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Original Article

Assessment of the effect of ketamine on cytochrome P450 isoforms activity in rats by cocktail method

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Abstract: Cocktail method was used to evaluate the influence of ketamine on the activities of CYP450 isoforms CYP1A2, CYP2D6, CYP3A4, CYP2C19, CYP2C9 and CYP2B6, which were reflected by the changes of pharmacokinetic parameters of six specific probe drugs phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion, respectively. The experimental rats were randomly divided into two group, control group and ketamine group. The ketamine group rats were given 50 mg/kg ketamine by continuous intragastric administration for 14 days. The mixture of six probes was given to rats through intragastric administration and the blood samples were obtained at a series of time-points through the caudal vein. The concentrations of probe drugs in rat plasma were measured by UPLC-MS/MS. In the experiment for ketamine and control group, there was statistical pharmacokinetics difference for phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion. Continuous intragastric administration for 14 days could induce the activities of CYP450 isoforms CYP1A2, CYP3A4 and CYP2B6 of rats.

Keywords: CYP450, ketamine, cocktail, UPLC-MS/MS, rat

Introduction

Ketamine, as a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, has a wide range of effects on human, including analgesia, anesthesia, hallucinations, arterial hypertension, and so on. In clinic, it is primarily used as an anesthetic, usually in combination with some sedative drugs [1]. While ketamine is a NMDA receptor antagonist, which can be actually for drug abuse. Ketamine is an active ingredient in a variety of tablets illegally sold for recreational purposes. The tablets make users feel a short yet intense 'rush' when the drug is initially administered and become addicted quickly, needing higher doses and more often. Although regarded as an addictive substance, ketamine was reported to have the ability to suppress the morphine-induced place preference, a paradigm for assessing rewarding or reinforcing effects of drugs [2].

Cytochrome P450 (CYP450) is one of the most important drug-metabolizing enzymes, with largest number and highest abundance of CYP isoforms present in the liver [3-5]. Cytochrome P450 enzymes comprise a superfamily of hemoproteins, and three families (CYP1, CYP2, and CYP3) are mainly involved in the metabolism of drugs in both humans and rats [6]. In order to assess various individual CYP450 activities, probe drugs have been widely used in many clinical investigations in the field of drug metabolism and pharmacogenetics [7-9]. Probe drug is one kind of compound specially catalyzed by CYP isoforms, and the activities of CYP isoforms can be reflected by the metabolic rate of probe drug. As several CYP isoforms involved in drug metabolism, the approach was developed.

At present, the study of ketamine toxicology mainly focuses on the central nervous system. To our knowledge, there are few reports about the hepatic toxicity of ketamine. In this paper, the cocktail probe drugs approach is used to evaluate the induction or inhibition effects of ketamine on the activities of rats CYP450 isoforms such as CYP1A2, CYP2D6, CYP3A4, CYP2C19, CYP2C9 and CYP2B6 in rats, which

are reflected by the changes of pharmacokinetic parameters of six specific probe drugs, then provide guidance for rational clinical drug use after administration of ketamine.

Material and methods

Chemicals and reagents

Phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion (all > 98%) and the internal standard diazepam (IS) were purchased from Sigma-Aldrich Company (St. Louis, USA). HPLC grade acetonitrile and methanol were purchased from Merck Company (Darmstadt, Germany). All other chemicals were of analytical grade. Ultra-pure water (resistance > 18 mΩ) were prepared by Millipore Milli-Q purification system (Bedford, USA).

Animals

Male Sprague-Dawley rats (250 ± 20 g) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd. The animal license number was SCXK (Shanghai) 2012-0005. All twenty rats were housed at Laboratory Animal Research Center of Wenzhou Medical University. Animals were housed under controlled conditions (22°C) with a natural light–dark cycle. All experimental procedures were conducted according to the Institutional Animal Care guidelines and approved ethically by the Administration Committee of Experimental Animals, Laboratory Animal Center of Wenzhou Medical University.

Instrumentation and conditions

UPLC-MS/MS with ACQUITY I-Class UPLC and a XEVO TQD triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) equipped with an electrospray ionization (ESI) interface were used to analyze the compounds. The UPLC system was comprised of a Binary Solvent Manager (BSM) and a Sample Manager with Flow-Through Needle (SM-FTN). The Masslynx 4.1 software (Waters Corp., Milford, MA, USA) was used for data acquisition and instrument control.

Phenacetin, metroprolol, midazolam, omeprazole, tolbutamide, bupropion and diazepam (IS) were separated using a UPLC® BEH C18 column (2.1 mm × 100 mm, 1.7 μm, Waters, USA) maintained at 40°C. The initial mobile phase consisted of acetonitrile and water (containing 0.1% formic acid) with gradient elution at a flow rate of 0.4 mL/min and an injection volume of 2 μL. Elution was in a linear gradient, with the acetonitrile content changing from 30 to 60% between 0.3 and 1.8 min and increasing up to 95% over 0.2 min. The acetonitrile content was maintained at 95% for 0.5 min and then decreased to 30% within 0.1 min, and maintained at 30% for 0.4 min. The total run time of the analytes was 3 min. After each injection, the sample manager underwent a needle wash process, including a strong wash (methanol-water, 50/50, V/V) and a weak wash (methanol-water, 10/90, V/V).

The mass spectrometric detection was performed on a triple-quadrupole mass spectrometer equipped with an ESI interface in a positive mode. Nitrogen was used as the desolvation gas (1000 L/h) and cone gas (50 L/h). The selected ion monitoring conditions were defined as follows: capillary voltage 2.5 kV; source temperature 150°C; desolvation temperature 500°C. The multiple reaction monitoring (MRM) mode of m/z 180.1 → 109.9 for phenacetin, m/z 268.1 → 115.8 for metroprolol, m/z 326.0 → 291.0 for midazolam, m/z 346.1 → 197.8 for omeprazole, m/z 271.2 → 155.1 for tolbutamide, m/z 240.1 → 184.1 for bupropion and m/z 285.1 → 193.1 for IS was used as quantitative analysis.

Preparation of standard solutions

Stock solutions of 1.0 mg/mL each of phenacetin, metroprolol, midazolam, omeprazole, tolbutamide, bupropion and IS were prepared in methanol. The working standard solutions of each analyte were prepared by serial dilution of the stock solution with methanol. All of the solutions were stored at 4°C and brought to room temperature before use.

The calibration standards were prepared by spiking blank rat plasma with appropriate amounts of phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion. Calibration plots of each probe drug were constructed in the range 10-2000 ng/mL for plasma (2, 10, 20, 50, 100, 200, 500, 1000 and 2000 ng/mL).

Pharmacokinetic study

Twenty male Sprague-Dawley rats (250 ± 20 g) were randomly divided to control group and ket-

Table 1. Pharmacokinetic parameters of phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion in control-group and ketamine-group rats (mean ± SD, n = 10)

Compound	Group	$AUC_{(0-t)}$	$AUC_{(0-\infty)}$	t1/2z	T_{max}	CLz/F	Vz/F	C_{max}
		μg/L*h	μg/L*h	h	h	L/h/kg	L/kg	ug/L
Phenacetin	Control	1193.4 ± 670.6**	1198.2 ± 672.4**	0.7 ± 0.4	0.2 ± 0.2	11.5 ± 7.1*	11.7 ± 8.2	1251.9 ± 562.0**
	Ketamine	447.2 ± 282.8	448.6 ± 284.0	0.6 ± 0.4	0.1 ± 0.1	29.7 ± 14.5	26.4 ± 22.9	573.7 ± 218.4
Metroprolol	Control	1696.2 ± 697.4	1724.0 ± 704.1	2.1 ± 0.6	0.5	6.5 ± 2.0	19.6 ± 9.7	820.9 ± 354.7
	Ketamine	2109.4 ± 1767.8	2167.8 ± 1831.6	2.0 ± 0.6	0.5 ± 0.1	7.1 ± 4.3	17.6 ± 6.8	760.7 ± 295.3
Midazolam	Control	204.3 ± 192.9*	226.9 ± 221.2*	0.9 ± 0.3	0.3 ±0.2	59.9 ± 54.3**	61.5 ± 44.5*	131.1 ± 107.4*
	Ketamine	15.7 ± 7.1	16.4 ± 6.9	0.9 ± 0.4	0.3 ± 0.2	355.2 ± 151.5	480.7 ± 346.8	14.9 ± 10.5
Omeprazole	Control	103.5 ± 95.6	105.6 ± 95.6	0.7 ± 0.3	0.2 ± 0.2	135.8 ± 62.1*	144.5 ± 80.9	126.4 ± 114.1
	Ketamine	64.0 ± 48.3	70.8 ± 50.4	1.1 ± 0.6	0.4 ± 0.5	198.3 ± 115.4	276.9 ± 150.5	62.5 ± 45.2
Tolbutamide	Control	63308.6 ± 16700.4	124720.9 ± 91266.1	41.1 ± 35.4	11.9 ± 8.7**	0.013 ± 0.01	0.5 ± 0.2	1893.6 ± 560.8
	Ketamine	66421.8 ± 33542.7	87418.2 ± 51053.0	21.6 ± 13.6	4.5 ± 3.0	0.014 ± 0.006	0.4 ± 0.2	2351.3 ± 1059.9
Bupropion	Control	109.4 ± 56.6*	118.3 ± 59.6*	3.8 ± 1.8	0.5 ± 0.1	101.3 ± 43.0	607.2 ± 570.6*	53.5 ± 25.8*
	Ketamine	101.6 ± 35.3	108.1 ± 36.2	3.3 ± 1.1	0.6 ± 0.2	102.2 ± 32.8	496.5 ± 246.4	38.3 ± 13.0

Compared ketamine group with the control group, *: $P < 0.05$, **: $P < 0.01$.

amine group (n = 10). Control group were give saline by continuous intragastric administration for 14 days; while ketamine group were give ketamine (50 mg/kg) by continuous intragastric administration for 14 days. After two days, the ketamine and control group were given the mixtured six probe drugs (phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion were 10 mg/kg, 10 mg/kg, 10 mg/kg, 10 mg/kg, 1 mg/kg and 10 mg/kg) by oral administration.

Blood samples (0.3 mL) were collected from the tail vein into heparinized 1.5 mL polythene tubes at 0.0833, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 h after oral administration of probe drugs. The samples were immediately centrifuged at 8000 r/min for 5 min, and 100 μL plasma was obtained for each sample.

The plasma samples were extracted and measured by UPLC-MS/MS. In a 1.5 mL centrifuge tube, an aliquot of 10 μL of the internal standard working solution (0.5 μg/mL) was added to 0.1 mL of collected plasma sample followed by the addition of 0.2 mL of acetonitrile. After the tube was vortex-mixed for 1.0 min, the sample was centrifuged at 15000 rmp for 10 min. The supernatant (2 μL) was injected into the UPLC-MS/MS system for analysis.

Plasma probe drugs concentration versus time data for each rat was analyzed by DAS software (Version 3.0, Drug Clinical Research Center of Shanghai University of T.C.M and Shanghai BioGuider Medicinal Technology Co., Ltd., China). The pharmacokinetic parameters of the test group and control group probe drugs with the t-test inspection were analyzed by SPSS I8.0 statistical software. A $P < 0.05$ was considered as statistically significant.

Results

Method validation

The concentration of phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion in rat plasma was simultaneously determined by a sensitive and simple UPLC-MS/MS method. Calibration curves for six probe drugs were generated by linear regression of peak area ratios against concentrations, respectively. The calibration plot of the probe drugs in the range of 2-2000 ng/mL ($r > 0.995$). Each probe drug peak area ratio with concentration has a good linear relationship in the range of concentration. The LLOQ for each probe drug in plasma was 2 ng/mL. The relative standard deviation (RSD%) of the six probe drugs in low, medium and high three concentrations were less than 13%. The intra-day and inter-day relative error (RE%) ranged from -8% to 11%. The results demonstrate that the values were within the acceptable range and the method was accurate and precise. The extraction recoveries were ranged from 87.7% to 98.4%. The results of matrix effect, the percent nominal concentration were more than 87% or less than 114%.

Pharmacokinetic study

The main pharmacokinetic parameters after administration of phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion from non-compartment model analysis were summarized in **Table 1**. The representative phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion concentration vs. time profiles of 20 rats were presented in **Figure 1**. As could be seen from **Figure 1**, the AUC and C_{max} of phenacetin, midazolam and bupropion in ketamine group is lower than the control group, this result is consistent with the **Table 1**.

As can be seen from **Table 1**, compared ketamine group with the control group, the pharmacokinetic parameters of phenacetin have changed, $AUC_{(0-t)}$ from the 1193.4 reduced to 447.2 ng/mL*h with significant difference ($P < 0.01$); CL from 11.5 increased to 29.7 L/h/kg with significant difference ($P < 0.05$); C_{max} varied from 1251.9 to 573.7 ng/mL with significant difference ($P < 0.01$). Compared to the control group, the ketamine group, $AUC_{(0-t)}$ reduced, CL increased, C_{max} becomes lower, and it indicate that the continuous administration of ketamine may induce the activity of CYP1A2 enzyme of rats. The similar results were found in midazolam and bpropion, the pharmacokinetic parameters of midazolam and bpropion have changed between control group and ketamine group, but there was significant difference for AUC, CL and C_{max}, AUC reduced ($P < 0.01$ or $P < 0.05$), CL increased ($P < 0.01$) and C_{max} becomes lower ($P < 0.01$ or $P < 0.05$), it show that the ketamine may induce the activity of CYP1A2, CYP3A4 and CYP2B6 enzyme. Compared ketamine group with the control group, there were no significant difference for

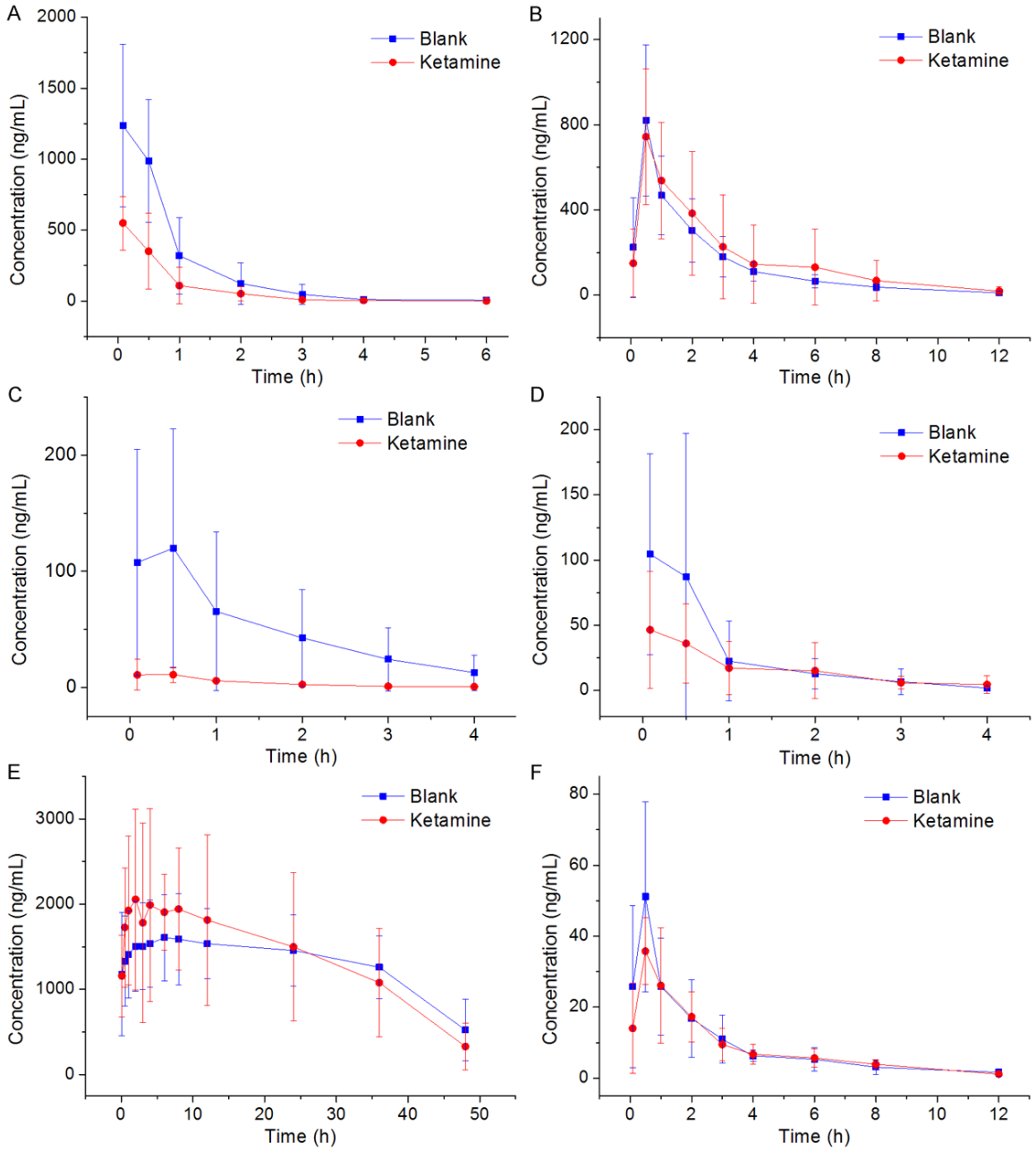

Figure 1. The pharmacokinetics profiles of phenacetin (A), metroprolol (B), omeprazole (C), midazolam (D), tolbutamide (E) and bupropion (F) in control-group and ketamine-group rats (n = 10).

AUC of metroprolol, omeprazole and tolbutamide, it show that the ketamine may not induce or inhibit the activity of CYP1A2, CYP3A4 and CYP2B6 enzyme.

Discussion

Ketamine is an anesthetic and analgesic regularly used in veterinary patients. As ketamine is almost always administered in combination with other drugs, interactions between ketamine and other drugs bear the risk of either adverse effects or diminished efficacy [10]. Since cytochrome P450 enzymes (CYPs) play a pivotal role in the phase I metabolism of the majority of all marketed drugs, drug–drug interactions often occur at the active site of these enzymes.

In general, changes in pharmacokinetics are thought to be caused by drug-drug or drug-food interactions [11]. In pharmacokinetic interac-

tions, approximately 65% of drug-drug interactions occur in metabolic sites [12], and drug metabolic enzymes are considered to be the most important interactive sites [13]. A large number of drugs are metabolized by CYP enzymes in the liver, and more than 90% of drug-drug interactions occur at the CYP-catalyzed step. Similarly, supplement-drug interactions involving CYP activity are occasionally found to cause considerable adverse events. For these reasons, we evaluated the effects of intragastric administration of ketamine for 14 days on the activity of CYP enzymes in *vivo*. We selected CYP isoforms CYP1A2, CYP2D6, CYP3A4, CYP2C19, CYP2C9 and CYP2B6 because more than 90% of drugs are known to be metabolized by these 6 CYP enzymes [14].

Ketamine is N-demethylated into norketamine, mainly by cytochrome P450 (CYP) 2C9, CYP2B6 and CYP3A4 in the liver, and then excreted by the kidneys [15]. When biotransformed in the liver, ketamine was found to induce hepatotoxicity following a prolonged surgical operation in a clinical study [16]. In our study, continuous intragastric administration of ketamine for 14 days may induce the activities of CYP450 isoforms CYP1A2, CYP3A4 and CYP2B6 of rats. These results would give us valuable information regarding the interactions of ketamine with drugs, induction of drug metabolizing enzyme reduces the efficacy of drug.

Conclusion

The concentrations of probe drugs (phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion) in rat plasma were measured by UPLC-MS/MS. There was statistical pharmacokinetics difference for six probe drugs between ketamine and control group. Continuous intragastric administration for 14 days could induce the activities of CYP450 isoforms CYP1A2, CYP3A4 and CYP2B6 of rats.

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Disclosure of conflict of interest

None.

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