Supplementary Information 1: Description of the original drug-drug interaction studies and drug analysis

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 study designs, and the summary of the studies are shown in Supplementary tables 1 and 2. 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 entering a 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.

Exclusion 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.

* For the sake of simplicity, the study participants were modelled as separate individuals in order to avoid over-parametrization issue in the final model due to the addition of inter-occasion variability.

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.

Study designs

All five studies were conducted using a randomized, placebo controlled, balanced, cross-over design. Pretreatments in Studies I - IV were double blinded, whereas the grapefruit juice (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 2). In Study III ticlopidine (Ticid^{R}, 250 mg tablet. Roche, Nutley, New Yersey) was ingested two times daily at 8:00 a.m. and 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

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.

Intravenous *S*-ketamine dose in Study IV was given as an injection in two minutes. 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.

Table S1. Design of studies I-V

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

Data from the phases coloured with gray were used in the current analysis.
*Same S-ketamine dose was administered in all phases of a single study.

Table S2. Summary of the clinical trials used to gather data for our population modeling study

Study	n	n samples/ individual	Gender (f/m)	Age (yrs)*	Weight (kg)*	Sampling period	Analytical technique	LLOQ[*] (ng/mL)	Interday %CV[§]	Reference
I	10	13	7f/3m	22.5 (19-27)	64.5 (52-73)	0.33-24 h	LC-MS[**]	0.1	2.3-12.6 2.9-6.9	1
II	12	13	6f/6m	26 (20-31)	70 (55-88)	0.33-24 h	LC-MS[**]	0.1	1.5-3.9 3.2-10.2	2
III	11[‡]	13	5f/6m	27.5 (20-35)	62 (50-84)	0.33-24 h 1.33-12 h[††]	LC-MS[**]	0.025 10[***]	<10 -	3
IV	12	13	6f/6m	22.5 (20-28)	59 (50-79)	0.33-24 h	LC-MS[**]	0.025	1.4-2.7 2.2-4.6	4
V	11[Π]	13	4f/7m	22 (20-27)	62 (50-85)	0.33-24 h	LC-MS[**]	0.025	<5 -	5

LLOQ, Lower Limit of Quantification; CV, coefficient of variation

*mean and range

[**] Agilent 1100 series HPLC (Agilent Technologies, Waldbronn, Germany) and API 2000 QTRAP-tandem Mass Spectrometer (Sciex Division of MDS Inc., Toronto, Ontario, Canada.

[†] Ticlopidine dose, [††] Sampling period for Ticlopidine, [***] LLOQ for Ticlopidine

[‡] Modelled as 22 individuals, 11 for placebo phase and 11 for Ticlopidine pre-dosing phase

[Π] Modelled as 22 individuals, 11 from PO (per oral) S-ketamine administration and 11 from IV administration

[§] Interday CV for *S*-Ketamine (top value) and Norketamine (bottom value)

In Studies I - III, the last pretreatment was taken at 8:00 a.m. and S-ketamine was administered at 9:00 a.m. In Studies I, II and IV during the placebo phase the 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, during the placebo phase, pre-treatment with 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 150 ml of water.

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 III, an additional blood sample was drawn before administration of any drugs in the morning of the study day. Plasma was separated within 30 min and stored at -70ºC until analysis.

Determination of plasma drug concentrations

Determination of S-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 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.

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.

References

1. Hagelberg NM, Peltoniemi MA, Saari TI, et al. Clarithromycin, a potent inhibitor of CYP3A, greatly increases exposure to oral S-ketamine. *Eur J Pain*. 2010;14(6):625-629. doi:10.1016/j.ejpain.2009.10.003.
2. 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(6):743-750. doi:10.1111/j.1472-8206.2011.00954.x.
3. Peltoniemi MA, Saari TI, Hagelberg NM, et al. Exposure to Oral S-ketamine is unaffected by itraconazole but greatly increased by ticlopidine. *Clin Pharmacol Ther*. 2011;90(2):296-302. doi:10.1038/clpt.2011.140.
4. Peltoniemi MA, Saari TI, Hagelberg NM, et al. Rifampicin has a Profound Effect on the Pharmacokinetics of Oral S-Ketamine and Less on Intravenous S-Ketamine. *Basic Clin Pharmacol Toxicol*. 2012;111(5):325-332. doi:10.1111/j.1742-7843.2012.00908.x.
5. 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(6):979-986. doi:10.1007/s00228-012-1214-9.