JOURNAL OF FOOD AND DRUG ANALYSIS XXX (2017) 1–10

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

The time-dependent effects of St John's wort on cytochrome P450, uridine diphosphate-glucuronosyltransferase, glutathione S-transferase, and NAD(P)H-quinone oxidoreductase in mice

Jin-Fu Yang [a,b], *Yue-Rong Liu* [a], *Chiung-Chiao Huang* [a], *Yune-Fang Ueng* [a,b,c,d,*]

[a] *National Research Institute of Chinese Medicine, Taipei, Taiwan, ROC*
[b] *Department and Institute of Pharmacology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, ROC*
[c] *Institute of Biological Pharmacy, School of Pharmacy, National Yang-Ming University, Taipei, Taiwan, ROC*
[d] *Institute of Medical Sciences, School of Medicine, Taipei Medical University, Taipei, Taiwan, ROC*

ARTICLE INFO

Article history:
Received 12 September 2016
Received in revised form
21 December 2016
Accepted 18 January 2017
Available online xxx

Keywords:
cytochrome P450 2C
cytochrome P450 3A
Hypericum perforatum
liver
kidney

ABSTRACT

Hypericum perforatum [St. John's wort (SJW)] is known to cause a drug interaction with the substrates of cytochrome P450 (P450, CYP) isoforms, mainly CYP3A. This study aims to determine the dose response and time course of the effects of SJW extract on P450s, UDP-glucuronosyltransferase (UGT), glutathione *S*-transferase (GST), and NAD(P)H-quinone oxidoreductase (NQO) in mice. The oral administration of SJW extract to male mice at 0.6 g/kg/d for 21 days increased hepatic oxidation activity toward a Cyp3a substrate nifedipine. By extending the SJW treatment to 28 days, hepatic nifedipine oxidation (NFO) and warfarin 7-hydroxylation (WOH) (Cyp2c) activities were increased by 95% and 34%, respectively. Immunoblot analysis of liver microsomal proteins revealed that the Cyp2c protein level was elevated by the 28-day treatment. However, the liver microsomal activities of the oxidation of the respective substrates of Cyp1a, Cyp2a, Cyp2b, Cyp2d, and Cyp2e1 remained unchanged. In the kidney, SJW increased the NFO, but not the WOH activity. The extended 28-day treatment did not alter mouse hepatic and renal UGT, GST, and NQO activities. These findings demonstrate that SJW stimulates hepatic and renal Cyp3a activity and hepatic Cyp2c activity and expression. The induction of hepatic Cyp2c requires repeated treatment for a period longer than the initial induction of Cyp3a.

* *Corresponding author*. National Research Institute of Chinese Medicine, 155-1 Li-Nong Street, Section 2, Taipei 112, Taiwan, ROC.
E-mail address: ueng@nricm.edu.tw (Y.-F. Ueng).

http://dx.doi.org/10.1016/j.jfda.2017.01.004

1. Introduction

The drug-metabolizing cytochrome P450 (P450, CYP)-dependent monooxygenase system primarily localizes at the membranes of endoplasmic reticulum and participates in the metabolism of about 96% of drugs under development or on the market [1]. Microsomal P450 isoforms catalyze the oxidation of structurally diverse drugs with broad substrate specificity. The oxidations catalyzed by P450s require NADPH-P450 reductase (CPR) to carry out the two-electron transfer to the P450 substrate and the oxygen-inserted P450-substrate complexes [2]. A hemoprotein cytochrome b_5 can initiate a second electron transfer. Cytochrome b_5 stimulates the activities of certain P450 isoforms, including CYP3A4; the stimulation of CYP3A4 activity is possibly through a protein–protein interaction without the direct influence of the electron transfer [3]. Alteration of the electron transfer partners may be involved in the changes of P450 activity. In human livers, CYP1, CYP2, and CYP3 are the main P450 families responsible for drug oxidations [1]. CYP3A4 is the most abundant human hepatic P450 and oxidizes about 27% of the drugs under development and used in patients. The CYP2C subfamily occupies about 20% of the total P450 content [4] and oxidizes about 24% of the drugs [1]. The functional changes of P450s, especially the CYP3A and CYP2C subfamilies, can be crucial for herb–drug interaction.

The extract of St. John's wort (SJW; *Hypericum perforatum*) is an over-the-counter botanical supplement widely used for the treatment of mild to moderate depression and sleep disorders in Europe and the United States [5].The use of SJW in patients has been reported to decrease the therapeutic efficacy of a variety of drugs, including tolbutamide, warfarin, and indinavir, which are mainly metabolized by some P450 isoforms, including CYP2C9 and CYP3A4 [5]. In the *in vitro* system of the primary culture of human hepatocytes, it has been demonstrated that SJW extract induced CYP3A4 through the activation of the human pregnane X receptor (PXR), and the SJW ingredient hyperforin is identified as the primary PXR activator and induced CYP3A4 [6]. However, inconsistency exists in reports regarding the changes of the other P450 isoforms, such as CYP2C9. In a study involving 12 participants, treatment with SJW extract (25 mg hyperforin/g extract) at a daily dose of 0.9 g for 14 days caused a 52% decrease in the mean AUC (area under the curve of a plot of plasma drug concentration vs. time after administration) value of the CYP3A4 substrate midazolam [7]. This SJW treatment did not change the pharmacokinetic parameters of caffeine and tolbutamide, which are primarily metabolized by CYP1A2 and CYP2C9, respectively. However, in another pharmacokinetic study on 21 participants taking 0.9 g/d SJW extract (hyperforin content not shown) for 15 days prior to a CYP2C9/19 substrate gliclazide, the intrinsic clearance of gliclazide was elevated [8], suggesting that the SJW extract stimulated the CYP2C-mediated drug metabolism in humans. In the primary culture of human hepatocytes, a study of the effect of 48-hour exposure showed that hyperforin at 0.2μM and 1μM concurrently elevated the activities and/or expression of CYP3A4 and CYP2C9, whereas hyperforin had no influence on the mRNA and protein levels of CYP1A2 or CYP2D6 [9]. Reports regarding human samples suggested that CYP2C9, but not CYP1A2 and CYP2D6, might be induced by SJW. Although the polymorphic expression of CYP2C9 can be associated with different activities [8], the inconsistency in the metabolic changes of CYP2C9 substrates revealed the importance of the information regarding the dose- and time-dependent functional changes of P450 isoforms by SJW extract *in vivo* or *ex vivo*.

Experimental animals provide an alternative biological system to assess the changes of drug-metabolizing enzymes *in vivo* and *ex vivo*, and a significant variation caused by genetic polymorphism can be excluded. In a study of drug-perfused livers in Wistar rats, unlike the potential induction of CYP2C by SJW in humans, the AUC value of a CYP2C substrate tolbutamide in perfusate was significantly increased by 80% in SJW (unknown hyperforin content; 100 mg/kg/d intraperitoneally for 10 days)-treated rats, suggesting a decrease in rat CYP2C activity [10]. In contrast, this treatment decreased the AUC values of dextromethorphan and midazolam, which are the substrates of CYP2D and CYP3A, respectively. In a study of 7-day, 14-day, and 21-day treatments, only the 21-day oral treatment of Swiss Webster mice with 0.14 g/kg and 0.28 g/kg SJW (2.3% hyperforin) induced the expression and activities of CYP3A and CYP2E1, whereas CYP1A activity remained unchanged [11]. However, although the oral treatment of Wistar rats with 0.4 g/kg SJW extract (unknown hyperforin content) for 10 consecutive days transiently increased the expression level of hepatic CYP1A2, the increase was eliminated after a 30-day consecutive treatment [12]. The time-dependent functional change of CYP1A2 remains unclear. In a study on rat livers perfused with saline containing a CYP1A2 substrate, phenacetin, the concentration of phenacetin in the perfusate was significantly higher after SJW treatment (100 mg/kg/d, 10 days), suggesting that CYP1A2 activity was decreased by SJW [13]. The changes in CYP1A2 and CYP2C activities/expression by SJW extract show inconsistent results in different treatment regimens and biological systems. The information on hyperforin content in the SJW extracts has not been fully provided, which reveals the difficulty of making a conclusion regarding the subchronic/chronic effects of SJW on P450s from the present reports of animal studies.

The oxidation metabolites, as generated from the oxidation by a P450, can be further subjected to conjugative metabolism. Among the varied conjugative metabolisms, glucuronidation catalyzed by the microsomal UDP-glucuronosyltransferase (UGT) family is quantitatively the most important metabolic process [14]. UGT participates in the glucuronidation of drugs or the metabolites of drugs, such as acetaminophen and warfarin. SJW extract stimulated the pGL_3-UGT1A1 reporter activity in CV-1 cells expressing human or mouse PXR [15]. However, hepatic UGT activity was not affected by 21-day treatment with SJW extract in Swiss Webster mice, whereas CYP3A was induced [10]. The importance of the cytosolic glutathione S-transferase (GST) family has been recognized in the detoxification of environmental toxins, such as benzo(*a*)pyrene and aflatoxin B_1 through the glutathione conjugation of their active metabolites [16]. The hepatic protein level of one type of GST, GST-P, can be increased in rats treated with 0.4 g/kg SJW extract daily for 10 consecutive days, and the induced level was decreased to a basal level after a 30-day treatment [12]. The functional change of GST remains unclear. The oxidative stress inducible

Please cite this article in press as: Yang J-F, et al., The time-dependent effects of St John's wort on cytochrome P450, uridine diphosphate-glucuronosyltransferase, glutathione S-transferase, and NAD(P)H-quinone oxidoreductase in mice, Journal of Food and Drug Analysis (2017), http://dx.doi.org/10.1016/j.jfda.2017.01.004