Screening of Thai plants for Inhibition of CYP2D6 Enzyme Activity

Benjabhorn Sethabouppha¹, Suparat Chanluang², Le Hoang Lam⁴, and Suttasinee Suwannakul³*

¹Department of Pharmaceutical Chemistry and Pharmaceutical Technology
²Department of Biopharmacy
³Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Warinchamrab, Ubon Ratchathani, Thailand
⁴Department of Quality Management, School of Biotechnology and Food Technology, Hanoi University of Science and Technology, Hanoi, Vietnam.

E-mail: ssuwannakul@gmail.com

Abstract

The aim of this study was to assess the inhibitory potential of aqueous extracts from four Thai plants on CYP2D6 activity. The plant material tested included holy basil or Ka Prao (Ocimum sanctum L.), ivy gourd or Tam Leung (Coccinia grandis), bamboo grass or Ya Nang (Tiliacora triandra), and Indian mulberry leaf or Bai Yoe (Morinda citrifolia). Metabolic tests were carried out in vitro using rat liver microsome with and without the plants’ aqueous extracts. The amount of dextorphan, a metabolite of dextromethorphan, formed from a CYP2D6-catalyzed enzymatic reaction, was measured by using High Performance Liquid Chromatography (HPLC).

Keywords: CYP2D6; Cytochrome P450; Food-drug interaction; Thai plants; In vitro

Introduction

Cytochrome P450 are major enzymes responsible for oxidation reactions in phase I metabolism in vivo [1]. The CYP2D6 is an isozyme of the CYP2D subfamily of P450 and plays an important role in biotransformation of many drugs such as amitryptiline, clomipramine, dextromethorphan, propanolol, and tamoxifen [2]. In Thailand, holy basil, Indian mulberry leaf, ivy gourd, and bamboo grass are common plants found in daily diets. When taken orally as both drug and plant material, as a vegetable, food-drug interaction may occur, affecting the CYP2D6 enzymatic activities. In this study, four extracts of
the above plants were tested in vitro using hepatic enzyme system for food-drug interaction.

Materials and methods

Four fresh Thai plants were purchased from a local market in Ubon Ratchathani. Pooled rat liver microsome was prepared using Wistar rats’ livers and total protein content of rat liver microsome (RLM) was determined by Lowry assay following the method described previously [3], [4]. Metabolic tests were carried out with RLM in triplicate in vitro using dextromethorphan (DMP) as a specific CYP2D6 probe substrate. The amount of dextorphan (DTP), a major metabolite of DMP, obtained from each metabolic reaction with or without plant aqueous extract, was determined using High Performance Liquid Chromatography (HPLC) technique [5]. The amount of DTP found was converted into percentage of inhibition compared to those obtained from the reaction without the plant aqueous extract.

Results and discussion

The HPLC condition used in this study was well-separated DTP (RT=3.42 min) from DMP (RT = 6.75 min). The peak area of DTP was linear over the concentration of 0.60 to 50 x10⁻³ mM (R² = 0.99).

This study did not detect the DTP from metabolic reaction without NADPH and metabolic reaction with positive control (cimetidine) because NADPH-cytochrome P450 reductase (CPR), the electron donor protein, is required in most cytochrome P450-mediated metabolic reaction whereas cimetidine is a well known CYP2D6 inhibitor.

The researchers measured the DTP in the rest of the reactions that included fractions of aqueous herbal extracts ranging between 2.85 to 11.06 x10⁻³ mM. When the amount of DTP was converted into percentage of inhibition, there was a wide range of 3.35 to 54.25%. These numbers reflected the unequal potential of each plant to be a CYP2D6 inhibitor. The amounts of DTP found, percentages found, and percentages of inhibition of all reactions are shown in Table 1, Figure 1, and Figure 2.

The compound concentration has a strong impact on CYP2D6 inhibition. In vivo, when food passes through the gastrointestinal tract, digestion and absorption occur in the stomach, intestine, and bowel. All chemical constituents of each plant then pass through the hepatic vein into the hepatic cells where the CYP2D6 mainly localize and contribute to the biotransformation processes [1]. At this point, these chemicals may not affect CYP2D6 function due to the dilution by blood or body fluids. Thus the in vivo test may not significantly alter the blood levels of these compounds. The in vitro test should be conducted if high inhibition potency in vitro is found. Besides the biological factors, cultivated location and harvested seasons also cause variations in the chemical quantities of each plant [7], [8]. Harvesting time, temperature, and drying periods of plants also affect the chemical contents [9], [10], [11]. Even heat in the cooking processes accelerates the chemical degradation [12], [13]. All these reasons may reduce the risk of food-drug interaction.

The metabolic drug-drug interaction was defined as a change of normal metabolism of a certain drug when co-administered with the other drug via the same drug-metabolizing enzyme.
Likewise, food-drug interaction may occur with the same explanation since foods contain numerous plants and these plants contain numerous compounds [1]. An example of metabolic food-drug interaction is grapefruit juice and felodipine. A major component of this fruit juice inhibits CYP3A4-catalyzed biotransformation [14] and change felodipine oral availability in humans [15]. With CYP2D6, goldenseal (Hydrastis canadensis) shows ~50% inhibition of CYP2D6 activity whereas milk thistle (Silybum marianum) and black cohosh (Cimicifuga racemosa), kava kava (Piper methysticum), St. John’s wort (Hypericum perforatum), and Echinacea (Echinacea purpurea) had no significant effect on CYP2D6 activity [16].

In humans, CYP2D6 hepatic expression was found in only around 2-5% of total P450 content [2]. The expression varies from person to person, from undetectable in poor metabolizers (PMs), very low in intermediate metabolizers (IMs), to more than 100-fold higher level in most active extensive metabolizers (Ems), in relation to their genetic factor [2], [17]. This genetic polymorphism may also influence CYP2D6-mediated food-drug interactions in humans [17].

The results of the study revealed that the aqueous extracts of the four plants affected CYP2D6 enzyme function to different degrees of inhibition, for example, holy basil (54.29%), Indian mulberry leaf (30.30%), ivy gourd (15.84%), and bamboo grass (3.42%). The alcoholic extracts of Pak Wan Ban (Sauropus androgynus) and these four herbs also inhibited CYP2D6 at different levels (4.86 to 65.63%) as reported in a previous work [5]. Apart from CYP2D6, the study also found that the alcoholic extracts of these four herbs had some potential to inhibit CYP3A4 (1.05 to 57.91%) [6]. The possible mechanisms of inhibition are destruction of hepatic cytochrome P450, metal ion content in plant extracts, or the forming of inactive complexes with hepatic cytochrome P450 [2]. To clarify the mechanism of action, more experiments should be carried out with individual plant extracts that show strong inhibition.

Table 1 Quantity of dextorphan obtained from each metabolic reaction and inhibitory effect of each plant extract on CYP2D6 enzyme activity.

<table>
<thead>
<tr>
<th>Metabolic reaction</th>
<th>Dextorphan x10⁻³mM (± SD)</th>
<th>% found*</th>
<th>% inhibition**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control reaction</td>
<td>11.06 (±2.69)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With holy basil or Ka Prao (Ocimum sanctum L.)</td>
<td>5.06 (±0.82)</td>
<td>45.75</td>
<td>54.25</td>
</tr>
<tr>
<td>Control reaction</td>
<td>10.07 (±0.56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With ivy gourd or Tam Leung (Coccinia grandis)</td>
<td>8.48 (±0.98)</td>
<td>84.21</td>
<td>15.79</td>
</tr>
<tr>
<td>Control reaction</td>
<td>7.46 (±0.97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With bamboo grass or Ya Nang (Tiliacora triandra)</td>
<td>7.21 (±0.85)</td>
<td>96.65</td>
<td>3.35</td>
</tr>
<tr>
<td>Control reaction</td>
<td>4.08 (±0.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indian mulberry leaf or Bai Yoe (Morinda citrifolia)</td>
<td>2.85 (±0.08)</td>
<td>69.85</td>
<td>30.15</td>
</tr>
</tbody>
</table>

*Compared to control reaction without plant extract

** Compared to positive control reaction (cimetidine)
Figure 1 Comparative amounts of dextorphan obtained from each metabolic reaction

Figure 2 Percentage of inhibition of four Thai plant aqueous extracts on CYP2D6 enzyme activity

Conclusion

It can be concluded that all four Thai plant aqueous extracts inhibited CYP2D6 function from a low to moderate degree (3.35 to 54.25%). The same test using hexane extracts of these vegetable should be completed in the future.

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