Pharmacogenetics of Ketamine Metabolism and Immunopharmacology of Ketamine

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Abstract

Ketamine is an anaesthetic agent that is being increasingly used at sub-anaesthetic doses as an analgesic or co-analgesic in the management of postoperative pain and chronic pain. In most countries, ketamine is administered as a racemic compound consisting of two enantiomers: *(S)*- and *(R)*-ketamine at a ratio of 1:1. Ketamine analgesia is frequently restricted by the low efficacy and large interindividual variability in drug response, which may be associated with the differences in the plasma pharmacokinetics. Previous *in vitro* studies suggested that ketamine is primarily cleared to its active metabolite, norketamine, by two hepatic CYP enzymes: CYP2B6 and CYP3A4, whose expression and catalytic activities show vary large variability in humans due to genetic and environmental influences. Therefore, it is logical that the variability in these enzymes contributes to the variability in ketamine pharmacokinetics. Additional variability in analgesic response may arise from the heterogeneous nature of pain, as ketamine is expected to be more effective against hyperalgesia and allodynia (neuropathic pain). Although these anti-hyperalgesic and anti-allodynic mechanisms have been primarily associated with the non-competitive antagonism of neuronal NMDA receptors, it has been speculated that the attenuation of proinflammatory response may also contribute to ketamine analgesia, since there is evidence to suggest important roles of proinflammatory cytokines in the pathogenesis of neuropathic pain.

Thus, the major aims of this thesis were to examine the influence of variability in enzyme activity, especially that due to *CYP2B6* genetic polymorphisms, on ketamine pharmacokinetics *in vitro* and *ex vivo* in chronic pain patients. The secondary aims of this thesis were to examine the effects of ketamine and norketamine enantiomers on proinflammatory cytokine production *in vitro*, using interleukin-6 (IL-6) as a marker of cytokine production; and to explore the mechanistic characterisation of the drug actions using both *in silico* docking simulations and *in vitro* experiments.

The *in vitro* experiments showed that, at clinically relevant concentrations, CYP2B6 but not CYP3A4 is the major isoform responsible for ketamine metabolism to norketamine in human liver microsomes (HLM). Moreover, the presence of the *CYP2B6*6* allele, the most common allelic variant of the *CYP2B6* gene, reduced the intrinsic clearance of both ketamine enantiomers in HLMs and cDNA-expressed proteins by at least 62%. This substantial *CYP2B6*6* allele-induced decrease in ketamine intrinsic clearance *in vitro* was also observed *ex vivo* in chronic pain patients who received 24 h continuous subcutaneous infusions of 100 mg to 500 mg ketamine. The impact of the *CYP2B6*6* allele, by itself and in combined with the age of the patient, explained approximately 40% and 60% of interindividual variation in plasma ketamine concentrations at steady-state, respectively, whereas sex, disease and other medications had no significant influences. The decrease of ketamine clearance may be associated with the adverse effects of ketamine, as patients who experienced adverse effects showed approximately 15% lower steady-state plasma clearance of ketamine than those who did not. However, no evidence linking the plasma pharmacokinetics of ketamine and norketamine and the analgesic efficacy was found. One possible explanation for this lack of concentration-response relationship is the overwhelming effect of heterogeneous nature of pain on analgesic response, since ketamine analgesic efficacy was higher in patients suffering from neuropathic pain than other pain types. This finding may reflect the fact that the analgesic activity of ketamine is more likely due to the attenuation of pain hypersensitivity rather than the direct suppression of nociceptive transmission.

The *in vitro* experiments on the inhibition of IL-6 by ketamine and norketamine enantiomers showed that pre-incubation with these drugs, at biologically relevant concentrations (1 to 100 μM), stereoselectively attenuated stimulated IL-6 production in recombinant cells in a concentration- and time-dependent manner. *(R)*-ketamine inhibited stimulated IL-6 production by approximately 60% at all exposure duration, an inhibitory effects that were up to 2-fold

greater than *(S)*-ketamine after short term exposure (less than 2 h). However *(S)*-ketamine was as potent as *(R)*-ketamine after long-term exposure (4 to 8 h), as its inhibitory effects were significantly enhanced with exposure duration. In addition, *(S)*-norketamine also attenuated IL-6 response in a time-dependent manner with approximately half the potency of *(S)*-ketamine. Further *in vitro* experiments and *in silico* docking simulation suggested that this time-dependent effects of *(S)*-ketamine and *(S)*-norketamine may indicates a mechanistically-based difference between acute and chronic effects of *(S)*-enantiomers on IL-6 production. This findings extend the current knowledge of the innate immune pharmacology of ketamine that may lead to a new direction for future research into ketamine analgesia.

In summary, this thesis demonstrates a substantial impact of the *CYP2B6*6* allelic variant on the clearance of ketamine, which may contribute to the interindividual variability in drug concentration. However, other factors such as the heterogeneity in the nature of pain and the inflammatory state should be taken into consideration to provide a more accurate prediction on ketamine analgesic response.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis resides with the copyright holders of these works.

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Yibai Li 24 Nov 2014

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Abbreviations

AAG	α_1-acid glycoprotein
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP-1	Activator protein 1
AUC	Area under the concentration-time curve
BPI	Brief Pain Inventory
CaMKII	Calmodulin dependent protein kinase II
CAR	Constitutive androstane receptor
CD	Cluster of differentiation
CL_{int}	Intrinsic clearance
CREB	cAMP response element-binding protein
CRPS	Complex regional pain syndrome
CSCI	Continuous subcutaneous infusion
CYP	Cytochrome P450 enzyme
DMEM	Dulbecco's Modified Eagle Medium
DHNK	Dehydronorketamine
DXO	Dextrorphan
EAAT	Excitatory amino-acid transporters
ERK	Extracellular signal-regulated kinases
GABA	γ-Aminobutyric acid
HEK293	Human embryonic kidney 293 cell line
HLM	Human liver microsome
HNK	Hydroxynorketamine
HPLC	High performance liquid chromatography
IC_{50}	50% inhibitory concentration
IL	Interleukin
i.m.	Intramuscular
IRF	Interferon regulatory factors
it	Intrathecal
i.v.	Intravenous
JNKs	c-Jun N-terminal kinases
K_i	Dissociation constant
K_m	Michaelis constant

LBP	Lipopolysaccharide binding protein
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinases
MD-2	Myeloid differentiation protein
mGluRs	Metabotropic glutamate receptors
MK-801	Dizocilpine
NF-κB	Nuclear factor kappa B
NK	Norketamine
NMDA	N-methyl-$_D$-aspartate
NO	Nitric oxide
NRS	Numeric Rating Scale
PBS	Phosphate buffered saline
PCA	Patient controlled analgesia
PCP	Phencyclidine
PKC	Protein kinase C
PXR	Pregnane X receptor
RacK	Racemic ketamine
RCT	Randomised controlled trial
RK	*(R)*-ketamine
RPMI	Roswell Park Memorial Institute medium
s.c.	Subcutaneous
SK	*(S)*-ketamine
SNP	Single nucleotide polymorphism
ThioTEPA	N,N'N'-triethylenethiophosphoramide
TLR	Toll-like receptor
TNF	Tumour necrosis factors
TrkB	Tyrosine receptor kinase B
VAS	Visual analogue scale
UV	Ultraviolet