Original Article

Effect on CYP450 isoforms activity of rats after acute methomyl poisoning

Zhiyi Wang1*, Yanyan Xu2*, Mengzhi Xu3, Yingying Lin3, Suping Yang3, Congcong Wen3, Xianqin Wang3, Chan Chen4

1The Second Affiliated Hospital & Yuying Children’s Hospital, Wenzhou Medical University, Wenzhou 325000, China; 2Department of Pharmacy, Lishui Central Hospital, Lishui 323000, China; 3Analytical and Testing Center of Wenzhou Medical University, Wenzhou 325035, China; 4The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China. *Equal contributors.

Received November 5, 2015; Accepted February 10, 2016; Epub March 15, 2016; Published March 30, 2016

Abstract: In order to investigate the effects of methomyl poisoning 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 methomyl group and control group. The methomyl group rats were given 4 mg/kg methomyl by intraperitoneal administration. After 7 days, five probe drugs bupropion, metaprolol, 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 metaprolol, phenacetin, testosterone and tolbutamide in rats were observed by comparing methomyl group with control group. Methomyl poisoning inhibits the activities of CYP2D1 and CYP1A2 of rats. Enzyme inhibition by methomyl poisoning can increase the risk of adverse reactions. Induction of drug metabolizing enzyme CYP3A2 and CYP2C11 by methomyl poisoning would reduce the efficacy of other drug. Additionally, methomyl poisoning may cause hepatotoxicity.

Keywords: CYP450, methomyl, poisoning, cocktail, rat

Introduction

Methomyl [S-methyl N-((methylcarbamoyl)oxy) thioacetimidate], a carbamate compound that functions as an AChE-inhibiting systemic insecticide, has been classified as a highly toxic pesticide for aquatic organisms [1, 2]. Because of its broad biological activity, relatively rapid disappearance and high efficiency against insects, methomyl is widely used in many agricultural countries for crop protection and soil or plant treatment [3-7]. Its acute toxicity to various freshwater fish, with 96-h LC50 values ranging from 0.9 mg/L (bluegill sunfish) to 3.4 mg/L (rainbow trout), was reported in several studies [8].

Cytochrome P450 (CYP) enzymes are responsible for most biotransformation steps of xenobiotics and endogenous molecules [9]. 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 [10] or reduce the desired effect [11]. Such drug-drug interactions were described as a major reason for hospitalization or even death [12].

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

Material and methods

Chemicals

Bupropion, metaprolol, phenacetin, testosterone and tolbutamide (all > 98%) and the inter-
Effect of methomyl on CYP450 isoforms activity of rats

Animals

Sprague-Dawley rats (male, 220 ± 20 g) purchased from Shanghai SLAC Laboratory Animal Co., Ltd. Animals were reared under a natural light-dark cycle conditions with controlled temperature (22°C). All 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

Sixteen rats (220 ± 20 g) were randomly divided into methomyl groups and control group. Methomyl was dissolved in water concentrations (4 mg/mL). The methomyl group was given methomyl 4 mg/kg by intraperitoneal administration. Control group were given saline by same administration method. At 8 days morning, five probe drugs bupropion, metoprolol, phenacetin, testosterone and tolbutamide were mixed in corn oil and given to the rats of methomyl groups and control group by intragastrical administration at a single dosage 10 mg/kg for bupropion, metoprolol, 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 intragastrical 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 methomyl group and control group were analyzed by SPSS 18.0 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, metoprolol, phenacetin, testosterone and tolbutamide in rat plasma were simultaneously determined by a sensitive and simple UPLC-MS/MS method [13]. 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 15%. 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 115%. The matrix effects were more than 80% or less than 115%. The extraction recoveries were better than 85%.

Histopathology

After pharmacokinetic properties analysis, rats were deeply anesthetized with 10% chloral hydrate (i.p., 20 mg/kg). The some liver and kidney of control group and methomyl 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, metoprolol, phenacetin, testosterone and tolbutamide calculated from non-compartment model analysis were summarized in Table 1. The representative profiles of concentration of drugs (bupropion, metoprolol, phen-
Effect of methomyl on CYP450 isoforms activity of rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AUC_{(0-t)}</th>
<th>AUC_{(0-∞)}</th>
<th>t_{1/2z}</th>
<th>CL_{z/F}</th>
<th>V_{z/F}</th>
<th>C_{max}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/mL*h</td>
<td>ng/mL*h</td>
<td>h</td>
<td>L/h/kg</td>
<td>L/kg</td>
<td>ng/mL</td>
</tr>
<tr>
<td>Bupropion (CYP2B1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>113.0 ± 78.7</td>
<td>115.6 ± 78.8</td>
<td>0.7 ± 0.3</td>
<td>124.0 ± 69.3</td>
<td>140.6 ± 98.7</td>
<td>91.1 ± 63.3</td>
</tr>
<tr>
<td>Methomyl</td>
<td>110.6 ± 84.7</td>
<td>136.1 ± 101.6</td>
<td>1.7 ± 0.3**</td>
<td>108.7 ± 73.4</td>
<td>273.0 ± 200.4</td>
<td>53.3 ± 37.0</td>
</tr>
<tr>
<td>Metroprolol (CYP2D1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>209.9 ± 90.1</td>
<td>243.4 ± 104.2</td>
<td>2.1 ± 2.8</td>
<td>48.5 ± 20.2</td>
<td>111.3 ± 102.2</td>
<td>129.2 ± 54.0</td>
</tr>
<tr>
<td>Methomyl</td>
<td>470.7 ± 253.3*</td>
<td>603.8 ± 265.1**</td>
<td>1.3 ± 0.4</td>
<td>20.9 ± 12.9**</td>
<td>38.8 ± 19.9</td>
<td>135.9 ± 70.6</td>
</tr>
<tr>
<td>Phenacetin (CYP1A2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1944.3 ± 1204.7</td>
<td>1968.5 ± 1201.6</td>
<td>0.5 ± 0.3</td>
<td>7.1 ± 4.5</td>
<td>5.7 ± 4.0</td>
<td>1916.0 ± 1078.5</td>
</tr>
<tr>
<td>Methomyl</td>
<td>3779.9 ± 2150.0*</td>
<td>3786.2 ± 2148.7*</td>
<td>0.4 ± 0.1</td>
<td>3.8 ± 2.6*</td>
<td>2.5 ± 2.0</td>
<td>2721.4 ± 1065.0*</td>
</tr>
<tr>
<td>Testosterone (CYP3A2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>110.1 ± 26.0</td>
<td>144.4 ± 33.3</td>
<td>3.5 ± 3.7</td>
<td>72.4 ± 16.8</td>
<td>322.4 ± 260.6</td>
<td>44.8 ± 7.8</td>
</tr>
<tr>
<td>Methomyl</td>
<td>69.6 ± 36.1*</td>
<td>72.5 ± 37.5**</td>
<td>1.2 ± 0.6</td>
<td>166.6 ± 73.2*</td>
<td>285.3 ± 200.1</td>
<td>47.9 ± 21.8</td>
</tr>
<tr>
<td>Tolbutamidee (CYP2C11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>17666.7 ± 2520.0</td>
<td>26535.9 ± 12464.4</td>
<td>15.1 ± 12.5</td>
<td>0.004 ± 0.001</td>
<td>0.076 ± 0.020</td>
<td>1099.1 ± 202.7</td>
</tr>
<tr>
<td>Methomyl</td>
<td>15042.2 ± 1054.0*</td>
<td>16574.8 ± 1719.8*</td>
<td>6.3 ± 1.7*</td>
<td>0.006 ± 0.001**</td>
<td>0.054 ± 0.010</td>
<td>1462.1 ± 129.9**</td>
</tr>
</tbody>
</table>

Methomyl group was compared with the control group, *: P < 0.05, **: P < 0.01.
acetin, testosterone and tolbutamide) vs. time were presented in Figure 1.

From the Table 1, no difference in pharmacokinetic behaviors can be observed between methomyl group and control group for bupropion. While for phenacetin, compared with the control group, AUC(0-t) increased (P < 0.05), CL decreased (P < 0.05), Cmax increased (P < 0.05). The similar pharmacokinetic behaviors were found for tolbutamide. Compared with the control group, AUC(0-t) decreased (P < 0.05), CL increased (P < 0.05) for testosterone. The similar pharmacokinetic behaviors were found for metoprolol.

**Morphological changes of liver**

The morphological changes of liver were shown in Figure 2. There was significant morphological difference in the liver tissues stained with hematoxylin and eosin (HE). In control group,
the liver lobules were intact, hepatocytic plates were separated by sinusoids, and liver cells were arranged tightly along with central veins. While, there was diffusive lesions in methomyl group. The shapes of hepatic cell were changeable, the cell nucleus was condensed, and the regular arrangements of liver cells along with central veins were disappeared. That indicated methomyl was high toxicity to liver.

In the kidney tissues, there is no significant morphological difference was observed. The glomerulus and its tubule can be recognized clearly in methomyl group. There was no glomerular lesion, renal tubular necrosis; glomerulus extracellular matrix accumulating, basement membrane thickening or capillary adheres.

Discussion

In general, changes in pharmacokinetics are thought to be caused by drug-drug or drug-food interactions [14]. 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 [15, 16]. Similarly, supplement-drug interactions involving CYP activity are occasionally found to cause considerable adverse events. For these reasons, we evaluated the effects of acute methomyl poisoning on the activity of CYP enzymes in vivo. We selected CYP isoforms CYP1A2, CYP2D1/CYP2D6, CYP3A2/CYP3A4, CYP2C11/CYP2C9 and CYP2B1/CYP2B6 because more than 90% of drugs are known to be metabolized by these 6 CYP enzymes [17, 18].

There no significant difference for AUC, CL and C_{max} of bupropion (P > 0.05) between the methomyl group and control group was observed. It suggested that the methomyl was
not able to induce or inhibit the activity of CYP2B1 enzyme. The pharmacokinetic parameters of metropolol and phenacetin experienced change with increased \(AUC_{0-t}\) (\(P < 0.05\)) and decreased CL (\(P < 0.05\)). This result indicates that the acute methomyl poisoning could inhibit the metabolism of metropolol (CYP2D1) and phenacetin (CYP1A2) in rat. The pharmacokinetic parameters of tolbutamide and testosterone experienced change with decreased \(AUC_{0-t}\) (\(P < 0.05\)) and increased CL (\(P < 0.05\)). It indicates that the acute methomyl poisoning could induce the activity of the metabolism of testosterone (CYP3A2) and tolbutamide (CYP2C11) in rat.

After the pharmacokinetic profiles evaluation by cocktail method, we also investigated the hepatotoxicity of methomyl by observing the pathological changes of liver after methomyl administration. The methomyl poisoning causes hepatotoxicity. A more systematic and comprehensive study to investigate the hepatotoxicity of methomyl will be carried out.

Conclusion

The results observed in this study would provide us valuable information regarding the interactions of methomyl poisoning with other drugs. Inhibit of drug metabolizing enzyme CYP2D1 and CYP1A2 by methomyl 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. Induction of drug metabolizing enzyme CYP3A2 and CYP2C11 by methomyl poisoning would reduce the efficacy of other drug. Additionally, methomyl poisoning causes hepatotoxicity.

Acknowledgements

This study was supported by grants from the incubator project of Zhejiang Provincial Natural Science Foundation of China, No. LY15H150008; the Zhejiang Medicines Health Science and Technology Program, No. 2014-KYA144 and 2014KYS153; Wenzhou Municipal Science and Technology Bureau, No. Y2014-0688 and Y20140493; the First Affiliated Hospital of Wenzhou Medical University, No. FHY2014035.

Disclosure of conflict of interest

None.
Effect of methomyl on CYP450 isoforms activity of rats


