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Original Article

Effect of ademetionine on cytochrome P450 isoforms activity in rats

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Abstract: Cocktail method was used to evaluate the influence of ademetionine 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 ademetionine group. The ademetionine group rats were given 50 mg/kg ademetionine by continuous oral administration for 7 days. The mixture of six probes was given to rats through oral 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 ademetionine and control group, there was statistical pharmacokinetics difference for phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion. Continuous oral administration for 7 days could induce the activities of CYP450 isoforms CYP1A2 of rats, while it may inhibit the activities of CYP2D6, CYP3A4, CYP2C19 and CYP2C9.

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

Introduction

Ademetionine, is usually used to prevent the occurrence of cholestasis in the liver in patients with cirrhosis. Ademetionine may also play an important role in reducing the mortality of amanita verna poisoning [1]. The active ingredient of ademetionine is S-Adenosyl-L-methionine (SAMe, SAM, AdoMet, ademetionine), which involves in three important metabolic processes transmethylation, transsulfuration, and aminopropylation [2, 3]. These metabolic reactions occur mostly in the liver [4]. After partial hepatectomy, ademetionine levels are dramatically reduced shortly afterward in liver [5]. Deficiency of hepatic ademetionine can lead to steatohepatitis, increased hepatocyte apoptosis, fibrosis, and hepatocellular carcinoma [6-8]. Ademetionine has protective action in the liver, which includes regulation of hepatocyte growth [9] and apoptotic response [10], increased GSH levels [11], improvement of membrane fluidity [12], and inhibition of collagen I production [11].

Cytochrome P450 (CYP) enzymes consist of numerous families and subfamilies with similar amino acid sequences. Among these CYP enzymes, five human hepatic CYP isoforms CYP2A6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 have been proved to play an important role in more than 90% of drug metabolism [13, 14], which may lead to many clinically important drug interactions [15]. CYP inhibition by one drug could lead to an increase in plasma concentrations of another drug, known as drug–drug interactions, and cause adverse events or even death [16]. Therefore, it is very important to study the inhibitory effect of drugs in order to reduce adverse events [17].

However, up to now, few study focus on the influence of ademetionine on these other CYP isoforms. The goal of this report is to evaluate

the effects of continuous administration of ademetionine on other CYP 450 metabolism.

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

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

Compound	Group	AUC (0-t)	AUC (0-∞)	t1/2z	Tmax	CLz/F	Vz/F	Cmax
		ug/L*h	ug/L*h	h	h	L/h/kg	L/kg	ug/L
Phenacetin	Ademetionine	6597.5 ± 2608.8**	6598.5 ± 2607.8**	1.0 ± 0.9	0.3 ± 0.2	1.8 ± 0.8*	3.2 ± 4.7	3331.7 ± 903.8**
	Control	11343.9 ± 3627.7	11353.2 ± 3624.7	1.4 ± 0.6	0.5	1.0 ± 0.3	2.0 ± 1.3	7530.6 ± 1850.9
Metroprolol	Ademetionine	5817.0 ± 1448.0**	6189.9 ± 1276.0**	23.3 ± 13.4	0.7 ± 0.3	1.7 ± 0.4**	61.1 ± 45.5	1311.8 ± 427.1**
	Control	2095.2 ± 731.1	2214.3 ± 780.7	15.2 ± 8.2	0.6 ± 0.2	5.0 ± 1.5	102.3 ± 63.9	660.8 ± 216.1
Midazolam	Ademetionine	3597.5 ± 840.6**	3599.8 ± 840.6**	1.0 ± 0.2	0.5 ± 0.3	2.9 ± 0.8**	4.0 ± 1.2**	1047.8 ± 317.4**
	Control	183.0 ± 46.6	188.7 ± 42.7	2.9 ± 1.3	0.6 ± 0.2	55.7 ± 14.1	243.1 ± 179.6	97.9 ± 38.9
Omeprazole	Ademetionine	1508.3 ± 645.4*	1518.3 ± 636.0*	2.1 ± 1.2	0.1	7.7 ± 3.3**	27.5 ± 24.3**	1293.9 ± 585.6**
	Control	576.8 ± 91.6	764.1 ± 418.2	7.6 ± 11.2	0.2 ± 0.2	15.1 ± 4.5	111.3 ± 78.2	399.9 ± 107.0
Tolbutamide	Ademetionine	301972.6 ± 45360.5**	590192.8 ± 382702.1**	39.9 ± 28.6	13.6 ± 7.4	0.002 ± 0.001**	0.09 ± 0.02	9525.1 ± 1378.1*
	Control	198514.0 ± 51667.1	239166.6 ± 52242.8	19.8 ± 8.3	3.3 ± 2.8	0.004 ± 0.001	0.12 ± 0.05	7263.2± 2219.3
Bupropion	Ademetionine	1007.9 ± 403.4	1045.2 ± 442.2	2.1 ± 0.6	0.7 ± 0.4	10.9 ± 3.9**	32.9 ± 16.1**	219.5 ± 86.8*
	Control	78.6 ± 16.2	103.9 ± 35.9	6.0 ± 3.8	0.4 ± 0.3	105.8 ± 32.5	811.2 ± 328.4	22.7 ± 5.2

(Compared ademetionine group with the control group, *: $P < 0.05$, **: $P < 0.01$).

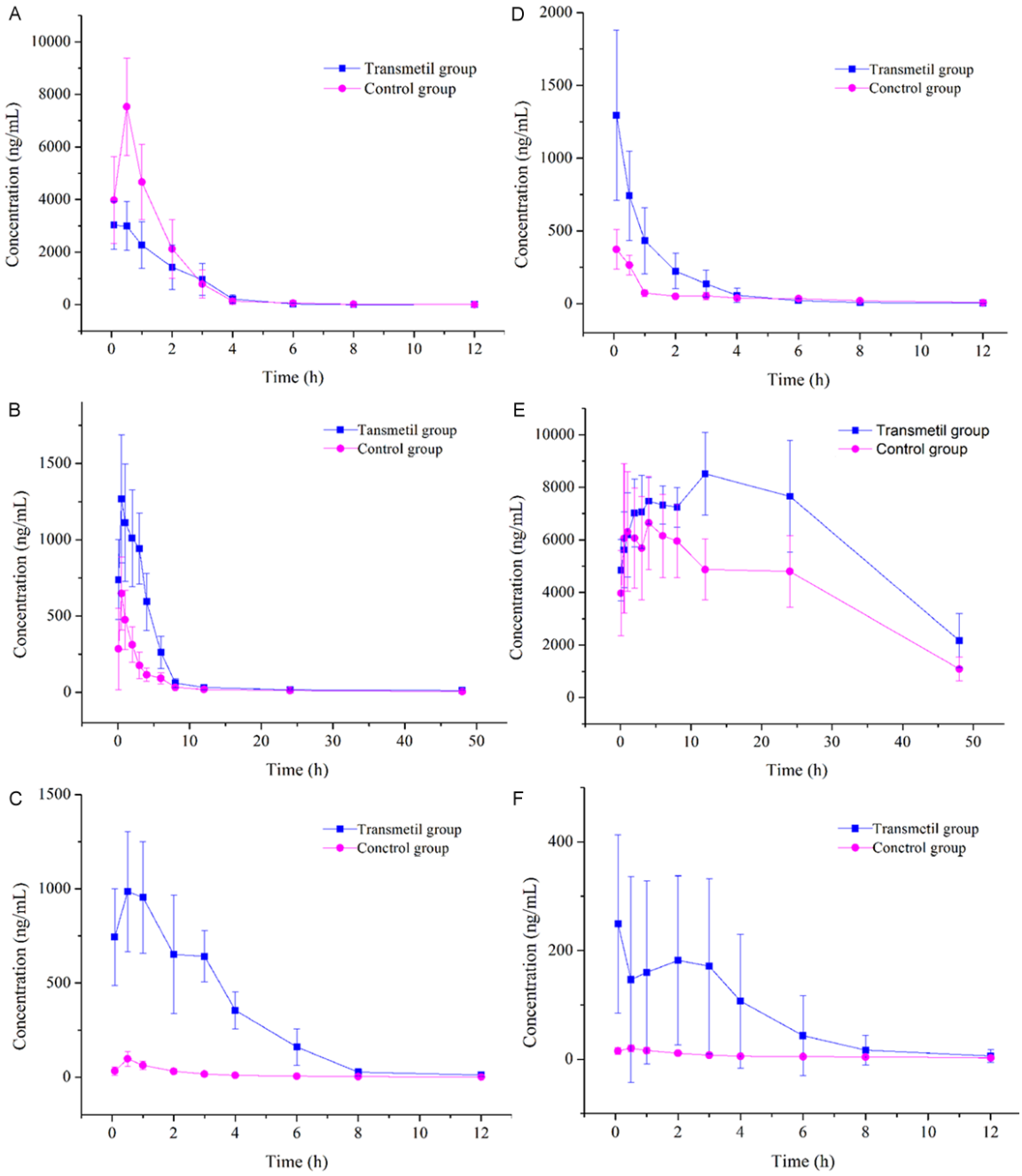

Figure 1. The pharmacokinetics profiles of phenacetin (A), metroprolol (B), omeprazole (C), midazolam (D), tolbutamide (E) and bupropion (F) in control-group and ademetionine-group rats (n = 10).

ademetionine group (n = 10). Control group were give saline by continuous oral administration for 7 days; while ademetionine group were give ademetionine (50 mg/kg) by continuous oral administration for 7 days. After two days, the ademetionine and control group were given the mixture of 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 and discussion

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 [18]. 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%.

The developed UPLC-MS/MS, which was used for the subsequent quantitation of the six probe drugs, showed faster analysis time than conventional LC-MS [19-22] and tremendously enhanced signal intensity. It took only 3 min to finish analyzing a plasma sample, which can save much time in experimental studies with hundreds of samples. In addition, the LLOQ for the six probe drugs was comparatively low (2 ng/mL), which is satisfactory for determining a lower plasma concentration in the last sampling time point, it is more sensitive than LC-MS with LLOQ of 10 ng/mL for determination of same six probe drugs [19, 23].

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 metroprolol, midazolam, omeprazole, tolbutamide and bupropion in ademetionine group is higher than the control group, this result is consistent with the **Table 1**.

As can be seen from **Table 1**, compared ademetionine group with the control group, the pharmacokinetic parameters of phenacetin have changed, $AUC_{(0-t)}$ from the 11343.9 reduced to 6597.5 ng/mL*h with significant difference ($P < 0.01$); CL from 1.0 increased to 1.8 L/h/kg with significant difference ($P < 0.05$); C_{max} varied from 7530.6 to 3331.7 ng/mL with significant difference ($P < 0.01$). Compared to the control group, the ademetionine group, $AUC_{(0-t)}$ reduced, CL increased, C_{max} becomes lower, it indicate that the continuous administration of ademetionine may induce the activity of CYP1A2 enzyme of rats.

Compared ademetionine group with the control group, the pharmacokinetic parameters of bupropion have changed, $AUC_{(0-t)}$ from the 78.6 increased to 1007.9 ng/mL*h, there was no significant difference ($P > 0.05$); CL from 105.8 reduced to 10.9 L/h/kg, there was significant difference ($P < 0.01$); C_{max} varied from 22.7 to 219.5 ng/mL, there was significant difference

($P < 0.05$). Compared to the control group, the ademetionine group, CL reduced, C_{max} becomes higher, there was significant difference for these pharmacokinetic parameters ($P < 0.01$), it indicate that the ademetionine might inhibit the activity of CYP2B6 enzyme.

Compared ademetionine group with the control group, the pharmacokinetic parameters of midazolam have changed, $AUC_{(0-t)}$ from the 183.0 increased to 3597.5 ng/mL*h, there was significant difference ($P < 0.01$); CL from 55.7reduced to 2.9 L/h/kg, there was significant difference ($P < 0.01$); C_{max} varied from 97.9 to 1047.8 ng/mL, there was significant difference ($P < 0.01$). Compared to the control group, the ademetionine group, $AUC_{(0-t)}$ increased, CL reduced, C_{max} becomes higher, there was significant difference for these pharmacokinetic parameters ($P < 0.01$), it indicate that the ademetionine may inhibit the activity of CYP3A4 enzyme.

The similar results were found in metroprolol, omeprazole and tolbutamide, the pharmacokinetic parameters of metoprolol have changed between control group and ademetionine group, but there was significant difference for AUC, CL and C_{max}, AUC increased ($P < 0.01$ or $P < 0.05$), CL reduced ($P < 0.01$) and C_{max} ($P < 0.01$ or $P < 0.05$), it show that the ademetionine may inhibit the activity of CYP2D6, CYP2C19 and CYP2C9 enzyme.

In general, changes in pharmacokinetics are thought to be caused by drug–drug or drug-food interactions [24]. In pharmacokinetic interactions, approximately 65% of drug-drug interactions occur in metabolic sites, and drug metabolic enzymes are considered to be the most important interactive sites [25]. 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 continuous oral administration of ademetionine for 7 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 [26]. In present work, 7 days continuous oral administration of ademetionine may induce the activities of CYP1A2, and inhibit the activities of CYP2D6, CYP3A4, CYP2C19 and CYP2C9 on rats. It is different from our previous work oral administration of ademetionine inhibit the activities of CYP3A4 on rats [27], it may due to longer time continuous oral administration more changes in enzyme activity.

Conclusion

Continuous oral administration of ademetionine for 7 days may induce the activities of CYP450 isoforms CYP1A2 of rats, while it may inhibit the activities of CYP2D6, CYP3A4, CYP2C19 and CYP2C9. These results would give us valuable information regarding the interactions of ademetionine with drugs, induction of drug metabolizing enzyme reduces the efficacy of drugs, and inhibition of drug metabolizing enzyme increases the plasma concentration of drugs.

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Disclosure of conflict of interest

None.

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