AUC_m/AUC_p ratio was detected between the phases. Poor compliance with itraconazole or occasional reduction of its absorption was excluded by measuring the concentrations of itraconazole in the morning of the study day. Intestinal transporter proteins, which are located on the apical (gut lumen) or basolateral (portal blood flow) membranes of enterocytes, can produce the movement of drugs either into the cell (influx) or out of the cell (efflux). Oral bioavailability depends on these flux dynamics - influx reduces while efflux increaces this bioavailability (Hanley et al. 2011). Our findings were surprising, because itraconazole is a potent intestinal and hepatic CYP3A, P-gp, BCRP and even CYP2B6 inhibitor *in vitro* (Wang et al. 2002b; Walsky et al. 2006; Gupta et al. 2007; Yasui-Furukori et al. 2007). The above-mentioned properties of itraconazole would be expected to restrict the

metabolism of ketamine, augment intestinal absorption and increase the bioavailability of ketamine. The absence of DDI by itraconazole on the pharmacokinetics of ketamine may be explained by action of other transporter proteins than P-gp or BCRP. These modulators, such as OATPs, may mask the possible inhibitory effect of CYP3A on the elimination of S-ketamine. OATPs are well known influx transporters and could theoretically transfer ketamine from the intestinal lumen to portal circulation. The profound inhibition of OATPs by itraconazole could reduce the absorption of ketamine from the gut lumen and reduce its bioavailability. However, as with S-ketamine, there is no evidence to support itraconazole being an OATP inhibitor.

6.2.2. Effect of CYP3A induction

Rifampicin and St. John´s wort were the inducers of CYP3A4 in our studies. Rifampicin can also act as an unselective inducer of multiple CYPs such as CYP2C9 and CYP2B6 (Niemi et al. 2003). As expected, both drugs reduced the plasma concentrations of S-ketamine. In Study IV, ketamine was administered orally and intravenously. The interaction of rifampicin with S-ketamine was more profound when ketamine was ingested and exposed to first-pass metabolism reflecting the strong enhancement of induction of CYP3A, P-gp and/or CYP2B6 by rifampicin (Greiner et al. 1999; Kanebratt et al. 2008). Rifampicin decreased the mean $AUC_{0-\infty}$ of intravenous S-ketamine only by 14%, while reduction after oral S-ketamine was 86%. The peak plasma concentrations of intravenous S-ketamine remained unchanged, whereas oral S-ketamine concentrations were decreased by 81% by rifampicin. The oral bioavailability of ketamine has previously been estimated to be between 17-24% (Grant et al. 1981; Clements et al. 1982; Yanagihara et al. 2003). In our study, the mean oral bioavailability was only 11% and was decreased as low as 2% by rifampicin. This means that concomitant use of rifampicin and oral S-ketamine will definitely cause clinically inadequate concentrations of S-ketamine unless its dose is not substantially raised. The mean $t_{1/2}$ of S-ketamine and norketamine were moderately shortened by CYP3A inducer rifampicin. This may weaken the effect of S-ketamine in the concomitant use with rifampicin in clinical use.

The 72% decrease in the production of norketamine by rifampicin was unexpectedly high after intravenous S-ketamine, because there was only 14% reduction in the $AUC_{0-\infty}$ of ketamine. This could be due to further induction of norketamine metabolism by rifampicin. Besides being a CYP inducer, rifampicin is a competitive inhibitor of CYP3A4 enzyme *in vitro* (Kajosaari et al. 2005). Although rifampicin has is a powerful inducer of hepatic and intestinal CYP3A enzyme, it can also induce or inhibit several drug transporters (Vavricka et al. 2002; Niemi et al. 2003). In fact, rifampicin

reduces the plasma concentrations of digoxin due to P-gp induction (Greiner et al. 1999). However, the role of different transporters in the elimination of S-ketamine and norketamine is unknown. The clearance of intravenous S-ketamine was enhanced only by 19% by rifampicin. According to the results of our studies, intestinal first-pass metabolism of S-ketamine is induced greater than the hepatic rifampicin metabolism.

On the basis of our studies, rifampicin enhances the metabolism of S-ketamine greater than that of St. John´s wort. This may be due to an additional CYP2B6 induction by rifampicin. Two weeks pretreatment with the CYP3A4 inducer, St. John´s wort, decreased the mean $AUC_{0-\infty}$ of ketamine by 58% and the mean C_{max} by 66%. These findings were expected because other substrates of CYP3A besides S-ketamine are affected similarly by St. John´s wort (Sugimoto et al. 2001; Bauer et al. 2003; Nieminen et al. 2010a). The mean AUC_m/AUC_p increased by 2.2-fold after treatment with St. John´s wort supporting the role of CYP3A4-mediated N-demethylation of S-ketamine during the first-pass metabolism, both in the intestine and liver. St. John´s wort is not known to be an inducer of CYP2B6. Many drugs can act as a substrate for both CYP3A4 and P-gp (Bruyere et al. 2010). In fact, St. John´s wort has the potency to induce P-gp activity (Durr et al. 2000; Johne et al. 1999) making it difficult to specify the exact induction mechanism of St. John´s wort with various substrates. The definitive role of P-gp in the elimination of drugs can be studied only with substrates that are not dependent on CYP enzymes but are substrates of P-gp, like digoxin (Johne et al. 1999). As discussed above, in humans, there are minimal studies on ketamine showing that it is a substrate for P-gp or other transporters. In any case, the role of P-gp in the elimination of ketamine remains unclear after our studies because ketamine is strongly dependent on CYP-mediated metabolism *in vitro* (Yanagihara et al. 2001; Hijazi and Boulieu 2002).

6.2.3. Effect of CYP2B6 inhibition

CYP2B6 is a major enzyme in the metabolism of ketamine at low therapeutic concentrations *in vitro* (Yanagihara et al. 2001; Hijazi and Boulieu 2002). In Study III, we demonstrated that the metabolism of oral S-ketamine is impaired when CYP2B6 route is inhibited by ticlopidine. The mean increase in the $AUC_{0-\infty}$ of ketamine was 2.4-fold and the greatest individual increase was 10.4-fold. Reduced metabolism of ketamine led to 49% decrease in the mean AUC_m/AUC_p between the phases. This confirmed the significant role of CYP2B6 in the clearance of S-ketamine also *in vivo*. The findings in Study III are in agreement with a previous study demonstrating the effect of ticlopidine on the pharmacokinetics of bupropion that have CYP2B6-dependent metabolism (Turpeinen et al. 2005).

Although ticlopidine increased the $AUC_{0-\infty}$ of ketamine in all eleven subjects, the effect on the individual C_{max} of ketamine was not constant. The mean increase of C_{max} was 1.7-fold, but in three subjects, the effect was opposite. This variability may indicate that ticlopidine influences the absorption of S-ketamine from the intestine *via* P-gp and/or other transporters. In fact, ticlopidine modulates OATP-B action *in vitro* (Lu et al. 2006). In addition, coadministration with ergoloid mesylates decrease the AUC on ticlopidine in healthy volunteers by inhibiting the intestinal OATP-B- mediated uptake of ticlopidine during the absorption phase (Lu et al. 2006). As mentioned before, there exists no published data on the effect on P-gp on the pharmacokinetics of S-ketamine. Based on our studies, absorption and the oral bioavailability of S-ketamine may be affected by modulating P-gp or other transporters. Another explanation for the variation in the mean C_{max} of ketamine by ticlopidine may be the well-known genetic polymorphism in the activity of CYP2B6 (Code et al. 1997; Turpeinen et al. 2006). We did not measure the individual level of CYP2B6 expression.

While ticlopidine significantly increased the exposure to plasma levels of oral ketamine in Study III, the CYP3A inhibitor itraconazole caused no alterations in the concentrations of ketamine compared to placebo. When interpreting only Study III, CYP2B6-mediated metabolism may be a major mechanism in the elimination of ketamine at low therapeutic plasma concentrations. However, in Studies I, II, IV and V, we demonstrated a profound effect of CYP3A inducers and inhibitors on the metabolism of oral S-ketamine with equivalent plasma concentrations to those used in the Study III. By combining the results from Studies I - V, it is evident that S-ketamine undergoes elimination *via* both CYP3A and CYP2B6 enzymes. Additional findings derived from our studies are that itraconazole has no effect on the pharmacokinetics of ketamine. One explanation for the absence of pharmacokinetic interactions between itraconazole and ketamine may be that itraconazole may modulate the activities of transporter proteins other than P-gp such as OATP and BCRP and mask the effect of CYP3A-mediated inhibition on the elimination of ketamine.

Prior to our studies, minimal systematic *in vivo* information regarding the effect of well-known CYP inducers and inhibitors on the pharmacokinetic profile of S-ketamine in humans was studied. Chosen pretreatment drugs are all in clinical use and also herbal medicinal products such as St. John´s wort and GFJ are widely used. Previous experiments with human liver microsomes suggested that CYP3A4 and CYP2B6 play a major role in the metabolism of ketamine (Hijazi and Boulieu 2002; Yanagihara et al. 2003**;** Niwa et al. 2005b). Before we finished our last DDI work with S-ketamine, one study

group published their results on the effect of rifampicin on the plasma concentrations of S-ketamine after two hours infusion (20 mg / 70 kg/h and 40 mg / 70 kg/h) of S-ketamine (Noppers et al. 2011). Our results were in line with this study where rifampicin decreased the AUC_{0-300} of ketamine by 14% and C_{max} by 17%. Also, as in our study, after rifampicin treatment, the plasma concentrations of norketamine decreased.

6.3. Pharmacodynamics

The power calculation for the pharmacodynamics in our studies was based on the change in the $AUC_{0-\infty}$ of S-ketamine. None of the drug response findings were the main focus of our study design. However, we wanted to collect secondary data to evaluate differences in the behavioral or analgesic effects between study phases when a small dose of S-ketamine was employed. The dose of S-ketamine was kept minimal because we studied healthy volunteers and wanted to minimize the risk of adverse effects. A single oral dose of S-ketamine was well tolerated in all studies, as expected. After S-ketamine ingestion, two participants reported the feeling of drunkenness within one hour after dosing. No serious adverse effects were observed or reported by the volunteers. Half of the volunteers experienced transient visual disturbances and altered perception of sounds a few minutes after intravenous S-ketamine administration.

Subjectively rated relaxation was significantly decreased by GFJ and the drug effect increased by clarithromycin compared to control. In addition, the performance in the DSST increased significantly by GFJ, ticlopidine and itraconazole, even if itraconazole produced no differences in the plasma concentrations of ketamine between the phases. The parameters of subjectively rated drowsiness were significantly higher in the ticlopidine and itraconazole phases compared to placebo. The pharmacodynamic findings on behavioral effects between the phases appeared to be purely coincidental. We detected no significant differences in the analgesic scores between the phases. The most sensitive VAS-score with S-ketamine appeared to be self-reported drug effect. We detected a significant linear correlation between the C_{max} of ketamine and drug effect in the Study III during the ticlopidine phase, where the exposure to ketamine was greatest. A significant linear correlation was found with the behavioral effects and C_{max} of ketamine in the ketamine-rifampicin study, where the dose of S-ketamine was equal to that in Study III. These findings demonstrated that our methodology was sensitive enough to measure the behavioral effects of low dose S-ketamine. However, a small dose of S-ketamine and/or a small sample size may be the reason that the pharmacodynamic differences between the phases were partly illogical.

The dose of S-ketamine to produce analgesic effects measured by immersion of hand in ice water was too low. High interindividual variability made the pharmacodynamic and analgesic results, inconclusive. Recently, another study group demonstrated that a two-hour S-ketamine infusion produced a C_{max} of approximately 373 ng/ml which eased the heat pain by 50% in healthy volunteers (Sigtermans et al. 2009a). In the same study, a high concentration of plasma S-ketamine concentrations (2.2 μg/ml) was needed to decrease the pain parameters by 50% for electrical pain. In our studies, the maximum mean C_{max} of ketamine remained at the level of 32.5 ng/ml, which was a low concentration of ketamine to ease pain during CPT in healthy volunteers. However, the cold pressor test is a valid method for studying the analgesic effects of opioids (Posner et al. 1985; Staahl et al. 2008). There is no reason for CPT not to work also with S-ketamine when the exposure to S-ketamine is adequate.

6.4. Limitations of the study and general discussion

The exact contribution of CYP3A4, CYP2B6 or other CYP enzymes to the metabolism of ketamine *in vitro* is unclear. The role of a specific enzyme may fluctuate depending on the concentration of ketamine at the site of action of CYP enzyme (Yanagihara et al. 2001; Hijazi and Boulieu 2002). Ketamine undergoes extensive first pass metabolism *in vivo* and it has an oral bioavailability of only 17-24%. To perform systematic *in vivo* DDI studies with ketamine is challenging because ketamine is metabolized at least by three CYP enzymes *in vitro* (Grant et al. 1981; Clements et al. 1982; Chong et al. 2009). In addition, the role of P-gp or other transporters, such as OATP and BCRP in the pharmacokinetics of ketamine is not known. However, our pre-medication drugs, including ticlopidine, GFJ and clarithromycin were all OATP modulators (Hirano et al. 2006; Lu et al. 2006; Hanley et al. 2011). In addition, itraconazole can act as an intestinal and hepatic P-gp and BCRP inhibitor (Wang et al. 2002a; Walsky et al. 2006; Gupta et al. 2007; Yasui-Furukori et al. 2007). In Study IV, the pretreatment drug was rifampicin, which can act *via* several CYP enzymes and P-gp likewise St. John´s wort in Study II. In conclusion, the precise role of transporters in the elimination of S-ketamine can not be determined by our studies.

St. John´s wort and ticlopidine were discontinued after S-ketamine ingestion in the study morning although the pretreatment schedule was twice or three times daily. The $t_{1/2}$ of ticlopidine after a single dose is only about 13 h and $t_{1/2}$ of active inducing components of St. John´s wort is not known. The steady state of ticlopidine occurs after 5-10 days of treatment (Knudsen et al. 1992). It is plausible that the interaction was constant during the elimination phase of S-ketamine and blood collection. In addition, the $t_{1/2}$ of ketamine was only about

5-7 h in all studies. The metabolism of clarithromycin is nonlinear and the inhibitor itself is metabolized by CYP3A making the prediction of interactions very difficult. As with St. John´s wort and ticlopidine, clarithromycin was not continued after S-ketamine ingestion. The $t_{1/2}$ of clarithromycin is five hours which is similar to the $t_{1/2}$ of ketamine. Therefore, the extent of the inhibition during the elimination phase of ketamine may be underestimated.

In Studies I - V, ketamine syrup (10-28 ml) was administered and rinsed with only 20 ml of water instead 150 ml of GFJ or water in the Study V. Ketamine may bypass first-pass metabolism and be absorbed directly from the oral cavity when less volume of water was ingested after S-ketamine syrup. However, there were no significant differences between the pharmacokinetic values of S-ketamine or formation of norketamine in control phases when different volume of water was ingested. This means that volume of water was adequate for S-ketamine syrup to reach duodenum. In case of possible excess residual S-ketamine in the oral cavity after low volyme water, pharmacokinetic profile of S-ketamine has showed to be similar after sublingual and oral administration (Chong et al. 2009).

6.5. Clinical implications

The use of low dose ketamine is becoming popular in the treatment of chronic- and perioperative pain (Bell et al. 2006). In addition, ketamine is effective in reducing perioperative opioid consumption (Bell et al. 2006). Perioperative opioids produce hyperalgesia and increase postoperative pain making the use of ketamine very useful (Guignard et al. 2000). Theoretically, ketamine could block the NMDA receptors in the central nervous system and reduce the chronic postoperative pain by modulating central sensitization. Previous pain management studies focused on the intravenous administration of ketamine. We demonstrated that there are drastic changes in the plasma concentrations of ingested S-ketamine when CYP3A4 or CYP2B6 enzymes are inhibited or induced. However, the plasma concentrations of ketamine unaffected by CYP3A4 inducer rifampicin when ketamine was administered intravenously. These findings suggest that the dose of oral S-ketamine should be carefully titrated in patients receiving drugs that affect CYP3A4 or CYP2B6 enzymes.

7. SUMMARY AND CONCLUSIONS

1. Inhibition of CYP3A by clarithromycin increased the exposure to ketamine by 2.6-fold and may inhibit its N-demethylation both during absorption and elimination. GFJ, which has inhibitory effects mainly on intestinal CYP3A, increased the exposure to ketamine by 3.0-fold. It indicates that the oral bioavailability of ketamine can be affected by the intestinal modulation of metabolism or transport. In contrast to our findings with clarithromycin and GFJ, another CYP3A inhibitor itraconazole had no effect on the pharmacokinetics of ketamine. Itraconazole may affect the absorption of ketamine *via* modulation of one or more transporter proteins and weaken the effect of CYP3A-mediated inhibition on the N-demethylation of ketamine.

2. Induction of CYP3A4 by St. John´s wort decreased the mean $AUC_{0-\infty}$ of oral S-ketamine by 58%. Another CYP3A inducer, rifampicin, greatly reduced the exposure to oral S-ketamine by 86%. The oral bioavailability of ketamine decreased from 11% to 2% by rifampicin. Despite the strong effect of rifampicin on oral S-ketamine, only a 14% decrease was observed after exposure to ketamine by intravenous administration. Our study showed that rifampicin induces intestinal first-pass metabolism of S-ketamine. These results confirm the role of CYP3A in the metabolism of oral S-ketamine *in vivo*.

3. The CYP2B6 inhibitor, ticlopidine, had a profound effect on the pharmacokinetics of ketamine. Ticlopidine treatment increased the mean $AUC_{0-\infty}$ of oral ketamine by 2.4-fold. The decrease in the mean AUC_m/AUC_p ratio was 49% suggesting that CYP2B6 has a significant role in the elimination of S-ketamine.

   The present results confirm previous *in vitro* findings that the metabolism of S-ketamine depends both on the CYP3A and CYP2B6 metabolic route. Because of the low dose of S-ketamine and high interindividual variability in the pharmacodynamic and analgesic measurements, the differences between the treatment phases remained inconclusive. However, concomitant use of drugs that affect CYP3A or CYP2B6 activity can make the ketamine treatment totally ineffective or raise the plasma concentrations of ketamine to unsafe levels.

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