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CYP3A4 and CYP2D6 inhibitory activities of Indonesian medicinal plants

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Abstract

Thirty samples of Indonesian medicinal plants were analyzed for their capacity to inhibit *in vitro* metabolism by human cytochrome P450 3A4 (CYP3A4) and CYP2D6 with a radiometric assay. The MeOH-soluble fractions of 25 samples, prepared from water extracts, demonstrated inhibitory activity more than 50% on the metabolism mediated by CYP3A4, and 21 samples on the metabolism mediated by CYP2D6. Among the MeOH-soluble fractions, *Piper nigrum* leaf showed the highest inhibitory activity against CYP3A4 (91.7%), and *Punica granatum* against CYP2D6 (98.1%). The water extracts of which MeOH-soluble fraction showed inhibitory activity more than 70% were fractionated with EtOAc. From the EtOAc-soluble fractions, *Curcuma heyneana* (67.0%), *Pi. cubeba* (75.0%), *Pi. nigrum* fruit (84.0%), *Pi. nigrum* leaf (85.8%), and *Zingiber aromaticum* (75.3%) demonstrated inhibitory activity more than 50% on the metabolism mediated by CYP3A4, but only *Pi. nigrum* fruit (72.8%) and *Pi. nigrum* leaf (69.1%) showed strong inhibitory activity against CYP2D6. For samples that showed more than 70% inhibition, their IC_{50} values were determined. The most potent inhibitory activity against CYP3A4 (IC_{50} value of 25 μg/ml) was found for the extract of *Pi. nigrum* leaf, while that of *Catharanthus roseus* showed the most potent inhibitory effect against CYP2D6 (IC_{50} value of 11 μg/ml). These results should indicate once more the possibility of potential medicinal plant–drug interactions.

Keywords: Cytochrome P450; Jamu; Drug interaction; Inhibition; CYP3A4; CYP2D6

Introduction

Cytochrome P450 (CYP) is the main enzyme which catalyzes the metabolism of drugs and other xenobiotics. CYP3A4, the major hepatic and intestinal CYP in humans, metabolizes more than 50% of clinically used drugs such as cyclosporine A, dihydropyridines, ethinylestradiol, midazolam, terfenadine, and triazolam (Rendic and DiCarlo, 1997). CYP2D6 catalyzes the metabolism of about 30% of all drugs including amitriptyline, imipramine, haloperidol, propranolol, and dextromethorphan (Clarke and Jones, 2002).

Recently, several reports have demonstrated that natural compounds and herbal products may cause pharmacokinetic interaction with western drugs used clinically when they are simultaneously administrated (Foster et al., 1999; Nebel et al., 1999; Taylor and Wilt,

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1999). The use of medicinal herbs has particularly increased over the past few years among specific patient populations including HIV-infected patients. St. John's wort altered pharmacokinetics of the HIV protease inhibitor, indinavir in individuals on retroviral therapy (Piscitelli et al., 2000). Indeed, plasma concentrations of the HIV protease inhibitor, saquinavir, have been decreased in individuals exposed long-term to garlic supplements (Piscitelli et al., 2002). The increased clearance of saquinavir may be due to induction of hepatic and/or intestinal CYP3A4. The most widely studied natural product is grapefruit juice, which has been found to increase the bioavailability and/or to prolong the metabolic elimination of many drugs such as dihydropyridine-type calcium channel blockers, histamine-1 receptor antagonists (e.g., terfenadine), quinidine, benzodiazepines (e.g., midazolam), 17β-estradiol, and caffeine (Ameer and Weintraub, 1997; Bailey et al., 1998).

Indonesia, a country in Southeast Asia, has many medicinal plants which are used as traditional medicines "Jamu" (Sastroamidjojo, 1997). Those medicinal plants have been used from the ancient time to now, and are largely consumed by people of different levels in villages and also in big cities. People could easily buy ready-made "Jamu" which is packed in the form of powder, pills, capsules, drinking liquid, and ointment. There are still "Jamu" shops to sell only ingredients or to prepare the "Jamu" on spot by request. It is a common view across the country that some women are roaming the street to sell "Jamu", and many Indonesians start their day with drinking "Jamu". These traditional medicines are almost unregulated, and many patients do not inform their physician about the traditional medicines they consume. Therefore, interactions between traditional medicines and drugs prescribed clinically are becoming a concern. To the best of our knowledge, there have been no reports on the inhibitory potential of Indonesian medicinal plants against CYP. Thus, we chose 30 medicinal plants which are generally used in Indonesian traditional medicines (Sastroamidjojo, 1997; PT Eisai Indonesia, 1995) (Table 1) and evaluated their inhibitory activity against CYP3A4 and CYP2D6.

Materials and methods

Medicinal plants

Indonesian medicinal plants were obtained at GORO traditional market, Jakarta, Indonesia, in May 2002 and voucher samples are preserved at the Museum of Materia Medica, Research Center for Ethnomedicines, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan (Table 1).

Extraction and preparation of test solutions

Each medicinal plant (25–150 g) was cut into small pieces and extracted with water (150–400 ml, reflux, 2 h, × 2). The water solution was concentrated under reduced pressure and lyophilized to give a water extract. A part of the water extract (0.5 g) from each medicinal plant was extracted with MeOH (15 ml), followed by centrifugation to facilitate removal of the supernatant, to give a MeOH-soluble fraction. The MeOH-soluble fraction was evaporated and redissolved in 1.5 ml of MeOH and used as a test solution. The medicinal plants on which the MeOH-soluble fraction showed strong inhibition against CYP3A4 and/or CYP2D6, an EtOAc-soluble fraction was prepared from the water extract by extracting with EtOAc (15 ml), followed by centrifugation. The EtOAc-soluble fraction was evaporated and dissolved in 1.5 ml of MeOH and used as a test solution.

Chemicals

Quinidine sulfate dihydrate and ketoconazole were obtained from Wako Pure Chemicals Industry, Ltd. (Osaka, Japan). [*N*-methyl-^{14}C]Erythromycin (55 mCi/mmol, >99% pure) and [*O*-methyl-^{14}C]dextromethorphan (55 mCi/mmol, >99% pure) were purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO, USA). Human liver microsomes (HLM) were obtained from Xenotech, LLC (Kansas, KS, USA) and stored at −80 °C prior to use. β-Nicotinamide adenine dinucleotide phosphate ($NADP^+$, oxidized form), glucose-6-phosphate (G-6-P), and G-6-P dehydrogenase were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). All other chemicals and solvents were of the highest grade available.

CYP inhibitory activity

Inhibitory activity against CYP3A4 was assayed by the method of Riley and Howbrook (1998) with a slight modification. Briefly, the assay was performed in a disposable culture tube of 13 × 100 mm (Iwaki, Tokyo, Japan). Tubes were designated as "control", "positive control", and "test". The control tube consisted of 2.5 µl of MeOH; positive control consisted of 2.5 µl of ketoconazole (100 µM); and test tubes consisted of 2.5 µl of samples (equivalent to 1.65 mg/ml of extract). To all tubes were added 150 µl of phosphate buffer (pH 7.4), 197.5 µl of ultrapure water, 50 µl of [*N*-methyl-^{14}C]erythromycin (0.1 µCi/incubation, 1 mM in 5% of MeOH), and 50 µl of HLM (4 mg/ml). The total incubation volume was 500 µl. After 5 min preincubation under shaking at 37 °C, the reaction was initiated by addition of 50 µl of NADPH-generating system (4.20 mg/ml of $NADP^+$, 100 mM of G-6-P, 100 mM of