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Original Article

Effect of paraquat on CYP450 isoforms activity of rats after intraperitoneal administration

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Abstract: Paraquat is an organic heterocyclic herbicide and defoliant agent used worldwide. However, paraquat is highly toxic to humans and animals and no effective antidote is available, which causes a high mortality rate. In order to investigate the effects of paraquat on the metabolic capacity of cytochrome P450 (CYP) enzymes, a cocktail method was employed to evaluate the activities of CYP2B1, CYP2D1, CYP1A2, CYP3A2, CYP2C11. The rats were randomly divided into paraquat group (Low, High) and control group. The paraquat group rats were given 9, 18 mg/kg (Low, High) paraquat by intraperitoneal administration. Five probe drugs (bupropion, metroprolol, phenacetin, testosterone and tolbutamide) were given to rats through intragastric administration, and the plasma concentrations were determined by UPLC-MS/MS. Statistical pharmacokinetics difference for metroprolol, testosterone and tolbutamide in rats were observed by comparing paraquat group with control group. Combined with PCR results, intraperitoneal administration of paraquat induces the activities of CYP2C11 of rats.

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

Introduction

Paraquat is a quaternary nitrogen, nonselective contact herbicide, which is used without restriction all over the world because of its high insecticidal efficiency and low residues in the plant [1, 2]. It was used in agriculture since 1962. However, owing to been extensively demonstrated highly toxic to multiorgans, paraquat has led to numerous poisoning or death accidents [3-5]. When absorbed through ingestion, skin contact or inhalation, and thousands of deaths due to intentional or accidental ingestion of paraquat have been reported [6]. Based on the report of Liu et al., paraquat poisoning is cause of the unbalanced reactive oxygen species (ROS) biological systems [7]. Mineo Matsubara et al. pointed out that paraquat caused S-phase arrest of rat liver cells in vivo [8]. There have been large quantities of researches about liver injury of paraquat, and liver is responsible for drug metabolism.

Cytochrome P450 (CYP450) exists mainly in the liver, the CYP450 isoenzymes are named according to the sequences of amino acids [9]. And it is the most important enzyme in the metabolism of drugs, which plays an important role in many bio-conversion of exogenous and endogenous chemical substances. Wherein CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A are involved in more than 90% of clinical drug metabolism [10, 11]. The rate-limiting enzyme in the implementation of I phase reaction of drug metabolism. Up until 2011, 51 CYP members have been identified.

"Cocktail" probe drugs is kind of a fast, high-throughput research methods, has been widely used in many researches, such as the evaluation of the effect of drugs on cytochrome P450 (CYP450), the confirmation of drug metabolism pathways, prediction of drug-drug interactions, phenotype analysis of drug metabolism, optimization of pharmaceutical administration and so

Table 1. The sequences of the primers used in polymerase chain reaction

Isoenzymes	Forward	Reverse
CYP1A2	GTCACCTCAGGGAATGCTGTG	GTTGACAATCTTCTCAGG
CYP3A2	CTTCACAAACCGGAGGCCTTTTGGT	ATCAGGGTGAGTGGCCAGTTCATAC
CYP2B1	GACAGAAGGATGAGGGAGGAA	CTCCCTCTGTCTTTCATTCTGT
CYP2C11	AAAAGCACAATCCGCAGTCT	GCATCTGGCTCCTGTCTTTC
CYP2D1	TGAGATGTCGCTTTGGGGAC	GAGGACCACACCTTGAGAGC

on. "Cocktail" probe drugs are combined with modern detection equipment, and have its unique advantages in the research and development of methodology. Such as liquid chromatography-mass spectrometry, matrix-assisted laser desorption ionization time of flight mass spectrometry, ultra performance liquid chromatography-quadrupole-TOF tandem mass spectrometry and the like. Stewart et al. [12] using UPLC-MS/MS and "Pittsburgh cocktail" Spectrometry Determination of 6 CYP enzymes metabolic probe drugs and product concentration in human plasma and urine. This paper employ cocktail probe, and choose bupropion, phenacetin, tolbutamide, metoprolol, midazolam, as probe drugs of CYP2B1, CYP1A2, CYP2C11, CYP2D1, CYP3A2, to study the impact of paraquat on cytochrome P450 isoforms CYP2B1, CYP1A2, CYP2C11, CYP2D1, CYP3A2, in rats to obtain their metabolic information, which is of high significance on the prevention of herbicide-drug interaction and potential toxicity of the drug ,and finally provide theoretical guidance and experimental basis for clinical rational drug administration [13, 14].

Material and methods

Chemicals

Bupropion, metroprolol, phenacetin, testosterone and tolbutamide (all >98%) and the internal standard diazepam (IS) were obtained from Sigma-Aldrich Company (St. Louis, USA). Ultrapure water was prepared by Millipore Milli-Q purification system (Bedford, USA). Methanol and acetonitrile (HPLC grade) were obtained from Merck Company (Darmstadt, Germany).

Animals

Sprague-Dawley rats (male, 220 ± 20 g) purchased from Shanghai SLAC Laboratory Animal Co., Ltd. Animals were housed under a natural light-dark cycle conditions with controlled temperature (22°C). All thirty rats were housed at Laboratory Animal Research Center of Wenzhou Medical University. All experimental procedures were approved ethically by the Wenzhou Medical University Administration Committee of Experimental Animals.

Pharmacokinetics

Thirty rats (220 ± 20 g) were randomly divided into four different dosages of paraquat groups (Low-group, High-group and control group with 10 rats in each group). Paraquat was dissolved in water at two different concentrations (9 and 18 mg/mL). Two different paraquat groups (Low-group, High-group) were respectively give paraquat 9 and 18 mg/kg one time by intraperitoneal administration. Control group were give saline by same administration method. The second day morning, five probe drugs bupropion, metroprolol, phenacetin, testosterone and tolbutamid were mixed in corn oil and given to the rats of three paraquat groups and control group by intragastric administration at a single dosage 10 mg/kg for bupropion, metroprolol, phenacetin, testosterone, 0.1 mg/kg for tolbutamide.

Blood (0.3 mL) samples were collected into heparinized 1.5 mL polythene tubes from the tail vein at 0.0833, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 h after intragastric administration of five probe drugs. Plasma (100 μL) was obtained from blood sample after centrifugation at 4000 *g* for 10 min. In a 1.5 mL centrifuge tube, 200 μL of acetonitrile (containing 50 ng/mL IS) was added into 100 μL of collected plasma sample. After vortex-mixing for 1.0 min, the sample was centrifuged at 13000 *g* for 15 min. Then supernatant (2 μL) was injected into the UPLC-MS/MS system for analysis. Concentration of plasma probe drugs versus time was analyzed by Version 3.0 Data Analysis System (Wenzhou Medical University, China).

Statistical analysis

The main pharmacokinetic parameters of the paraquat group and control group were ana-

Table 2. Pharmacokinetic parameters of probe drugs from control group and paraquat group rats (mean ± SD, n=10)

Parameters ng/mL*h		$AUC_{(0-t)}$ ng/mL *h	$AUC_{(0-\infty)}$ h	t1/2z L/h/kg	CLz/F L/kg	Vz/F ng/mL	C_{max}
Bupropion (CYP2B1)	Control	101.0 ± 78.5	102.9 ± 79.7	0.7 ± 0.2	149.1 ± 82.5	145.0 ± 98.3	83.3 ± 62.6
	Low	155.1 ± 29.9	167.3 ± 36.6	0.8 ± 0.3	62.1 ± 12.5*	70.0 ± 26.9	111.9 ± 29.2
	High	318.1 ± 345.1	327.3 ± 354.3	0.8 ± 0.1	69.2 ± 63.0	78.5 ± 72.5	187.8 ± 173.5
Metroprolol (CYP2D1)	Control	206.9 ± 89.8	242.4 ± 104.0	2.1 ± 2.8	48.8 ± 20.3	111.2 ± 102.2	129.2 ± 54.0
	Low	285.3 ± 52.6*	298.2 ± 42.4	1.2 ± 0.8	34.1 ± 4.7*	58.1 ± 43.8	179.7 ± 57.3
	High	317.9 ± 179.2	322.3 ± 182.3	0.8 ± 0.2	37.4 ± 17.4	37.5 ± 11.4	148.3 ± 50.9
Phenacetin (CYP1A2)	Control	2194.6 ± 1141.8	2196.5 ± 1141.6	0.5 ± 0.2	5.7 ± 3.0	4.8 ± 3.6	2125.9 ± 1012.8
	Low	2381.1 ± 1090.1	2383.7 ± 1090.5	0.5 ± 0.3	5.1 ± 2.6	3.6 ± 2.6	2615.0 ± 1032.3
	High	4597.4 ± 4700.2	4599.0 ± 4700.0	0.4 ± 0.1	4.0 ± 2.8	2.5 ± 1.7	3269.7 ± 2422.6
Testosterone (CYP3A2)	Control	132.5 ± 37.8	157.3 ± 30.9	4.4 ± 3.5	65.4 ± 11.5	399.1 ± 263.0	43.4 ± 7.6
	Low	230.4 ± 62.1*	287.3 ± 118.2*	5.5 ± 6.3	40.6 ± 18.5*	248.9 ± 160.7	102.6 ± 25.9**
	High	201.3 ± 139.5	328.7 ± 323.7	6.8 ± 4.8	52.5 ± 34.5	373.6 ± 165.1	87.6 ± 39.6*
Tolbutamide (CYP2C11)	Control	19792.2 ± 1684.0	27669.2 ± 7115.7	12.7 ± 7.2	0.004 ± 0.001	0.06 ± 0.02	1351.6 ± 240.7
	Low	17666.7 ± 2520.0	26535.9 ± 12464.4	15.1 ± 12.5	0.004 ± 0.001	0.08 ± 0.02	1099.1 ± 202.7*
	High	9732.0 ± 4134.0**	11600.8 ± 5671.0**	8.2 ± 4.2	0.011 ± 0.007**	0.11 ± 0.05	814.0 ± 251.5*

Paraquat group was compared with the control group, *: P<0.05, **: P<0.01.

lyzed by SPSS I8.0 statistical software; statistical significance was assessed by t-test (P<0.05 was considered as statistically significant).

UPLC-MS/MS determination of probe drugs

The concentration of bupropion, metroprolol, phenacetin, testosterone and tolbutamid in rat plasma were simultaneously determined by a sensitive and simple UPLC-MS/MS method [15]. The compounds were analyzed by a UPLC-MS/MS with ACQUITY I-Class UPLC and a XEVO TQD triple quadrupole mass spectrometer that equipped with an electrospray ionization (ESI) interface (Waters Corp., Milford, MA, USA). Data acquisition and instrument control were performed on the Masslynx 4.1 software (Waters Corp., Milford, MA, USA).

The LLOQ for each probe drug in plasma was 2 ng/mL. The RSD of the five probe drugs were less than 13%. The calibration plot of the probe drugs is in the range of 2-2000 ng/mL (r >0.995). The intra-day and inter-day accuracy ranged from 85% to 114%. The matrix effects were more than 85% or less than 112%. The extraction recoveries were better than 85%.

RT-PCR analysis

After pharmacokinetic properties analysis, rats were deeply anesthetized with 10% chloral hydrate (i.p., 20 mg/kg). The some liver of control group and paraquat treated groups was removed, frozen and store at 80°C.

The livers were processed for isolation of total RNA by using TRIzol reagent (Invitrogen, Calsbad, CA, USA) according to the instruction of the manufacturer. The RNA concentration was determined, and the quality of the isolated RNA was assessed using the 260/280 nm absorbance ratio (1.8-2.0 indicates a highly pure sample). RNA integrity was confirmed by running samples on 1% agarose gel. The RNA pellet was stored at -80°C until use [16, 17].

We have used 2 μL RNA in a 20 μL reaction mixture utilizing RevertAid™ M-MuLVRT (Fermentas, Hanover, MD, USA) according to the supplier's instructions. Resulting reverse transcription products were stored at -80°C until assay.

Reactions were performed in a final volume of 20 μL that contained Platinum SYBR Green qPCR SuperMix-UDG 12 μL, 2 μL cDNA, 0.6 μL each of specific oligonucleotide primer (10 μM), and 4.8 μL DEPC-treated autoclaved distilled water.

PCR was carried out using initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 58°C (CYP1A2), 55°C CYP2B1), 56°C (CYP2C11), 63°C (CYP3A2) for 30 s, extension at 72°C for 30 s and final extension at 72°C for 45 s. The sequences of the forward and reverse primers used in this experiment are summarized in **Table 1**.

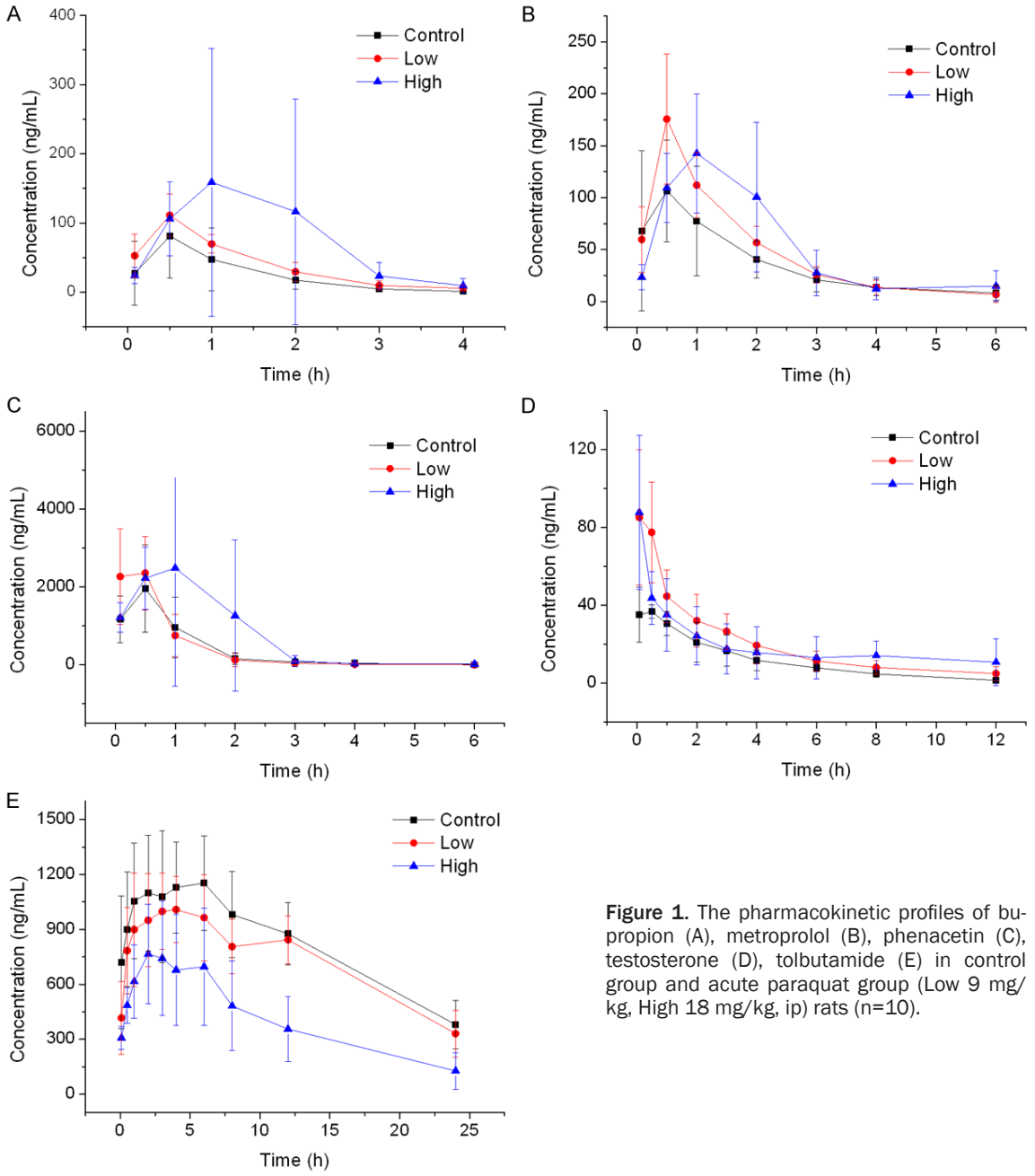

Figure 1. The pharmacokinetic profiles of bupropion (A), metroprolol (B), phenacetin (C), testosterone (D), tolbutamide (E) in control group and acute paraquat group (Low 9 mg/kg, High 18 mg/kg, ip) rats (n=10).

Results

Pharmacokinetics

The main pharmacokinetic parameters of bupropion, metroprolol, phenacetin, testosterone and tolbutamid calculated from non-compartment model analysis were summarized in **Table 2**. The representative profiles of concentration of drugs (bupropion, metroprolol, phenacetin, testosterone and tolbutamid) vs. time were presented in **Figure 1**.

From the **Table 2**, no difference in pharmacokinetic behaviors can be observed between paraquat group and control group for bupropion and phenacetin. While from the **Table 2**, the pharmacokinetic behaviors for metroprolol, compared with the control group, $AUC_{(0-t)}$ increased (Low, P<0.05 and High, *P*>0.05), CL decreased (Low, *P*<0.05; and High, P>0.05), C_{max} increased (Low, P>0.05; High, P>0.05). While for testosterone, compared with the control group, $AUC_{(0-t)}$ increased (Low, P<0.05 and High, *P*>0.05), CL decreased (Low, *P*<0.05; and High, P>0.05),

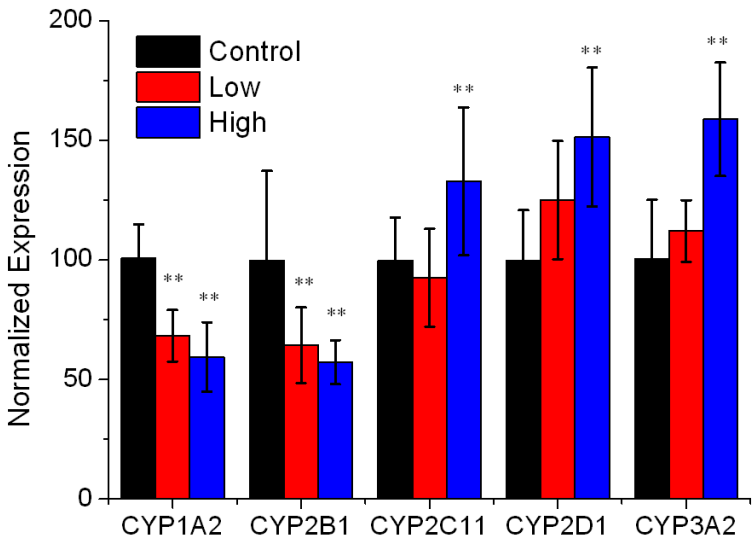

Figure 2. Effect of paraquat on mRNA expression of CYP1A2, CYP2B1, CYP2C11, CYP2D1 and CYP3A2 in liver of rat (n=6), *P<0.05 vs. Control; **P<0.01 vs. Control group.

C_{max} increased (Low, P<0.01; High, P<0.05). While for tolbutamide, compared with the control group, $AUC_{(0-t)}$ decreased (Low, P>0.05 and High, *P*<0.01), CL increased (Low, P>0.05; and High, *P*<0.01), C_{max} decreased (Low, P>0.05; High, *P*<0.01).

Effects of paraquat on the mRNA expression of CYP450 in rat liver

After intraperitoneal administration of paraquat (**Figure 2**), the levels of CYP1A2 and CYP2B1 in the paraquat group were decreased compared with the control group (P<0.01), the mRNA expression levels of CYP1A2 and CYP2B1 in the paraquat groups were obviously lower.

After intraperitoneal administration of paraquat (**Figure 2**), the mRNA expression levels of CYP2C11, CYP2D1 and CYP3A2 in the high paraquat group were increased compared with the control group (P<0.01); while no difference in the levels of CYP2C11, CYP2D1 and CYP3A2 in the low group can be observed.

Discussion

In general, changes in pharmacokinetics are thought to be caused by drug-drug or drug-food interactions [18]. 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 [19, 20]. For these reasons, we evaluated the effects of acute paraquat poisoning on the activity of CYP enzymes in *vivo*. We selected CYP isoforms CYP1A2, CYP2D1/CYP2D61, CYP3A2/CYP3A4, CYP2C11/CYP2C9 and CYP2B1/CYP2B6 because more than 90% of drugs are known to be metabolized by these 6 CYP enzymes [21-24].

There no significant difference for AUC, CL and C_{max} of bupropion and phenacetin (P>0.05) between the paraquat group (Low, High) and control group was observed. It suggested that intraperitoneal administration of paraquat was not able to induce or inhibit the activity of CYP2B1 and CYP1A2 enzyme.

The pharmacokinetic parameters of metroprolol and testosterone experienced obvious change with increased $AUC_{(0-t)}$ (P<0.05), C_{max} (P<0.05) and decreased CL (P<0.05) in low group, no significant difference found in high group. The mRNA expression levels of CYP2D1 and CYP3A2 in high paraquat groups were obviously increased. However, the mRNA expression results were not consistent with the pharmacokinetic results. It indicates that intraperitoneal administration of paraquat could not induce or inhibit the activity of the metabolism of CYP2D1 and CYP3A2 in rat. Enzymes activity or protein detection may prove the effect of paraquat better, the effect intraperitoneal administration of paraquat on CYP2D1 and CYP3A2 in rat should be further study.

The pharmacokinetic parameters of and tolbutamide experienced obvious change after the dosage increase. Compared with the control group, $AUC_{(0-t)}$ decreased (Low, P>0.05 and High, *P*<0.01), CL increased (Low, P>0.05; and High, *P*<0.01), C_{max} decreased (Low, P>0.05; High, *P*<0.01). The mRNA expression levels of CYP2C11 in high paraquat groups were obviously increased. The mRNA expression results were consistent with the pharmacokinetic results. This result indicates that intraperitone-

al administration of paraquat could induce the metabolism of CYP2C11 in rat.

Conclusion

The results observed in this study would provide us valuable information regarding the interactions of paraquat with other drugs. Induce of drug metabolizing enzyme CYP2C11 would decrease the plasm concentration of other drug.

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Disclosure of conflict of interest

None.

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