Original Article
Effect of dextromethorphan on CYP450 isoforms activity of rats

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Abstract: Dextromethorphan at high doses has phencyclidine-like effects on the NMDA receptor system; recreational use of high doses has been found to cause mania and hallucinations. In order to investigate the effects of dextromethorphan 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 dextromethorphan group (Low, Medium, High) and control group. The dextromethorphan group rats were given 12, 24, 48 mg/kg (Low, Medium, High) dextromethorphan by continuous intragastric administration for 7 days. Five probe drugs bupropion, metoprolol, 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 metoprolol, phenacetin and testosterone in rats were observed by comparing dextromethorphan group with control group. Continuous 7 days-intragastric administration of dextromethorphan inhibits the activities of CYP2D1 of rats. Enzyme inhibition by co-administered drugs and genetic variations of their expression can increase the risk of adverse reactions. Additionally, continuous 7 days-intragastric administration of dextromethorphan may not cause hepatotoxicity.

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

Introduction

Dextromethorphan is an over-the-counter antitussive medication present in many brands of cough, cold, and flu preparations, such as Robitussin, Coricidin, Benylin, and others [1]. It is also the subject of renewed therapeutic interest as an adjunct to opiate use in pain management [2] and has been proposed as a possible rapidly acting antidepressant. The antitussive effects are believed to be mediated through the sigma receptor group, which has affinity for opioid antitussives such as codeine [3]. When taken in significant excess, dextromethorphan produces hallucinogenic and dissociative effects similar in nature to phencyclidine and ketamine [4]. Dextromethorphan metabolism is mediated through the isoenzyme group CYP2D6, which is subject to genetic polymorphism, which may result in impaired metabolism and accumulation of the drug, leading to toxicity [5].

Cytochrome P450 (CYP) enzymes are responsible for most biotransformation steps of xenobiotics and endogenous molecules. Variations of their activity by inhibition or induction can influence the pharmacokinetics and thereby the effect of drugs (of abuse). Enzyme inhibition by co-administered drugs (of abuse) and/or genetic variations of their expression can increase the risk of adverse reactions [6] or reduce the desired effect [7]. Such drug-drug interactions were described as a major reason for hospitalization or even death [8].

So far, no study on the effects of dextromethorphan on the metabolic capacity of CYP enzyme
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was reported. Therefore, in this study, five probe drugs were employed to evaluate effect of dextromethorphan on the metabolic capacity of CYP2B1, CYP2D1, CYP1A2, CYP3A2, CYP2C11. The effects of dextromethorphan on rat CYP enzyme activity will be evaluated according to the pharmacokinetic parameters changes of five specific probe drugs (bupropion, metprolol, phenacetin, testosterone and tolbutamide).

Material and methods

Chemicals

Bupropion, metprolol, phenacetin, testosterone and tolbutamide (all >98%) and the internal standard diazepam (IS) 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) 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-two 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-two rats (220±20 g) were randomly divided into four different dosages of dextromethorphan groups (Low-group, Medium-group, High-group and control group with 8 rats in each group). Dextromethorphan was dissolved in corn oil as suspension at three different concentrations (12, 24, 48 mg/mL). Three different dextromethorphan group (Low-group, Medium-group, High-group) were respectively given dextromethorphan 12, 24, 48 mg/kg one time by intragastric administration at every morning, and last for 7 days. Control group were give saline by same administration method. At 8 days morning, five probe drugs bupropion, metprolol, phenacetin, testosterone and tolbutamide were mixed in corn oil and given to the rats of three dextromethorphan groups and control group by intragastric administration at a single dosage 10 mg/kg for bupropion, 24 mg/kg for metprolol, 80 mg/kg for phenacetin, 10 mg/kg for 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). The main pharmacokinetic parameters of the dextromethorphan group and control group were analyzed by SPSS l8.0 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, metprolol, phenacetin, testosterone and tolbutamide in rat plasma were simultaneously determined by a sensitive and simple UPLC-MS/MS method [9]. 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 14%. 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 93% to 114%. The matrix effects were more than 85% or less than 112%. The extraction recoveries were better than 86%.

Histopathology

After pharmacokinetic properties analysis, rats were deeply anesthetized with 10% chloral
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hydrate (i.p., 20 mg/kg). The some liver of control group and dextromethorphan treated groups were rapidly isolated and immersed in freshly prepared 4% w/v formaldehyde (0.1 M phosphate buffer, pH 7.2) for 48 h, and then embedded in paraffin. Then 5 µm-thick histologic sections were prepared and stained with routine HE method (hematoxylin and eosin). The morphological changes of liver were observed under light microscope.

Results

Pharmacokinetics

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

From the Table 1, no difference in pharmacokinetic behaviors can be observed between dextromethorphan group and control group for bupropion and tolbutamide. While for metprolol, compared with the control group, \( \text{AUC}_{(0-t)} \) increased (P<0.01), CL decreased (Low, P>0.05; medium, P<0.01; high, P<0.01), \( \text{C}_{\text{max}} \) increased (low, P>0.05; medium, P<0.01; high, P<0.05). While for phenacetin, compared with the control group, \( \text{AUC}_{(0-t)} \) decreased (Low, P<0.01; medium, P>0.05; high, P<0.01), \( \text{CL} \) increased (Low, P<0.05; medium, P>0.05; high, P>0.05), \( \text{C}_{\text{max}} \) decreased (Low, P<0.01; medium, P>0.05; high, P<0.01). There no difference in pharmacokinetic behaviors can be observed between dextromethorphan group (low and high) and control group for testosterone, while the pharmacokinetic behaviors of testosterone in medium dosage group compared with the control group, \( \text{AUC}_{(0-t)} \) decreased (P<0.01), \( \text{CL} \) increased (P<0.01), \( \text{C}_{\text{max}} \) decreased (P<0.05).

Morphological changes of liver

There was no obvious morphologic change observed between control group, low dose group, medium dose group and high dose group according to pathological examination of liver stained with hematoxylin and eosin (HE). The pathology slice examination showed: in control group, the liver lobules were intact, hepatocytic

Table 1. Pharmacokinetic parameters of probe drugs from control group and dextromethorphan group rats (mean ± SD, n=8)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>( \text{AUC}_{(0-t)} ) (ng/mL*h)</th>
<th>( \text{AUC}_{(0-\infty)} ) (ng/mL*h)</th>
<th>t1/2z (h)</th>
<th>( \text{CLz/F} ) (L/h/kg)</th>
<th>( \text{Vz/F} ) (L/kg)</th>
<th>( \text{C}_{\text{max}} ) (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupropion (CYP2B1)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>394.0±153.2</td>
<td>438.9±165.2</td>
<td>1.5±0.5</td>
<td>25.9±9.9</td>
<td>56.6±34.1</td>
<td>190.4±102.9</td>
</tr>
<tr>
<td>Low</td>
<td>241.0±88.1</td>
<td>282.1±163.8</td>
<td>1.7±1.5</td>
<td>37.5±11.1</td>
<td>89.5±77.0</td>
<td>79.0±35.2*</td>
</tr>
<tr>
<td>Medium</td>
<td>459.2±220.4</td>
<td>519.4±294.9</td>
<td>1.4±0.7</td>
<td>24.1±11.4</td>
<td>48.0±28.2</td>
<td>156.6±71.0</td>
</tr>
<tr>
<td>High</td>
<td>401.6±157.2</td>
<td>460.3±171.7</td>
<td>1.8±0.5</td>
<td>24.8±10.3</td>
<td>62.9±28.8</td>
<td>119.7±44.8</td>
</tr>
<tr>
<td>Metroprolol (CYP2D1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>1002.4±270.0</td>
<td>1009.5±276.1</td>
<td>0.7±0.1</td>
<td>10.5±2.5</td>
<td>31.0±11.3</td>
<td>458.1±124.1</td>
</tr>
<tr>
<td>Low</td>
<td>1479.0±334.8**</td>
<td>1505.0±353.5**</td>
<td>0.6±0.4</td>
<td>6.9±1.5**</td>
<td>6.3±4.6*</td>
<td>492.4±84.6</td>
</tr>
<tr>
<td>Medium</td>
<td>1822.1±357.3**</td>
<td>1885.1±505.6**</td>
<td>0.8±0.6</td>
<td>5.5±1.1**</td>
<td>5.3±2.1**</td>
<td>631.4±97.8*</td>
</tr>
<tr>
<td>High</td>
<td>1955.4±371.1**</td>
<td>2053.6±463.5**</td>
<td>1.1±0.3</td>
<td>5.1±1.0**</td>
<td>8.1±2.1</td>
<td>650.1±193.4*</td>
</tr>
<tr>
<td>Phenacetin (CYP1A2)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>7481.4±2117.8</td>
<td>7525.9±2176.2</td>
<td>0.4±0.1</td>
<td>1.4±0.5</td>
<td>0.8±0.4</td>
<td>5398.5±1002.0</td>
</tr>
<tr>
<td>Low</td>
<td>2418.3±1327.2**</td>
<td>2434.5±1336.5**</td>
<td>0.5±1.0</td>
<td>6.0±4.5*</td>
<td>4.3±3.2**</td>
<td>1821.9±1022.6**</td>
</tr>
<tr>
<td>Medium</td>
<td>6357.5±2656.6</td>
<td>6396.3±2682.1</td>
<td>0.4±0.1</td>
<td>1.8±0.8</td>
<td>1.1±0.5</td>
<td>5479.9±4932.8</td>
</tr>
<tr>
<td>High</td>
<td>3592.1±1890.0**</td>
<td>3606.3±1892.3**</td>
<td>0.5±1.0</td>
<td>5.1±6.3</td>
<td>3.8±5.1</td>
<td>2672.7±1358.8**</td>
</tr>
<tr>
<td>Testosterone (CYP3A)</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>114.2±41.9</td>
<td>121.8±44.6</td>
<td>0.9±0.3</td>
<td>93.1±35.7</td>
<td>118.8±59.2</td>
<td>83.4±35.7</td>
</tr>
<tr>
<td>Low</td>
<td>82.3±46.6</td>
<td>83.4±46.9</td>
<td>0.7±0.1</td>
<td>150.1±64.0</td>
<td>146.9±73.8</td>
<td>72.2±53.2</td>
</tr>
<tr>
<td>Medium</td>
<td>61.0±13.4**</td>
<td>67.9±16.5**</td>
<td>1.0±1.1</td>
<td>155.3±38.5**</td>
<td>197.5±159.7</td>
<td>48.6±20.9*</td>
</tr>
<tr>
<td>High</td>
<td>107.3±42.7</td>
<td>117.9±43.0</td>
<td>1.1±0.8</td>
<td>96.0±43.0</td>
<td>141.0±84.2</td>
<td>86.8±51.9</td>
</tr>
<tr>
<td>Tolbutamide (CYP2C11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>101950.5±12783.0</td>
<td>106587.1±13578.1</td>
<td>5.2±0.3</td>
<td>0.010±0.001</td>
<td>0.074±0.008</td>
<td>9556.1±1075.7</td>
</tr>
<tr>
<td>Low</td>
<td>111493.2±10194.4</td>
<td>118672.4±11743.0</td>
<td>5.7±0.7</td>
<td>0.008±0.001</td>
<td>0.069±0.007</td>
<td>10518.8±1426.4</td>
</tr>
<tr>
<td>Medium</td>
<td>113210.5±6280.4</td>
<td>123104.4±7378.1</td>
<td>6.4±0.7</td>
<td>0.008±0.001</td>
<td>0.075±0.009</td>
<td>10131.2±838.5</td>
</tr>
<tr>
<td>High</td>
<td>109777.7±5647.2</td>
<td>117088.7±7024.9</td>
<td>5.9±0.3</td>
<td>0.009±0.001</td>
<td>0.072±0.003</td>
<td>10790.9±2483.7</td>
</tr>
</tbody>
</table>

Dextromethorphan group was compared with the control group. *P<0.05, **P<0.01.
plates separated by sinusoids, liver cells attached to each other tightly and arranged radially along with central veins. These normal histological structures hadn’t damaged in three different dose groups after administrated dextromethorphan. In low dose group, medium dose group and high dose group, the hepatocytic plates were separated by sinusoids, liver lobules were intact and liver cells arranged tightly along with central veins which can be recognized clearly. There was no lobular inflammatory cell infiltration, liver cells swelling, dissolved, necrosis observed. The morphological changes of four groups were showed in Figure 2.

Discussion

In general, changes in pharmacokinetics are thought to be caused by drug-drug or drug-food interactions [10-15]. 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 liver, and more than 90% of drug-drug interactions occur at the CYP-catalyzed step [16, 17]. Similarly, supplement-drug interactions involving CYP activity are occasionally found to cause considerable adverse events. For these reasons, we evaluated the effects of dextromethorphan 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 [18, 19].

There no significant difference for AUC, CL and $C_{\text{max}}$ of bupropion and tolbutamide (P>0.05) between the dextromethorphan group (low, medium, high) and control group was observed. It suggested that the dextromethorphan was not able to induce or inhibit the activity of CYP2B1 and CYP2C11 enzyme. The pharmacokinetic parameters of metoprolol experienced obvious change with increased $AUC_{(0-t)}$ (P<0.05), $C_{\text{max}}$ (P<0.05) and decreased CL (P<0.05) after the dosage increase. This result indicates that the 7 days-intragastric administration of dextromethorphan could inhibit the metabolism of metoprolol (CYP2D1) in rat. The pharmacokinetic parameters of phenacetin and testosterone experienced obvious change after 7 days-intragastric administration of dextromethorphan. However, pharmacokinetic results were not consistent with the dosage increase. It indicates that the 7 days-intragastric administration of dextromethorphan could not induce or inhibit the activity of the metabolism of phenacetin (CYP1A2) and testosterone (CYP3A2) in rat.

As dextromethorphan is always administrated in combination with other drugs, interactions between dextromethorphan and other drugs would increase the risk of either diminished efficacy or adverse effects. In our study, we
found that 7 days-intragastric administration of dextromethorphan inhibit the metabolism of metprolol (CYP2D1). Therefore, the metabolism and elimination of drugs would change if they are administrated in combination with dextromethorphan.

After the pharmacokinetic profiles evaluation by cocktail method, we also investigated the hepatotoxicity of dextromethorphan by observing the pathological changes of liver after dextromethorphan administration. The pathological changes of liver were observed at three different dosages with no changes, Figure 2. A more systematic and comprehensive study to investigate the hepatotoxicity of dextromethorphan will be carried out.

Conclusion
The results observed in this study would provide us valuable information regarding the interactions of dextromethorphan with other drugs. Inhibit of drug metabolizing enzyme CYP2D1 by dextromethorphan would increase the plasm concentration of other drug. Enzyme inhibition by co-administered drugs and genetic variations of their expression can increase the risk of adverse reactions.

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Disclosure of conflict of interest
None.

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sulfide poisoning on cytochrome P450 iso-


