Screening of Thai plants for Inhibition of CYP2D6 Enzyme Activity

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บทคัดย่อ

การวิจัยนี้มีวัตถุประสงค์เพื่อประเมินความสามารถในการยับยั้งการทำงานของเอนไซม์ CYP2D6 ของส่วนสกัดน้ำของพืชไทย 4 ชนิด ได้แก่ กะเพรา (*Ocimum sanctum* L.) ตำลึง (*Coccinia grandis*) ย่านาง (*Tiliacora triandra*) และใบยอ (*Morinda citrifolia*) โดยศึกษาในหลอดทดลองจากปฏิกิริยาทางชีวเคมีของการเปลี่ยนแปลง dextromethorphan ไปเป็น dexthorphan ซึ่งอาศัยเอนไซม์ CYP2D6 ที่มีอยู่ในไมโครโซมที่เตรียมจากตับหนู ทำการทดลองทั้งในสภาวะที่เติมและไม่เติมส่วนสกัดน้ำของพืช และวิเคราะห์หาปริมาณ dexthorphan ที่เกิดขึ้นจากปฏิกิริยาโดยเทคนิค High Performance Liquid Chromatography (HPLC) ผลพบว่า ส่วนสกัดน้ำของพืชทั้ง 4 ชนิดยับยั้งการทำงานของ CYP2D6 ในระดับที่แตกต่างกัน

คำสำคัญ: CYP2D6 เอนไซม์ไซโตโครมพี450 ปฏิกิริยาระหว่างอาหารและยา พืชไทย การศึกษาในหลอดทดลอง

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 dexthorphan, 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 amitryptyline, 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 dexthorphan (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 (R^2 = 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 vivo* 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 changes 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 dexthorphan obtained from each metabolic reaction and inhibitory effect of each plant extract on CYP2D6 enzyme activity.

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

*Compared to control reaction without plant extract

** Compared to positive control reaction (cimetidine)

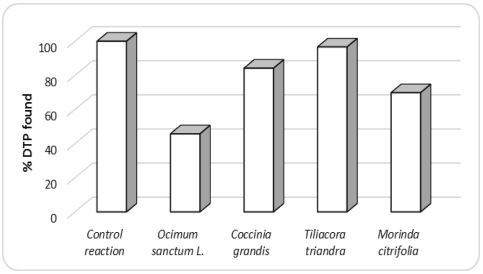

Figure 1 Comparative amounts of dexthorphan obtained from each metabolic reaction

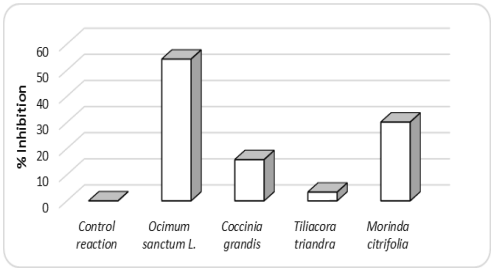

Figure 2 Percentage of inhibition of four Thai plant aqueous extracts on CYP2D6 enzyme activity

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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References

[1] Gibson, G.G. 1994. **Introduction to Drug Metabolism**. Glasgow: Blackie Academic & Professional.

[2] Zanger, U.M. and Eichelbaum, M. 2000. “Enzyme and Transporters”. In **Metabolic Drug Interactions**, edited by Levy, R.H. and et al. Philadelphia: Lippincott Williams & Wilkins.

[3] Hirunpanich, V., Sethabouppha, B. and Sato, H**.** 2007. **“**Inhibitory Effects of Saturated and Polyunsaturated Fatty Acids on the Cytochrome P450 3A Activity in Rat Liver Microsomes”. **Biol Pharm Bull**. 30 (8): 1586-1588.

[4] Sethabouppha, B. and et al. 2006. “Inter-individual Variation in CYP3A5-mediated Drug Metabolism: An In-vitro Study Using Alprazolam As a Probe Substrate”. In **Proceedings of the 9th European ISSX meeting,** 24-27 May, Manchester, UK.

[5] Janpirak, D. and et al. 2014. “Screening Test of 5 Thai Vegetable Alcoholic Extracts on Rat CYP2D6 Enzyme Activity *In-vitro*”. In **Proceedings of the 6th Annual National Conference of Northeast Pharmacy Research**, 2-3 February. Ubon Ratchathani, Thailand.

[6] Sitthiram, S. and et al. 2014. *“In vitro* Screening Test of Holy Basil and Other 3 Thai Vegetable Alcoholic Extracts on Rat CYP3A4 Enzyme Activity”. In **Proceedings of the 6th Annual**

National Conference of Northeast Pharmacy Research, 2-3 February. Ubon Ratchathani, Thailand.

[7] Jaakola, L. and Hohtola, A. 2010. "Effect of Latitude on Flavonoid Biosynthesis in Plants". **Plant, Cell and Environment**. 33: 1239-1247.

[8] Ribeiro, A.O. and et al. 2014. "Tree Crown Variation and Seasonal in the Phenolic Compounds Content of Stryphnodendron adstringens (Mart) Coville Leaves". **American J. of Plant Sciences.** 5: 2904-2912.

[9] Filho, C. and et al. 2006. "Influence of the Harvesting Time, Temperature and Drying Period on Basil (*Ocimum basilicum* L.) Essential Oil". **Brazilian J. of Pharmacognosy**. 16(1): 24-30.

[10] Senatore, F.1996. "Influence of Harvesting Time on Yield and Composition of the Essential Oil of a Thyme (*Thymus pulegioides* L.) Growing Wild in Campania (Southern Italy)". **J. Agric Food Chem.** 44 (5): 1327-1332.

[11] Saohin, W. and et al. 2007. "Effects of Drying Temperature and Residual Moisture Content of Fa-Tha-Li (Andrographis paniculata (Burm.f.) Neees) Crude Powder for Capsule Preparation". **Thai J. Pharm Sci.** 31: 28-35.

[12] Andlauer, W. and et al. 2003. "Influence of Cooking Process on Phenolic Marker Compounds of Vegetables". **Int J. Vitam Nutr Res**. 73(2):152-159.

[13] Kamel, S.M. 2013. "Effect of Microwave Treatments on Some Bioactive Compounds of Parsley (*Petroselinum Crispum*) and Dill (*Anethum graveolens*) Leaves". **J Food Process Technol**. 4:6.

[14] Edwards, D.J. III and Woster, P.M. 1996. "Identification of 6',7'-Dihydroxybergamottin, a Cytochrome P450 Inhibitor, in Grapefruit Juice". **Drug Metab Dispos**. 24: 1287-1290.

[15] Lown, K.S. and et al. 1997 "Grape Fruit Juice Increases Felodipine Oral Availability in humans by Increasing Intestinal CYP3A protein expression". **J. Clinical Invest**. 99: 2545-2553.

[16] Gurley BJ and et al. 2008. "Clinical Assessment of CYP2D6-mediated Herb-Drug Interactions in Humans: Effects of Milk Thistle, Black Cohosh, Goldenseal, Kava Kava, St. John's wort, and Echinacea" **Mol Nutr Food Res**. 52 (7): 755–763.

[17] Ingelman-Sundberg, M. 2005. "Genetic Polymorphisms of Cytochrome P450 2D6 (CYP2D6): Clinical Consequences, Evolutionary Aspects and Functional Diversity". **Pharmacogenomics J.** 5 (1): 6-13.