Original Article

Effect of acute paraquat poisoning on CYP450 isoforms activity in rats by cocktail method

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Abstract: Paraquat is a highly effective contact herbicide that is marketed worldwide as a fantastical, non-selective compound for broadleaf weed control. As compared to most pesticides, paraquat is extremely toxic to humans and the lack of strategies to manage paraquat poisoning has resulted in high fatality rates. The rats were randomly divided into acute paraquat poisoning group and control group. The paraquat group rats were given 36 mg/kg paraquat by intragastric administration. The influence of acute paraquat poisoning on the activities of CYP450 isoforms CYP2B6, CYP1A2, CYP2C9, CYP2D6, CYP3A4 and CYP2C19 were evaluated by cocktail method, they were responded by the changes of pharmacokinetic parameters of bupropion, phenacetin, tolbutamide, metoprolol, midazolam and omeprazole. The six probe drugs were given to rats through intragastric administration, and the plasma concentrations were determined by UPLC-MS/MS. In the results of paraquat group compared to control group, there was statistical pharmacokinetic difference for bupropion, tolbutamide, metoprolol, midazolam and omeprazole. Acute paraquat poisoning may induce the activities of CYP2C19, and inhibit of CYP2B6, CYP2C9, CYP2D6 and CYP3A4 in rats. This may give advising for reasonable drug use after acute paraquat poisoning.

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

Introduction

Paraquat (1,1-dimethyl-4-4-bipiridinium dichloride, PQ) is a potent herbicide which was used worldwide, especially in developing countries [1]. Since its introduction in agriculture in 1962, thousands of deaths have occurred yearly due to accidental or intentional ingestion of paraquat [2]. Paraquat is highly toxic to humans and animals and no effective antidote is available, which causes a high mortality rate [3, 4]. Although patient survival is enhanced by comprehensive therapy, mortality is not obviously improved [5]. The lung is an affected main target organ because paraquat is actively taken up by the alveolar epithelium. The toxicity of paraquat on the lung is dependent on a process of alternative reduction and re-oxidation process known as the redox cycling [6]. Paraquat is reduced primarily by nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P-450 reductase to form paraquat monocation free radical. The electron transferred to paraquat rapidly moves to oxygen with subsequent production of superoxide. Consequently, excessive oxidative damage occurs from reactive oxygen species (ROS) [7, 8].

Cytochrome P450 (CYP450) is the most important drug-metabolizing enzymes in liver with largest number and highest abundance of CYP isoforms [9-11]. CYP1, CYP2, and CYP3 are three kinds of isoenzymes mainly involved in the metabolism of many drugs in both humans and other animals such as rats [12]. Probe drug is a kind of compound specially catalyzed by CYP isoforms, and the metabolic rate of probe drug can be used to assess the activities of CYP isoforms. In order to assess these various individual CYP450 activities, various probe drugs have been found and widely used in many clini-
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In this paper, six probe drugs are used to evaluate the induction or inhibition effects of acute paraquat poisoning on the activities of rats CYP450 isoforms such as CYP1A2, CYP2B6, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 in rats. According to the changes in pharmacokinetic parameters of six specific probe drugs, it may provide rational clinical drug guidance use after acute paraquat poisoning.

Material and methods

Chemicals

Bupropion, phenacetin, tolbutamide, metoprolol, midazolam, omeprazole (all > 98%) and the internal standard diazepam were obtained from Sigma-Aldrich Company (St. Louis, USA). Ultra-pure 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) were purchased from Shanghai SLAC Laboratory Animal Co, Ltd. Animals were housed under a natural light-dark cycle conditions with controlled temperature (22°C). All forty rats were housed at Wenzhou Medical University Laboratory Animal Research Center. All experimental procedures were approved ethically by the Wenzhou Medical University Administration Committee of Experimental Animals.

UPLC-MS/MS conditions

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

Bupropion, phenacetin, tolbutamide, metoprolol, midazolam, omeprazole and diazepam (IS) were separated using a Waters BEH C18 column (2.1 mm × 100 mm, 1.7 μm) maintained at 40°C. The initial mobile phase consisted of 0.1% formic acid and acetonitrile 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 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 decreased to 30% within 0.1 min, and maintained at 30% for 0.4 min. The total run time of the analytes need 3 min.

The mass spectrometric detection was performed in a positive mode. Nitrogen was used as the cone gas (50 L/h) and desolation gas (1000 L/h). The mass conditions were set as follows: source temperature 150°C, capillary voltage 2.5 kV; desolation 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 metoprolol, 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.

Pharmacokinetics

Twenty rats (220 ± 20 g) were randomly divided to paraquat group and control group. Paraquat group were give paraquat (36 mg/kg) by intragastric administration. Control group were give saline by intragastric administration. After 20 days, the paraquat and control group intragastric administration of mixed six probe drugs (bupropion, phenacetin, tolbutamide, metoprolol, midazolam and omeprazole were 10, 10, 1, 10, 10 and 10 mg/kg).

Blood (0.3 mL) samples were collected at 0.083, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 h from the tail vein into heparinized 1.5 mL polythene tubes after intragastric administration of six probe drugs. The 100 μL plasma was obtained from blood sample after centrifuged at 4000 g for 10 min. In a 1.5 mL centrifuge tube, 100 μL of collected plasma sample followed by the addition of 200 μL of acetonitrile (containing 50 ng/mL IS). After vortex-mixed for 1.0 min, the sample was centrifuged at 13000 g for 15 min. Then the 2 μL supernatant was injected into the UPLC-MS/MS system for analysis.
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Table 1. Pharmacokinetic parameters of bupropion and omeprazole in control-group and paraquat-group rats (mean ± SD, n = 10)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AUC(0-t) ng/mL *h</th>
<th>AUC(0-∞) ng/mL *h</th>
<th>t1/2 h</th>
<th>CL L/h/kg</th>
<th>V L/kg</th>
<th>Cmax ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupropion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paraquat</td>
<td>584.8 ± 549.5*</td>
<td>590.3 ± 552.6*</td>
<td>4.7 ± 1.4**</td>
<td>25.3 ± 13.5**</td>
<td>167.8 ± 95.9**</td>
<td>146.6 ± 82.3**</td>
</tr>
<tr>
<td>Control</td>
<td>121.9 ± 32.6</td>
<td>143.1 ± 46.4</td>
<td>14.9 ± 7.4</td>
<td>78.4 ± 30.8</td>
<td>1496.8 ± 520.0</td>
<td>22.7 ± 5.2</td>
</tr>
<tr>
<td>Omeprazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paraquat</td>
<td>509.9 ± 189.5*</td>
<td>548.3 ± 229.5*</td>
<td>5.1 ± 4.5</td>
<td>21.1 ± 8.2*</td>
<td>123.1 ± 82.6</td>
<td>256.9 ± 70.5**</td>
</tr>
<tr>
<td>Control</td>
<td>703.3 ± 112.7</td>
<td>769.8 ± 127.2</td>
<td>7.7 ± 1.6</td>
<td>13.3 ± 2.3</td>
<td>147.4 ± 38.4</td>
<td>409.6 ± 111.6</td>
</tr>
</tbody>
</table>

Compared paraquat group with the control group, *P < 0.05, **P < 0.01.

Table 2. Pharmacokinetic parameters of and phenacetin and tolbutamide in control-group and paraquat-group rats (mean ± SD, n = 10)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AUC(0-t) ng/mL *h</th>
<th>AUC(0-∞) ng/mL *h</th>
<th>t1/2 h</th>
<th>CL L/h/kg</th>
<th>V L/kg</th>
<th>Cmax ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenacetin</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Paraquat</td>
<td>11527.9 ± 5060.0</td>
<td>11537.9 ± 5050.1</td>
<td>2.5 ± 3.6</td>
<td>1.1 ± 0.8</td>
<td>7.5 ± 20.4</td>
<td>4968.7 ± 1786.9</td>
</tr>
<tr>
<td>Control</td>
<td>11828.4 ± 3627.7</td>
<td>11834.0 ± 3621.7</td>
<td>2.5 ± 2.2</td>
<td>0.9 ± 0.3</td>
<td>3.7 ± 4.2</td>
<td>8172.9 ± 2590.3</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>352907.6 ± 45577.6**</td>
<td>466590.1 ± 116280.7**</td>
<td>22.7 ± 10.7*</td>
<td>0.002 ± 0.001**</td>
<td>0.068 ± 0.017**</td>
<td>11293.6 ± 2181.0**</td>
</tr>
<tr>
<td>Control</td>
<td>206997.8 ± 51809.1</td>
<td>234149.5 ± 62673.6</td>
<td>15.9 ± 4.3</td>
<td>0.005 ± 0.002</td>
<td>0.102 ± 0.024</td>
<td>7263.2 ± 2219.3</td>
</tr>
</tbody>
</table>

Compared paraquat group with the control group, *P < 0.05, **P < 0.01.

Table 3. Pharmacokinetic parameters of midazolam and Metoprolol in control-group and paraquat-group rats (mean ± SD, n = 10)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AUC(0-t) ng/mL *h</th>
<th>AUC(0-∞) ng/mL *h</th>
<th>t1/2 h</th>
<th>CL L/h/kg</th>
<th>V L/kg</th>
<th>Cmax ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midazolam</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Paraquat</td>
<td>644.7 ± 281.6**</td>
<td>940.3 ± 698.9**</td>
<td>17.6 ± 43.9</td>
<td>14.9 ± 7.3**</td>
<td>188.4 ± 247.3</td>
<td>192.5 ± 93.7*</td>
</tr>
<tr>
<td>Control</td>
<td>168.9 ± 35.1</td>
<td>172.2 ± 34.4</td>
<td>1.8 ± 2.0</td>
<td>60.2 ± 12.5</td>
<td>175.8 ± 235.8</td>
<td>93.2 ± 38.8</td>
</tr>
<tr>
<td>Metoprolol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paraquat</td>
<td>5026.9 ± 2937.8*</td>
<td>5301.0 ± 3208.8*</td>
<td>2.4 ± 0.6</td>
<td>2.7 ± 1.6**</td>
<td>8.2 ± 3.7**</td>
<td>1302.7 ± 526.2**</td>
</tr>
<tr>
<td>Control</td>
<td>1662.5 ± 570.7</td>
<td>1728.3 ± 581.1</td>
<td>2.5 ± 0.8</td>
<td>6.3 ± 1.9</td>
<td>22.4 ± 8.3</td>
<td>629.4 ± 214.1</td>
</tr>
</tbody>
</table>

Compared paraquat group with the control group, *P < 0.05, **P < 0.01.
Plasma probe drugs concentration versus time was analyzed by Version 3.0 Data Analysis System (Wenzhou Medical University, China). The main pharmacokinetic parameters of the paraquat group and control group were analyzed by SPSS 18.0 statistical software, the P < 0.05 was considered as statistically significant.

Results

Method validation

The concentration of bupropion, phenacetin, tolbutamide, metoprolol, midazolam and omeprazole in rat plasma was simultaneously determined by a sensitive and simple UPLC-MS/MS
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The LLOQ for each probe drug in plasma was 2 ng/mL. The RSD of the six probe drugs were less than 11%. 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 88% to 110%. The matrix effects were more than 85% or less than 113%. The extraction recoveries were better than 84%.

Pharmacokinetics

The main pharmacokinetic parameters of bu- propion, phenacetin, tolbutamide, metoprolol, midazolam and omeprazole were summarized from non-compartment model analysis in Tables 1-3. The representative bupropion, phenacetin, tolbutamide, metoprolol, midazolam and omeprazole concentration vs. time profiles were presented in Figure 1. As could be seen from Figure 1, the $C_{\text{max}}$ and AUC of bupropion, tolbutamide, metoprolol, midazolam in paraquat group is higher than the control group, while the $C_{\text{max}}$ and AUC of omeprazole is lower than the control group.

As can be seen from Table 1, the pharmacokinetic parameters of bupropion and omeprazole have changed, $AUC_{(0-t)}$ increased ($P < 0.05$), CL decreased ($P < 0.01$), $C_{\text{max}}$ increased ($P < 0.01$) for bupropion, compared paraquat group with the control group; $AUC_{(0-t)}$ decreased ($P < 0.05$), CL decreased ($P < 0.01$), $C_{\text{max}}$ increased ($P < 0.01$) for metoprolol, compared paraquat group with the control group. It indicates that the acute paraquat poisoning on rats may inhibit the activity of CYP3A4 and CYP2D6 enzyme of rats.

As can be seen from Table 2, the pharmacokinetic parameters of phenacetin and tolbutamide have changed, $AUC_{(0-t)}$ decreased ($P > 0.05$), CL increased ($P > 0.05$), $C_{\text{max}}$ decreased ($P > 0.05$) for phenacetin, compared paraquat group with the control group; $AUC_{(0-t)}$ decreased ($P < 0.01$), CL decreased ($P < 0.01$), $C_{\text{max}}$ increased ($P < 0.01$) for tolbutamide, compared paraquat group with the control group. It indicates that the acute paraquat poisoning on rats could not inhibit or induce CYP1A2, and it may inhibit the activity of CYP2C9 enzyme of rats.

As can be seen from Table 3, the pharmacokinetic parameters of midazolam and metoprolol have changed, $AUC_{(0-t)}$ decreased ($P < 0.01$), CL increased ($P < 0.01$), $C_{\text{max}}$ decreased ($P < 0.05$) for midazolam, compared paraquat group with the control group; $AUC_{(0-t)}$ decreased ($P < 0.05$), CL decreased ($P < 0.01$), $C_{\text{max}}$ increased ($P < 0.01$) for metoprolol, compared paraquat group with the control group. It indicates that the acute paraquat poisoning on rats may inhibit the activity of CYP2B6 enzyme and induce CYP2C19 enzyme of rats.

Discussion

Paraquat is a widely used herbicide that has caused many accidental and intentional deaths, particularly in Asia [18-21]. The two most important target organs of paraquat toxicity are lungs and kidneys [22]. Nephrotoxicity is very common in paraquat intoxication [23, 24]. Paraquat is mainly excreted unchanged by the kidney and acute renal failure is often the first systemic effect observed in paraquat toxicity [25, 26]. Renal dysfunction leads, in turn, to decreased renal paraquat clearance which promotes greater toxicity in other organs, and thus acute kidney injury increases these verity of pulmonary toxicity, multi-organ failure and death [27-29].

CYP450 mediated metabolism was found to be not a major elimination pathway for paraquat. This is not entirely bewildering due to prior work on similar agents that contain a benzamide moiety [30]. It is unlikely that CYP450-mediated oxidation is significant due to no changes in the chromatograms were paid attention and hence, the primary pathways of elimination for paraquat remain to be elucidated [31].

As other drugs are always used after acute paraquat poisoning, interactions between other drugs and paraquat undertake the risk of either diminished efficacy or adverse effects. Drug-drug interactions often occur at the active site of these enzymes since CYP450 enzymes play a key role in the phase I metabolism of the majority of all marketed drugs.

In general, changes in pharmacokinetics are thought to be caused by drug-drug or drug-food interactions [32]. 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. A large number of drugs are metabolized by CYP enzymes in the
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liver, and more than 90% of drug-drug interactions occur at the CYP-catalyzed step [33, 34]. Similarly, supplement-drug interactions involving CYP activity are occasionally found to cause considerable adverse events. For these reasons, we evaluated the effects of acute paraquat poisoning 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 [35, 36].

Conclusion

In our study, acute paraquat poisoning (36 mg/kg) may induce the activities of CYP450 isoforms CYP2C19 of rats, and may inhibit of CYP2B6, CYP2C9, CYP2D6 and CYP3A4 of rats. These results would give us valuable information regarding the interactions of paraquat with drugs, drugs used after acute paraquat poisoning might cause pharmacokinetic interactions, which required dose adjustment to avoid over dosage or reduced blood drug concentration.

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Disclosure of conflict of interest

None.

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