RESEARCH PROTOCOL

Role of CYP2B6 polymorphisms in ketamine metabolism and clearance

Evan D. Kharasch, M.D., Ph.D.
Russell D. and Mary B. Shelden Professor of Anesthesiology
Director, Division of Clinical and Translational Research
Washington University School of Medicine
Department of Anesthesiology
660 S. Euclid Ave., Campus Box 8054
St. Louis, Mo. 63110
Voice: (314) 362-8796
Fax: (314) 747-3371
E-mail: kharasch@wustl.edu

Original Version: July 9, 2013

1. SYNOPSIS

Study Title	Role of CYP2B6 polymorphisms in ketamine metabolism and clearance
Objective	To determine the effects of cytochrome P4502B6 (CYP2B6) genetic variants on ketamine metabolism and clearance
Study Design	Single-center, open label, single-session study to evaluate ketamine pharmaco-kinetics in subjects with known CYP2B6 genotype
Study Period	Planned enrollment duration: Approximately 3 months. Planned study duration: 1 day per subject
Number of Patients	Approximately 40 healthy volunteers identified by CYP2B6 genotyping by HRPO-approved screening
Inclusion and Exclusion Criteria	Inclusion Criteria a) 18-50 yr old b) *CYP2B6*1/*1, CYP2B6*1/*6* or *CYP2B6*6/*6* genotype c) Good general health with no remarkable medical conditions d) BMI < 33 e) Provide informed consent Exclusion Criteria a) Known history of liver or kidney disease b) Use of prescription or non-prescription medications, herbals, foods or chemicals known to be metabolized by or affecting CYP2B6 c) Females who are pregnant or nursing d) Known history of drug or alcohol addiction (prior or present addiction or treatment for addiction) e) Direct physical access to and routine handling of addicting drugs in the regular course of duty (a routine exclusion from studies of drugs with addiction potential)
Study Medication Administration	Subjects will be studied on one occasion at Washington University in St. Louis School of Medicine for administration of study drugs and blood collection. Study drug is oral ketamine HCl solution (0.4 mg/kg)
Measurements	Plasma samples are obtained for 12 hr after ketamine for measurement of parent drug and metabolite concentrations. A 24hr urine collection is also obtained. Subjective self-assessment of ketamine effect is performed using visual or verbal analog scales. Ketamine and metabolite concentrations are determined by stereoselective LC-MS/MS.
Outcomes	Primary: Plasma norketamine/ketamine AUC ratio Secondary: Plasma ketamine enantiomers and metabolite AUC, maximum concentration, apparent oral clearance, and metabolite formation clearances. Influence of *CYP2B6*6* hetero or homozygote genotype on above primary and secondary outcomes

2. STUDY PROTOCOL

2.1 Background and Significance

Ketamine is a noncompetitive NMDA receptor antagonist, originally developed in 1964 as a "dissociative" anesthetic, and FDA approved in 1970. It can be administered by intravenous, intramuscular, oral, sublingual, intranasal, and rectal routes. Ketamine has been widely used for decades in anesthesia, in part, because unlike most other intravenous anesthetics, it does not significantly depress the respiratory and circulatory systems. In addition, ketamine causes significant anti-nociceptive and anti-hyperalgesic (i.e. analgesic) effects at low doses – lower than those which cause loss sedation and loss of consciousness.

More recently, there has been marked interest in the use of ketamine for other applications. Oral ketamine has been evaluated extensively for use in chronic pain management.[1] Use in pediatric sedation, analgesia, and emergency room analgesia is also of interest.[2-5] More recently, has been the discovery that ketamine may be effective in the therapy of treatment-resistant depression, and with very fast response rates.[6-9] A review of clinicaltrials.gov reveals a plethora of recent studies on ketamine. Thus there is very active research and clinical interest in ketamine.

Ketamine is administered clinically as a racemic mixture (R- and S-ketamine). Ketamine undergoes extensive metabolism, primarily via N-demethylation to norketamine, and to several other metabolites.[10-13] First-pass metabolism of oral ketamine is considerable, thus it is only about 30% bioavailable. There are stereoselective differences in ketamine enantiomers metabolism and disposition. It has recently been established that cytochrome P4502B6 (CYP2B6) is the major isoform catalyzing ketamine N-demethylation *in vitro*, at therapeutic concentrations.[14-16] A recent clinical investigation confirmed the predominant role of CYP2B6, and lack of significant CYP3A involvement, in ketamine pharmacokinetics and metabolism.[17]

CYP2B6 is a highly polymorphic enzyme.[18] The most common variant allele, *CYP2B6*6*, found most commonly in Africans, Africans-Americans, and some Asian populations, is associated with diminished hepatic CYP2B6 enzyme expression. Compared with wild-type *CYP2B6*1/*1*. A recent *in vitro* investigation demonstrated that CYP2B6.6 (the protein encoded by the CYP2B6*6 allele) has diminished catalytic activity towards ketamine N-demethylation (compared with wild-type CYP2B.1, and liver microsomes from humans heterozygous or homozygous for the *CYP2B6*6* allele also had diminished catalytic activity towards ketamine N-demethylation, compared with *CYP2B6*1/*1*.[16]

Nonetheless, there is no formal evaluation of ketamine pharmacokinetics, metabolism or clearance in *CYP2B6*6* carriers. This investigation will formally address the role of *CYP2B6* polymorphism in ketamine disposition. The research proposed will test the hypothesis that *CYP2B6* variants (*CYP2B6*6* hetero or homozygotes) *in vivo* will have decreased ketamine metabolism and clearance.

2.2 Objective

Determine the influence of CYP2B6 genetic variation, specifically *CYP2B6*6* polymorphism, on ketamine metabolism and clearance. Other, rare genotypes may be enrolled as a secondary objective.

2.3 Patient Selection

Subjects will be recruited from the greater St. Louis community that has been identified as carriers of *CYP2B6* polymorphisms (identified by participation in Washington University HRPO approved study HRPO#201203033). Thirty evaluable, healthy male and non-pregnant female 18-50 yr volunteers, in approximately equal sex distribution, will be studied. Minorities will be actively sought and included, with minority composition to reflect that of the greater Washington University/St. Louis community.

2.3.1 Inclusion Criteria

Each subject must meet all of the following criteria:

a) 18-50 yr old
b) *CYP2B6*1/*1, CYP2B6*1/*6* or *CYP2B6*6/*6* genotype (see table) (Note: subjects of other rare genotype but with one or more 516G>T, 785A>G, 983T>C or 1459C>T polymorphism may be enrolled at PI's discretion)
c) Good general health with no remarkable medical conditions
d) BMI <33
e) Provided informed consent

Allele	Protein	cDNA Change	Amino acid change	Enzyme activity	
*CYP2B6*4*	CYP2B6.4	785A>G	K262R	Incr.	Lang *et al.*, 2001; Kirchheiner *et al.*, 2003
*CYP2B6*5*	CYP2B6.5	1459C>T	R487C		Lang *et al.*, 2001
*CYP2B6*6*	CYP2B6.6	516G>T; 785A>G	Q172H; K262R	Decr. expr	Lang *et al.*, 2001; Tsuchiya *et al.*, 2004; Hofmann *et al.*, 2008
*CYP2B6*7*	CYP2B6.7	516G>T; 785A>G; 1459C>T	Q172H; K262R; R487C		Lang *et al.*, 2001
*CYP2B6*9*	CYP2B6.9	516G>T	Q172H		Lamba *et al.*, 2003
*CYP2B6*13*	CYP2B6.13	415A>G; 516G>T; 785A>G	K139E; Q172H; K262R	Decr.?	Lang *et al.,* 2004
*CYP2B6*16*	CYP2B6.16	785A>G; 983T>C	K262R; I328T	Decr. expr.	Wang *et al.*, 2006
*CYP2B6*18*	CYP2B6.18	983T>C	I328T	Decr. expr.	Klein *et al.*, 2005
*CYP2B6*19*	CYP2B6.19	516G>T; 785A>G; 1006C>T	Q172H; K262R; R336C	Decr. expr.	Klein *et al.*, 2005
*CYP2B6*20*	CYP2B6.20	503C>T; 516G>T; 785A>G	T168I; Q172H; K262R	Decr. expr.	Klein *et al.*, 2005
*CYP2B6*26*	CYP2B6.26	499C>G; 516G>T; 785A>G	P167A; Q172H; K262R	Decr	Gatanaga *et al.*, 2007

2.3.2 Exclusion Criteria

Subjects will not be enrolled if any of the following criteria exist:

a) Known history of liver or kidney disease
b) Use of prescription or non prescription medications, herbals, foods or chemicals known to be metabolized by or affecting CYP2B6
c) Females who are pregnant or nursing
d) Known history of drug or alcohol addiction (prior or present addiction or treatment for addiction)
e) Direct physical access to and routine handling of addicting drugs in the regular course of duty (this is a routine exclusion from studies of drugs with addiction potential)

2.4 Design and Procedures

2.4.1 Study Design

Single-center, open-label, single-session study. The study population will be thirty evaluable healthy male and nonpregnant female 18-50 yr volunteers. All subjects will undergo the same study procedures.

2.4.2 Minimization of Bias

Placebo control and double-blinding are not required. Plasma drug concentration is not influenced by subjects' or investigators' knowledge of study aims and hypotheses. Results will be coded by enrollment number, such that pharmacokinetic data analysis will be blinded as to subjects' genotype group.

2.4.3 Pre-Study Period
Subjects who are potential candidates for the study will be educated as to the study procedures, benefits, and potential risks. They will fill out a health self-assessment, including a structured interview to screen for history of substance abuse or addiction. Each subject who qualifies for entry into the study on the basis of inclusion/exclusion criteria, prestudy evaluations and completion of an informed consent will be assigned the next available patient number. This indicates enrollment in the study. Weight will be recorded. A urine HCG pregnancy test for women of childbearing potential will be performed on the study day prior to study drug administration. Subjects who drop out of the study prior to completing the study will be replaced by using the next available patient number.

2.4.4 Study Period
Subjects will be required to refrain from: 1) alcohol for 48 hr prior to and during study days, 2) caffeine-containing beverages the day of study drug administration, 3) no grapefruit, oranges or apples or their juices for 5d prior to the study day, 4) food/liquids after midnight the day prior to drug administration (to eliminate food influence on oral drug absorption), 6) non-study medications (including over the counter and/or herbal), for 3d prior to study visit, without prior PI approval.

Subjects will be studied at Washington University in St. Louis School of Medicine. The study day will consist of an approximately 12.5-13 hr stay for drug administration, blood sampling, and urine collection. Subjects will be given a container to take home and continue their urine collection, and return the next morning to return their 24 hr urine collection.

Subjects will have a peripheral IV catheter inserted for blood sampling. Subjects are then administered 0.4 mg/kg oral racemic ketamine with 200cc water. At various time points, up to 12 hours after drug administration, venous blood samples will be drawn. Typical sampling (5cc) will be before, and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 hr after ketamine (13 samples, 65 ml). Coincident with blood sampling, subjective self-assessment of drug effect is performed using visual or verbal analog scales (scored from 0 to 100) for levels of alertness/sedation (almost asleep to wide awake), energy level (no energy to full of energy), confusion (confused to clear headed), clumsiness (extremely clumsy to well-coordinated), anxiety (calm/ relaxed to extremely nervous), and nausea (no nausea to worst nausea).

Two hours after dosing is completed subjects will be free to move around and be given a standard meal. Subjects will have free access to food and water during the study session and other meals will be provided during the day. At the end of the session the IV catheter is removed and subjects go home. Transportation home will be provided if required.

2.4.5 Study Drug and Doses
ketamine HCl solution (0.4 mg/kg administered orally). This is equivalent to about 0.1-0.15 mg/kg IV.

Drugs will be obtained from commercial sources or the Barnes-Jewish Hospital investigational pharmacy.

2.5 Observations and Measurements

2.5.1 Drug and metabolite concentrations
Plasma and urine concentrations of ketamine and metabolites are determined by LC-mass spectrometry using stereoselective methods developed and currently implemented in the PI's lab.

2.5.2 Calculations
Area under the curve (AUC) of plasma ketamine concentration is determined by noncompartmental methods using nonlinear regression analysis. Clearance is dose/AUC.

2.5.3 Primary Outcome Measure
Ketamine metabolism, measured as plasma norketamine/ketamine concentration-time $AUC_{0-\infty}$ ratio, by *CYP2B6*6* hetero or homozygote genotype.

2.5.4 Secondary Outcome Measures
1) ketamine enantiomers area under the plasma concentration curve ($AUC_{0-\infty}$), maximum plasma concentration, apparent oral clearance (and by *CYP2B6*6* hetero or homozygote genotype)
2) norketamine enantiomers formation clearances (and by genotype)

2.5.5 Statistical Methods
Plasma and urine ketamine and metabolite concentrations are analyzed by noncompartmental methods. Outcome measures in wild-type and *CYP2B6*6* carriers will be evaluated by ANOVA. If the hypothesis is correct, we will observe diminished ketamine metabolism and clearance in *CYP2B6*6* carriers.

2.5.6 Sample Size
This is a pilot study. We plan to study 30 evaluable subjects, assembled by *CYP2B6* genotyping, described previously (HRPO#201203033), to compose groups of 10 wild-type (*CYP2B6*1/*1*), 10 *CYP2B6* heterozygotes (*CYP2B6*1/*6)* and 10 *CYP2B6*6* homozygotes (CYP2B6**6/*6*).

3.0 Management of Intercurrent Events

3.1 Adverse Experiences
The investigator will closely monitor subjects for evidence of adverse events. All adverse events will be reported and followed until satisfactory resolution. The description of the adverse experience will include the time of onset, duration, intensity, etiology, relationship to the study drug (none, unlikely, possible, probable, highly probable), and any treatment required.

3.2 Premature Discontinuation
If a subject withdraws from the study, the subject will be replaced in order to provide the required number of subjects. Subjects will be withdrawn if the investigator decides that discontinuation is in the best interest of the subject, or the subject requests withdrawal from the study.

3.3 Potential Risks

3.3.1 Potential risks from ketamine
Oral ketamine has been reported to produce fewer and milder adverse effects than parenteral ketamine, because lower peak plasma levels are reached after oral intake, there is extensive first-pass metabolism, the main metabolite norketamine reaches higher plasma levels, and norketamine might have a more favorable safety profile than ketamine.[1] The most frequently observed adverse effects of oral ketamine reported (across a range of doses) were effects on the central nervous system, such as sedation, somnolence, dizziness, sensory illusions, hallucinations, nightmares, dissociative feeling and blurred vision. Qualitatively similar adverse effects in patients were seen with intramuscular (0.4 mg/kg) and oral (4 mg/kg) ketamine, but there were fewer side-effects in the latter group, suggesting that adverse effects of oral ketamine are about one tenth that of intravenous ketamine. No distinction was made between effects of a single dose and effects after long term dosing.[1] Adverse effects of ketamine (0.1 mg/kg IV) in healthy volunteers were minimal.[19] Based on prior experience with ketamine, the dose proposed (0.4 mg/kg oral, equivalent to 0.1-0.15 mg/kg IV), and prior published experience, some subjects may experience very mild drowsiness for about 1-2 hr after ketamine administration. As a precaution, volunteers are kept under observation if pharmacologic effects are present, and they are admonished to neither drive nor operate dangerous machinery for the remainder of the study day. Overall the risks associated with the use of ketamine in healthy volunteers are very small.

3.3.2 Other Potential Risks

Intravenous catheter placement can cause a bruise. The amount of blood drawn will not constitute a risk to subjects, since sessions are carefully timed to prevent phlebotomy from exceeding recommended limits. No psychological risks to subjects are envisioned.

3.4 Procedures to Minimize Potential Risks

The ketamine dose to be studied is subanesthetic, less than the recommended starting dose (0.5 mg/kg) for oral therapy in ketamine-naïve patients, and commensurate with that currently used in clinical studies (clinicaltrials.gov). Studies are conducted in the Washington University Department of Anesthesiology Research Unit under the general supervision of the PI, a board-certified anesthesiologist thoroughly trained and experienced in the administration of drugs, assessment of adverse effects, and their treatment. Subjects are monitored by trained (RN) nursing personnel. A urine pregnancy test will be performed on women of childbearing potential and subjects excluded if pregnant. Subjects will be informed that participation is voluntary and they may refuse to participate and may withdraw from the study at any time without penalty. Subjects will be told that in the event of a physical injury as the direct result of study procedures, they will be cared for by a member of the investigating team at no cost, within the limits of the Washington University compensation plan.

3.5 Data and Safety Monitoring Plan

The monitoring plan has been specifically developed to maximize research participant safety and for the protocols planned herein. In general, the PI has developed a specific set of Standard Operating Procedures (SOPs) for clinical research. All individuals working under the PI are required to read, be familiar with, and compliant with the SOPs. The specific monitoring plan for this investigation is commensurate with the risks and the size and complexity of the investigations planned. These risks, as described above, are small. The basic clinical protocol design has been used previously by the PI in hundreds of subjects, and the response to the drugs, adverse events (essentially only nausea and vomiting with opioids) and potential risks are well-characterized and well-known to the PI. Based on these considerations, we utilize a monitoring plan that involves:

(1) engaging a colleague not involved in the study to serve in a monitoring capacity. Based on the small size and low-risk, we envision only a third person (the colleague), not a full DSMB.
(2) a specific plan for submitting Adverse Event Reports to the IRB. That plan is detailed in the PI's SOP for Adverse Event Reporting (attached).

4. HUMAN SUBJECTS RESEARCH

4.1 Protection of Human Subjects

This study will involve normal subjects, whose participation will be strictly voluntary. This study is not a clinical trial which is designed to assess the safety and/or efficacy of drugs, devices, diagnostics, treatments, or preventive measures in humans for the treatment of any disease. The study will be covered under appropriate Washington University Institutional Review Board protocols and consent forms. The study will be conducted under the supervision of the PI, a Board-Certified and GCP-certified anesthesiologist with several years experience in the conduct of human volunteer studies.

4.2 Sources of Materials

For studies involving healthy volunteers, no medical records are used. Data on health and medication use are provided by subjects who fill out a health status questionnaire. Specimens include blood obtained exclusively for research purposes.

4.3 Recruitment and Informed Consent

Subjects will be approached for participation from a cohort of individuals previously genotyped for *CYP2B6* (HRPO approved protocol #201203033), in which subjects provide written consent for contact

and recruitment for additional research studies. Subjects with specific genotypes (*CYP2B6*1/*1*, *CYP2B6*1/*6* or *CYP2B6*6/*6*) will be invited to participate in this protocol. Subjects will be provided verbal and written descriptions of the study aims, procedures, risks, and benefits, and will be required to give written informed consent. A member of the investigative team provides all study descriptions, informed consent, and answers all questions. No deception is required for the purposes of this study. Subjects are informed verbally and in writing that participation is voluntary and they may refuse to participate and may withdraw from the study at any time without penalty.

4.4 Potential Benefits of the Proposed Research to the Subjects and Others

There is no benefit to individual subjects in this study. Society may benefit from a better understanding of ketamine metabolism, drug interactions and pharmacokinetics, leading to improved practice standards among clinicians.

4.5 Inclusion of Women

Studies and their advertisements actively encourage the participation of women in the research. As a matter of operational policy, our studies of healthy volunteers routinely and deliberately include equivalent numbers of women and men. To ensure sufficient enrollment of women, we typically close enrollment to men once their quota has been filled. This approach has been highly successful. Women of childbearing potential are not excluded from our research protocols. Pregnancy testing is performed as a matter of course to protect female research subjects, and subjects are instructed when appropriate to use adequate contraceptive measures.

4.6 Inclusion of Minorities

All of our studies and their advertisements actively encourage the participation of minorities in research. Our minority recruiting typically matches the demographic composition of the Washington University community from which subjects will be recruited (78% white, 21% Black, <1 % Hispanic). However, based on the higher incidence of *CYP2B6*6* allele in African-Americans, we anticipate a higher enrollment of minorities related to increased incidence of genotype.

4.7 Inclusion of Children

Children <18 yr will <u>not</u> be studied in this pilot investigation because there is no direct benefit to them, and the study aims address only adults.

5. REFERENCES

1. Blonk MI, Koder BG, van den Bemt PM, Huygen FJ: Use of oral ketamine in chronic pain management: a review. Eur J Pain 2010;14:466-72
2. Anand KJ: Pharmacological approaches to the management of pain in the neonatal intensive care unit. J Perinatol 2007;27 Suppl 1:S4-S11
3. Bleiberg AH, Salvaggio CA, Roy LC, Kassutto Z: Low-dose ketamine: efficacy in pediatric sedation. Pediatr Emerg Care 2007;23:158-62
4. Dallimore D, Herd DW, Short T, Anderson BJ: Dosing ketamine for pediatric procedural sedation in the emergency department. Pediatr Emerg Care 2008;24:529-33
5. Galinski M, Dolveck F, Combes X, Limoges V, Smail N, Pommier V, Templier F, Catineau J, Lapostolle F, Adnet F: Management of severe acute pain in emergency settings: ketamine reduces morphine consumption. Am J Emerg Med 2007;25:385-90
6. Katalinic N, Lai R, Somogyi A, Mitchell PB, Glue P, Loo CK: Ketamine as a new treatment for depression: A review of its efficacy and adverse effects. Aust N Z J Psychiatry 2013:in press
7. Mathews DC, Zarate CA, Jr.: Current status of ketamine and related compounds for depression. J Clin Psychiatry 2013;74:516-7

8. Murrough JW: Ketamine as a novel antidepressant: from synapse to behavior. Clin Pharmacol Ther 2012;91:303-9
9. Murrough JW, Perez AM, Pillemer S, Stern J, Parides MK, Aan Het Rot M, Collins KA, Mathew SJ, Charney DS, Iosifescu DV: Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment-resistant major depression. Biol Psychiatry 2013:in press
10. Trevor AJ: Biotransformation of ketamine, Status of ketamine in Anesthesiology. Edited by Domino EF. Ann Arbor, NPP Books, 1990, pp 93-100
11. Turfus SC, Parkin MC, Cowan DA, Halket JM, Smith NW, Braithwaite RA, Elliot SP, Steventon GB, Kicman AT: Use of human microsomes and deuterated substrates: an alternative approach for the identification of novel metabolites of ketamine by mass spectrometry. Drug Metab Dispos 2009;37:1769-78
12. Desta Z, Moaddel R, Ogburn ET, Xu C, Ramamoorthy A, Venkata SL, Sanghvi M, Goldberg ME, Torjman MC, Wainer IW: Stereoselective and regiospecific hydroxylation of ketamine and norketamine. Xenobiotica 2012;42:1076-87
13. Zhao X, Venkata SL, Moaddel R, Luckenbaugh DA, Brutsche NE, Ibrahim L, Zarate CA, Jr., Mager DE, Wainer IW: Simultaneous population pharmacokinetic modelling of ketamine and three major metabolites in patients with treatment-resistant bipolar depression. Br J Clin Pharmacol 2012;74:304-14
14. Yanagihara Y, Kariya S, Ohtani M, Uchino K, Aoyama T, Yamamura Y, Iga T: Involvement of CYP2B6 in N-demethylation of ketamine in human liver microsomes. Drug Metab. Dispos. 2001;29:887-90
15. Hijazi Y, Boulieu R: Contribution of CYP3A4, CYP2B6, and CYP2C9 isoforms to N-demethylation of ketamine in human liver microsomes. Drug Metab Dispos 2002;30:853-8
16. Li Y, Coller JK, Hutchinson MR, Klein K, Zanger UM, Stanley NJ, Abell AD, Somogyi AA: The CYP2B6*6 allele significantly alters the N-demethylation of ketamine enantiomers in vitro. Drug Metab Dispos 2013;41:1264-72
17. 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
18. Zanger UM, Klein K: Pharmacogenetics of cytochrome P450 2B6 (CYP2B6): advances on polymorphisms, mechanisms, and clinical relevance. Front Genet 2013;4:24
19. Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB, Jr., Charney DS: Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry 1994;51:199-214