Journal of Ethnopharmacology 130 (2010) 275–283

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Ethnopharmacological communication

In vitro modulatory effects on three major human cytochrome P450 enzymes by multiple active constituents and extracts of *Centella asiatica*

Yan Pan[a], Badrul Amini Abd-Rashid[b], Zakiah Ismail[b], Rusli Ismail[c], Joon Wah Mak[a], Peter C.K. Pook[a], Hui Meng Er[a], Chin Eng Ong[d,*]

[a] *School of Pharmacy and Health Sciences, International Medical University, 126, Jalan 19/155B, Bukit Jalil, 57000 Kuala Lumpur, Malaysia*
[b] *Herbal Medicine Research Unit, Division of Biochemistry, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia*
[c] *Pharmacogenetics Research Group, Institute for Research in Molecular Medicine, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia*
[d] *School of Medicine and Health Sciences, Monash University Sunway Campus, Jalan Lagoon Selatan, 46150 Bandar Sunway, Selangor Darul Ehsan, Malaysia*

ARTICLE INFO

Article history:
Received 14 December 2009
Received in revised form 26 April 2010
Accepted 3 May 2010
Available online 8 May 2010

Keywords:
Centella asiatica
Apiaceae
Cytochrome P450
Drug–herb interaction
Enzyme inhibition

ABSTRACT

Ethnopharmacological relevance: Centella asiatica (CA) has been widely cultivated as a vegetable or spice in China, Southeast Asia, India, Sri Lanka, Africa, and Oceanic countries and traditionally used for wound healing and maintaining normal blood pressure.
Aim of the study: The present study was carried out to examine the potential modulatory effects of three commercially available active components (asiaticoside, asiatic acid and madecassic acid) and four extracts (aqueous, ethanol, dichloromethane and hexane) of CA on three major cDNA-expressed human cytochrome P450 (CYP) isoforms.
Materials and methods: High-performance liquid chromatography (HPLC)-based enzyme assays, namely tolbutamide 4-methyhydroxylase, dextromethorphan *O*-demethylase and testosterone 6β-hydroxylase assays were developed to probe activities of CYP2C9, CYP2D6 and CYP3A4, respectively. Probe substrates were incubated with or without each active component and extract for each isoform, followed by examination of the kinetics parameters, IC_{50} and K_i, to characterize modulatory effects.
Results: CYP2C9 was more susceptible to inhibitory effects by CA extracts compared to CYP2D6 and CYP3A4. Moderate degree of inhibition was observed in ethanol (K_i = 39.1 μg/ml) and dichloromethane (K_i = 26.6 μg/ml) extracts implying potential risk of interaction when CYP2C9 substrates are consumed with CA products. The two extracts however showed negligible inhibition towards CYP2D6 and CYP3A4 (IC_{50}'s of 123.3 μg/ml and above). Similarly CA aqueous and hexane extracts did not significantly inhibit all three isoforms investigated (IC_{50}'s of 117.9 μg/ml and above). Among the active constituents investigated, asiatic acid and madecassic acid appeared to selectively inhibit CYP2C9 and CYP2D6 more than CYP3A4. Of particular interest is the potent inhibitory effect of asiatic acid on CYP2C9 (K_i = 9.1 μg/ml). This signifies potential risk of interaction when substrates for this isoform are taken together with CA products with high asiatic acid content. Inhibitions of asiatic acid with the other isoforms and that of madecassic acid with all isoforms were only moderate (K_i's ranged from 17.2 to 84.4 μg/ml). On the other hand, the IC_{50} values for asiaticoside were high (1070.2 μg/ml or above) for all three isoforms, indicating negligible or low potential of this compound to modulate CYP enzymatic activity.
Conclusion: Centella asiatica extracts and active constituents inhibited CYP2C9, CYP2D6 and CYP3A4 activities with varying potency with CYP2C9 being the most susceptible isoform to inhibition. Significant inhibition was observed for asiatic acid and CA ethanol and dichloromethane extracts, implying involvement of semipolar constituents from CA in the effect. This study suggested that CA could cause drug–herb interactions through CYP2C9 inhibition.

1. Introduction

Cytochrome P450 (CYP) is a mixed-function oxygenase system, which was first discovered in 1954 (Klingenberg, 1958). In humans, CYP enzymes are involved in the metabolism of exogenous substances (drugs, alcohols, anti-oxidants, organic solvents, anesthetic agents, dyes, environmental pollutants and chemicals)

* Corresponding author. Tel.: +60 3 55144918; fax: +60 3 55146323.
E-mail address: ceong98@hotmail.com (C.E. Ong).

0378-8741/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2010.05.002

producing metabolites which may be inactive, toxic or carcinogenic (Guengerich, 1992; Isin and Guengerich, 2007). They are also important in the metabolism of endogenous physiological compounds such as steroids, bile acids, fatty acids, prostaglandins, biogenic amines and retinoids (Slaughter and Edwards, 1995; Kerremans, 1996). Three major human CYP isoforms, CYP2C9, CYP2D6 and CYP3A4, well known for their vital roles in human drug metabolism were chosen for this study. CYP2C9, the major member of the CYP2C subfamily in human liver, metabolizes more than 16% of clinically used drugs, including hypoglycemic agents tolbutamide and glipizide, anticonvulsant phenytoin, anticoagulant warfarin, nonsteroidal anti-inflammatory drugs such as fluriprofen, diclofenac as well as some newly developed drugs such as antihypertensive losartan (Goldstein and de Morais, 1994; Goldstein, 2001). Despite its low hepatic expression level (about 2% of total CYP content), CYP2D6 is reported to be involved in the metabolism of the most commonly prescribed pharmaceuticals including debrisoquine, tricyclic antidepressants, selective serotonin re-uptake (SSRI) inhibitors such as fluoxetine, various amphetamine analogs, and dextromethorphan (Ramamoorthy et al., 2001; Jannetto et al., 2002; Wienkers and Heath, 2005). By far CYP3A4 is the most abundant CYP protein (up to 50% of total CYP content) in human liver (Danielson, 2002), responsible for the metabolism of a wide variety of substrates including nifedipine, erythromycin, troleandomycin, quinidine, cyclosporine A, 17α-ethynylestradiol, lidocaine and diltiazem (Peyronneau et al., 1993).

So far, a good number of literature and anecdotal reports suggest that concomitant administration of herbal products and pharmaceuticals may affect human drug metabolism and significantly increase the risk of serious adverse reactions (Budzinski et al., 2000). For example, the interaction between St. John's wort (a herbal preparation used in the treatment of depression) and several pharmaceuticals such as warfarin have been reported and the mechanism by which St. John's wort activates human cytochrome P450 (CYP) enzymes is possibly the most thoroughly researched topic to date (Obach, 2000). A comprehensive summary of the clinical relevance of herb interactions has been reported in 2006 by Williamson, touching on interactions of Berberis, Cinchona bark, Dan Shen, Dong quai, Echinacea, Garlic, Ginkgo, Goldenseal, Kavakava, Red clover, St. John's wort, and Valerian with various CYP isoforms (Williamson, 2006).

Centella asiatica (CA), belonging to the family Apiaceae, has been widely cultivated as a vegetable or spice in China, Southeast Asia, India, Sri Lanka, Africa, and Oceanic countries for centuries (Miyako et al., 2005). CA has a historical reputation for boosting mental activity and for helping a variety of systemic illnesses, such as high blood pressure, rheumatism, fever, and nervous disorders (Brinkhaus et al., 2000). In Malaysia, it is known as 'pegaga' and commonly consumed as a vegetable among Malays. It is also blended into a drink and used as a cooling drink. Despite the benefits of the CA preparations to human health, they are products of complex mixtures of bioactive entities, which are able to serve as substrates, inhibitors or inducers of CYP. As a result, co-administration of CA preparations and conventional drugs may have potential drug–herb interaction problems. Phytochemical analyses showed that *Centella asiatica* contains active ingredients such as triterpenoid glycosides (including asiaticoside), vallerin, tannins, alkaloid, volatile oil and pectin (Brinkhaus et al., 2000).

In light of the widespread use of CA products and lacking knowledge regarding the effects of CA preparations on CYPs, it is necessary to have a detailed study to examine the effects of hydrophilic and lipophilic components of CA extract on human CYPs. In this study, four CA extracts (aqueous, ethanol, dichloromethane and hexane) and three important commercially available active constituents (asiaticoside, asiatic acid and madecassic acid) were chosen for examination of their modulatory effects on activities of CYP2C9, CYP2D6, and CYP3A4. The purpose of this study was to establish the potential for CA to affect metabolism of common substrates by important CYP isoforms, and thus to assess the probability of drug–herb interactions.

2. Materials and methods

2.1. Chemicals and reagents

Asiaticoside and asiatic acid were purchased from Sigma–Aldrich (Madison, WI, USA). Madecassic acid was purchased from LKT laboratories, Inc. (St. Paul, MN, USA). The purity of all three active constituents was above 99%, and their structures are shown in Fig. 1. Phosphoric acid, orthophosphoric acid and all HPLC-grade solvents (acetonitrile, chloroform, dichloromethane, diethyl ether, ethanol, hexane fraction from petroleum, and methanol) were purchased from Fisher Scientific (Loughborough, Leicestershire, UK). Luria–Bertani (LB) media, isopropyl β-D-1-thiogalactopyranoside (IPTG), phenylemethanesulfonyl fluoride (PMSF), Tris-base, EDTA, DTT, glycerol were purchased from Promega (Madison, WI, USA). Terrific broth (TB) was purchased from Invitrogen Corporation (Carlsbad, CA, USA). All the other chemicals (tolbutamide, 4-hydroxytolbutamide, testosterone, 6β-hydroxytestosterone, dextromethorphan hydrobromide, dextrophan tartarte, β-nicotinamide adenine dinucleotide phosphate (NADP), D-glucose 6-phosphate (G-6-P), glucose-6-phosphate dehydrogenase, magnesium chloride, chlorpropamide, 5,5-diphenylhydantoin, corticosteron, demethyl sulfoxide (DMSO), δ-aminolevulinic acid, lysozyme, sucrose) were purchased from Sigma–Aldrich (St. Louis, MI, USA).

2.2. Centella asiatica extracts

CA aqueous, ethanol, dichloromethane and hexane crude extracts were prepared as ready-use stocks by the Institute for Medical Research (IMR), Malaysia. Fresh whole plant of *Centella asiatica* was collected from MARDI Melaka, Malaysia in August 2006 and botanically identified at IMR. CA extracts were prepared following an established procedure. Dried raw material of CA was ground, which was subsequently placed in conical flask and soaked for 3 days in hexane at room temperature. The homogenized suspension was then filtered through a Whatman No. 1 paper (Lawrence, Kansas, USA). The volume of the filtrate was then reduced by rotary evaporator at 40 °C to make the hexane extract. The yield of the extract was typically 0.65–0.9% (w/w) in terms of dried starting materials. The residue was air dried in room temperature, followed by re-extraction with dichloromethane for 3 days at room temperature. The filtration was done as mentioned above. The solvent of the filtrate was removed using rotary evaporator at 45 °C to make the dichloromethane extract. The yield of the extract was normally 0.90–1.05% (w/w). The residue was air dried again at room temperature, which was re-extracted by ethanol at room temperature for 3 days, and filtered. Ethanol was removed from the filtrate by rotary evaporator at 45 °C to form the ethanol extract (2.50–3.05%, w/w). The residue was subsequently extracted using distilled water at room temperature overnight and later filtrated. The residue was discarded and the filtrate was spray dried by Buchi Spray Drier (BÜCHI Labortechnik AG, Postfach, Switzerland) to obtain aqueous extract (4.10–4.90%, w/w). All the extracts were stored at 4 °C until further analysis.

2.3. cDNA expression of CYP isoforms

Each pCWori+ plasmid containing cDNA of CYP2C9, CYP2D6 and CYP3A4 was co-transformed with pACYC plasmid containing the