Journal of Chromatography A, 1217 (2010) 7942–7948

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Enantioselective capillary electrophoresis for identification and characterization of human cytochrome P450 enzymes which metabolize ketamine and norketamine *in vitro*

Simone Portmann[a], Hiu Ying Kwan[a], Regula Theurillat[a], Andrea Schmitz[b], Meike Mevissen[b], Wolfgang Thormann[a,*]

[a] Department of Clinical Pharmacology and Visceral Research, University of Bern, Murtenstrasse 35, CH-3010 Bern, Switzerland
[b] Division of Veterinary Pharmacology and Toxicology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

ARTICLE INFO

Article history:
Available online 16 June 2010

Keywords:
Capillary electrophoresis
Ketamine
Norketamine
CYP3A4
CYP2A6
CYP2B6
Pharmacokinetics
Stereoselective metabolism

ABSTRACT

Ketamine, a phencyclidine derivative, is used for induction of anesthesia, as an anesthetic drug for short term surgical interventions and in subanesthetic doses for postoperative pain relief. Ketamine undergoes extensive hepatic first-pass metabolism. Enantioselective capillary electrophoresis with multiple isomer sulfated β-cyclodextrin as chiral selector was used to identify cytochrome P450 enzymes involved in hepatic ketamine and norketamine biotransformation *in vitro*. The N-demethylation of ketamine to norketamine and subsequently the biotransformation of norketamine to other metabolites were studied via analysis of alkaline extracts of *in vitro* incubations of racemic ketamine and racemic norketamine with nine recombinantly expressed human cytochrome P450 enzymes and human liver microsomes. Norketamine was formed by CYP3A4, CYP2C19, CYP2B6, CYP2A6, CYP2D6 and CYP2C9, whereas CYP2B6 and CYP2A6 were identified to be the only enzymes which enable the hydroxylation of norketamine. The latter two enzymes produced metabolic patterns similar to those found in incubations with human liver microsomes. The kinetic data of ketamine N-demethylation with CYP3A4 and CYP2B6 were best described with the Michaelis–Menten model and the Hill equation, respectively. This is the first study elucidating the individual enzymes responsible for hydroxylation of norketamine. The obtained data suggest that *in vitro* biotransformation of ketamine and norketamine is stereoselective.

1. Introduction

Ketamine ((R,S-2-(2-chlorophenyl)-2-methylamino)cyclohexanon, for chemical structure see Fig. 1), is a phencyclidine derivative that is used in human and veterinary clinical practice since 1970. Ketamine's mechanism of action has not been fully elucidated yet, but it is considered that the most important neuropharmacological effects of ketamine are mediated through its non-competitive antagonism at the N-methyl-D-aspartate (NMDA) receptor. Interactions of ketamine with opioid receptors, muscarinic acetylcholine receptors and different voltage-gated channels have been described. Because of rapid onset and short duration of action, ketamine is frequently used for induction of anesthesia and for short term surgical procedures. Due to its hallucinogenic effects even at subanesthetic doses it is abused by medical personnel and ketamine (also known as special K) became popular among the European party scenes as a drug of abuse where it is taken intranasally, injected, smoked, or ingested as part of a drink. Ketamine consists of a racemic mixture of two enantiomers, S-ketamine and R-ketamine. The S-enantiomer has a four times higher affinity for the NMDA receptor than the R-enantiomer and also binds to the μ and κ opioid receptors. The anesthetic potency of S-ketamine is two to three times higher than that of the racemic mixture. The incidence of unwanted side-effects at equal plasma concentrations is identical for both enantiomers, but since lower doses of the S-enantiomer are needed to maintain an equal state of anesthesia, fewer side-effects and shorter recovery times are seen with the single enantiomer preparation. The pK_a value of ketamine is 7.5 and it is therefore positively charged at physiological pH. The partition coefficient, also named the log *P* (octanol/water) value, accounts for 3.1. Due to its high lipid solubility and low protein binding (20–50% is bound to plasma proteins), ketamine is extensively distributed throughout the body. The half-life of the parent compound has been reported to be about 3 h and it can be administered intravenously, intramuscularly, orally, rectally, subcutaneously, epidurally and on the transnasal route [1–8].

* Corresponding author. Tel.: +41 31 632 3288; fax: +41 31 632 4997.
E-mail address: wolfgang.thormann@ikp.unibe.ch (W. Thormann).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.06.028

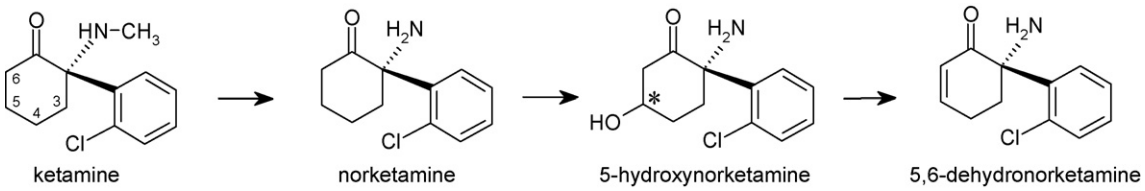

Fig. 1. Chemical structures for S-ketamine, S-norketamine, 5-hydroxynorketamine and S-5,6-dehydronorketamine. The asterisk marks the formation of a carbon stereogenic center which is formed upon hydroxylation of norketamine at the cyclohexanone ring.

The metabolism of ketamine has been studied in humans and various animal species. It was found that ketamine, incubated with human liver microsomes (HLM), is metabolized by the hepatic cytochrome P450 (CYP) enzyme system through N-demethylation to norketamine followed by hydroxylation of norketamine at various locations at the cyclohexanone and chlorophenyl rings and the formation of 5,6-dehydronorketamine [8–14]. The major metabolic phase I pathway for S-ketamine is depicted in Fig. 1. Direct hydroxylation of ketamine prior to N-demethylation is also possible but occurs to a marginal extent. The same metabolites are observed for other species *in vitro* [14–17], as well as *in vivo* in humans and animals [15,18–23].

The pharmacological activities of the metabolites have not been well studied in humans. In view of the growing interest concerning ketamine as a therapeutic agent and a drug of abuse, knowledge of the metabolism of ketamine in humans and the involved cytochrome P450 enzymes is of importance. Previous studies with lymphoblast-expressed CYP enzymes evidenced that CYP3A4, CYP2B6 and CYP2C9 are mainly responsible for the biotransformation of ketamine to its active metabolite norketamine [11,12]. Furthermore, incubation of the two ketamine enantiomers with 12 single CYP enzymes revealed also N-demethylation activities for CYP2A6, CYP2C8, CYP2C19 and CYP2D6 [11]. *In vitro* studies with individual enzymes concerning metabolites other than norketamine could not be found in the literature. Thus, efforts in elucidating the CYP enzymes involved in the metabolism of ketamine and norketamine were undertaken. In addition the stereoselectivity of each metabolic pathway was investigated. This work was executed in the context of a multidisciplinary research cooperation elucidating the metabolism and the pharmacokinetics of ketamine in different species. The project includes studies *in vitro* [14,15,17,24] and *in vivo* [15,22,23,25–29] for which enantioselective capillary electrophoresis (CE) with multiple isomer sulfated β-cyclodextrin (β-CD) as chiral selector [15,22,23] was employed to analyze the stereoisomers of ketamine and its metabolites in equine, canine and human biosamples.

The main goal of this project was to identify human CYP enzymes which are involved in the biotransformation of ketamine to norketamine *in vitro* and to elucidate which of these enzymes catalyze the formation of further metabolites. Individual recombinant human CYP enzymes from a baculovirus expression system (referred to as SUPERSOMES) were used for that task. Special emphasis was put on the stereoselective aspect of ketamine biotransformation by using enantioselective CE for detection of analytes. Racemic ketamine and norketamine were incubated with CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP2E1 SUPERSOMES, and data were compared to those obtained with incubation of the same compounds with HLM and human liver cytosol. Furthermore, the kinetics of ketamine to norketamine N-demethylation were examined for CYP3A4 and CYP2B6 and compared to those obtained with HLM. Two kinetic models (Michaelis–Menten and Hill) were fitted to the experimental data.

2. Materials and methods

2.1. Chemicals, reagents and solutions

Racemic ketamine hydrochloride was obtained from the pharmacy of the Inselspital (Bern, Switzerland). Norketamine as hydrochloride solution in methanol (1 mg/mL of the free base) was purchased from Cerilliant (Round Rock, USA) and (+)-pseudoephedrine hydrochloride was from Fluka (Buchs, Switzerland). Sulfated β-CD (7–11 mol sulfate/mol β-CD) was obtained from Sigma–Aldrich Chemie (Schnelldorf, Germany). Tris and HCl (37%) were from Merck (Darmstadt, Germany), H_3PO_4 (85%), ethylacetate and diammonium hydrogenphosphate from Fluka (Buchs, Switzerland), and dichloromethane from Biosolve (Valkenswaard, The Netherlands). Calibrator and control samples used for quantification of ketamine and norketamine enantiomers were prepared in 100 mM phosphate buffer (pH 7.4).

Baculovirus-insect-cell-expressed human CYP3A4 + P450 reductase + cytochrome b5 SUPERSOMES™, human CYP2C19 + P450 reductase + cytochrome b5 SUPERSOMES™, human CYP2D6*1 + P450 reductase SUPERSOMES™, human CYP1A1 + P450 reductase SUPERSOMES™, human CYP1A2 + P450 reductase SUPERSOMES™, human CYP2C9*1 (Arg_{144}) + P450 reductase + cytochrome b5 SUPERSOMES™, human CYP2B6 + P450 reductase + cytochrome b5 SUPERSOMES™, human CYP2A6 + P450 reductase + cytochrome b5 SUPERSOMES™, and human CYP2E1 + P450 reductase + cytochrome b5 SUPERSOMES™ were purchased from Gentest (Woburn, MA, USA, distributed through Anawa Trading, Wangen, Switzerland). The mixed gender pool of HLM, containing CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP4A and flavin monooxygenase (FMO), with a protein concentration of 20 mg/mL in 250 mM sucrose, pooled human liver cytosol and the nicotinamide adenine dinucleotide phosphate (NADPH) regenerating system were also from Gentest. The regenerating system comprises two solutions, solution A composed of 31.0 mM $NADP^+$, 66 mM glucose-6-phosphate (G-6-P) and 66 mM $MgCl_2$ and solution B containing 40 U/mL glucose-6-phophate dehydrogenase in 5 mM sodium citrate. The microsomes were stored in aliquots at −80 °C and the NADPH regenerating system was kept at −18 °C until use.

2.2. In vitro reactions and sample preparation for metabolic studies

A mixture containing substrate (either 50 μM racemic ketamine or 50 μM racemic norketamine) and NADPH regenerating system (1.55 mM $NADP^+$, 3.3 mM G-6-P, 0.4 U/mL G-6-P dehydrogenase, 3.3 mM $MgCl_2$) in 100 mM potassium phosphate buffer (pH 7.4) was preincubated at 37 °C for 3 min. In case of CYP2C9, a 100 mM Tris buffer (pH 7.5) was used instead of the phosphate buffer. For most CYP enzymes (1A1, 1A2, 3A4, 2C19, 2D6 and 2C9 with CYP content of 1000 pmol/mL), the enzymatic reaction was started at 37 °C after addition of a 16.3 μL aliquot of SUPERSOMES to a 760 μL reaction solution, which provided microsomal incubation mixtures