Saudi Pharmaceutical Journal (2014) 22, 564–569

King Saud University

Saudi Pharmaceutical Journal

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ORIGINAL ARTICLE

Modulation of CYP2D6 and CYP3A4 metabolic activities by *Ferula asafetida* resin

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Received 3 March 2014; accepted 26 March 2014
Available online 3 April 2014

KEYWORDS

Asafetida;
Dextromethorphan;
CYP3A4;
CYP2D6;
Microsomes;
Metabolism;
Interaction

Abstract Present study investigated the potential effects of *Ferula asafetida* resin on metabolic activities of human drug metabolizing enzymes: CYP2D6 and CYP3A4. Dextromethorphan (DEX) was used as a marker to assess metabolic activities of these enzymes, based on its CYP2D6 and CYP3A4 mediated metabolism to dextrorphan (DOR) and 3-methoxymorphinan (3-MM), respectively. *In vitro* study was conducted by incubating DEX with human liver microsomes and NADPH in the presence or absence of Asafetida alcoholic extract. For clinical study, healthy human volunteers received a single dose of DEX alone (phase-I) and repeated the same dose after a washout period and four-day Asafetida treatment (phase-II). Asafetida showed a concentration dependent inhibition on DOR formation (*in vitro*) and a 33% increase in DEX/DOR urinary metabolic ratio in clinical study. For CYP3A4, formation of 3-MM in microsomes was increased at low Asafetida concentrations (10, 25 and 50 μg/ml) but slightly inhibited at the concentration of 100 μg/ml. On the other hand, *in vivo* observations revealed that Asafetida significantly increased DEX/3-MM urinary metabolic ratio. The findings of this study suggest that Asafetida may have a significant effect on CYP3A4 metabolic activity. Therefore, using *Ferula asafetida* with CYP3A4

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Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jsps.2014.03.004

drug substrates should be cautioned especially those with narrow therapeutic index such as cyclosporine, tacrolimus and carbamazepine.

1. Introduction

Numerous studies have reported that traditional medicines may modulate the metabolic activity of cytochrome P-450 enzymes (CYP). Since, the use of traditional medicines is widespread in developing countries, so the extent of modulation of metabolizing enzymes needs to be assessed (Bhattaram et al., 2002; Mukherjee and Wahile, 2006; Müller and Kanfer, 2011). CYP modulating herbs may also influence the metabolism of co-administered CYP drug substrates. Such incidences may lead to altered bioavailability and pharmacokinetics of co-administered substrates (Müller and Kanfer, 2011; Izzo and Ernst, 2001). Further, the consequences will be more serious with narrow therapeutic index drugs (Barone et al., 2001). Several incidences of herb-drug interactions based on modulation of CYP enzymes are reported (Fang et al., 2011; Ho et al., 2011; Han et al., 2011; Boullata, 2005; Doehmer and Eisenbraun, 2012). In humans most of the drugs are metabolized by CYP3A4, CYP2D6, CYP2C19, CYP2C9, CYP2E1 and CYP1A2 (Zhou, 2008; Lee et al., 2013a,b). Geum chiloense (“hierba del clavo”) increases the blood concentration of cyclosporine, while ginger has the opposite effect (Duclos and Goecke, 2001; Chiang et al., 2006). Myricetin inhibited the drug metabolizing enzymes CYP3A4 and CYP2C9 (Choi et al., 2010). St John’s wort induces CYP3A4 isoenzyme and P-glycoprotein; and has been reported to reduce the blood level of digoxin, cyclosporine, tacrolimus, amitriptyline, midazolam, warfarin, indinavir, phenprocoumon and theophylline (Barone et al., 2001; Hu et al., 2005; Izzo, 2005). Septilin significantly decreased the $T_{1/2a}$, $T_{1/2e}$, $AUC_{0\text{-inf}}$, C_{max} and AUC_{0-24} of carbamazepine (Garg et al., 1998). Sho-seiryu-to extract powder delays the absorption and accelerates carbamazepine metabolism (Ohnishi et al., 1999). Ginkgo flavonoids increased the activity of CYP3A4 (Diamond et al., 2000).

Ferula asafetida L. belongs to family apiaceae. Its oleo-gum resin is obtained by incising or cutting the living roots and rhizomes. The resin is solid or semisolid with alliaceous odor and a bitter acrid taste. The resinous material comprises ferulic acid, umbelliferone, asaresinotannols, umbelliferone ethers, gums and volatile oils. The sulfides of volatile oils are responsible for the characteristic flavor of Asafetida (Sadraei et al., 2003; Kajimoto et al., 1989). Asafetida has been used as a folk medication for various ailments including flatulence, cough, asthma, bronchitis, hysteria, and convulsions (Eigner and Scholz, 1999).

Present study assesses the potential effects of *F. asafetida* on the metabolic activities of hepatic metabolizing enzymes CYP3A4 and CYP2D6 using dextromethorphan (DEX) as a probe. *In vitro* investigations were carried out by using human liver microsomes, while an *in vivo* study was conducted in healthy human subjects. In liver, DEX is mainly metabolized through N- and O-demethylations. The O-demethylation of DEX to dextrorphan (DOR) is predominantly mediated by CYP2D6 (Barnhart, 1980; Schadel et al., 1995). Therefore, DEX is commonly used as a marker for CYP2D6 activity (Kerry et al., 1994; Hu et al., 1998; Takashima et al., 2005; Wojtczak et al., 2007). The N-demethylation of DEX to 3-methoxymorphinan (3-MM) is mediated by CYP3A4 (Gorski et al., 1994). So, DEX has been used as a common probe for both CYP3A and CYP2D6 activities (Spanakis et al., 2009; Yu and Haining, 2001), as well as *in vivo* in several studies (Ducharme et al., 1996; Funck-Brentano et al., 2005; Jones et al., 1996).

2. Materials and methods

Nicotinamide adenine dinucleotide phosphate (NADPH) and β-glucuronidase (76,800 U/ml) were purchased from Helix Pomatia, ICN Biomedicals Inc., Costa Mesa, AC, USA. The syrup of dextromethorphan hydrobromide was obtained from Riyadh Pharma, Medical and Cosmetic products Co. Ltd., Riyadh, Saudi Arabia. Dextromethorphan (DEX) hydrobromide, dextrorphan hydrobromide and 3-methoxymorphinan hydrobromide were purchased from ICN Biomedicals Inc., Warrenale, USA. Human liver microsomes having a protein concentration of 20 mg/ml were purchased from Human Biologics International LLC (HBI, Scottsdale, USA), shipped in small vials with dry ice and stored in a deep freezer at −80 °C. *F. asafetida* dried resin was purchased from the local Saudi Market. The internal standards codeine and betaxolol were of USP reference standard. General-purpose reagents (GPR) were used for extraction processes, while HPLC grade solvents were used for HPLC determinations. All other materials were of analytical grade.

2.1. Extract of plant material

Dried Asafetida resin was purchased from the Saudi market and grounded to a fine powder. This dried resin powder was exhaustively extracted with ethanol for five days by the cold maceration process. The ethanolic extract of the resin was filtered and concentrated under reduced pressure using a rotatory evaporator. The extract was weighed and its serial dilutions were made with ethyl alcohol (96%) to prepare stock solutions of 1.25, 2.5, 5, 25 and 50 mg/ml concentrations. These stock solutions were stored in a refrigerator until used for incubation.

2.2. Microsomal incubation

Methanolic solution of dextromethorphan (25 μM concentration per final incubation mixture) was transferred into eppendorf tubes and dried with the help of nitrogen. The human liver microsomes (comprising 0.25 mg protein/ml) and appropriate volume of potassium phosphate buffer (0.1 M, pH 7.4) were added to the DEX loaded tubes and gently mixed. This mixture of DEX, microsomes and buffer was pre-incubated at 37 °C for 3 min in a shaker water bath. The metabolic reaction was initiated by adding 1 mM NADPH in a final volume of 0.5 ml. Reaction was allowed for 30 min in the absence