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Induction of cytochromes P450 1A1 and 1A2 by tanshinones in human HepG2 hepatoma cell line

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ABSTRACT

Diterpenoid tanshinones including tanshinone IIA (TIIA), cryptotanshinone (CTS), tanshinone I (TI) and dihydrotanshinone I (DHTI) are the major bioactive components from Danshen. The major aim of our present study was to investigate the induction potential of these four main components of tanshinones (TIIA, CTS, TI, and DHTI) on the expression of CYP1A1 and CYP1A2 in HepG2 cells. Our results showed that all of these four tanshinones caused a significant time- and concentration-dependent increase in the amount of CYP1A1/2 expression in HepG2 cells. These induction effects were further characterized through transcriptional regulation: the induction of CYP1A1/2 mRNA level by tanshinones was completely blocked by the transcription inhibitor actinomycin D; the expression of CYP1A1/2 heterogeneous nuclear RNA was induced by tanshinone treatment; and CYP1A1 mRNA stability was not influenced by these tanshinones. Interestingly, tanshinones plus B[a]P produced additive/synergistic effect on CYP1A1/2 induction. In addition, the tanshinone-induced CYP1A1/2 expression was abolished by the aryl hydrocarbon receptor (AhR) antagonist resveratrol, suggesting an AhR dependent transcription mechanism. In the reporter gene assay, while TI and DHTI significantly induced AhR-dependent luciferase activity, TIIA and CTS failed to induce this activity. Collectively, the tanshinones could induce CYP1A1 and CYP1A2 expression through transcriptional activation mechanism and exert differential effects on activating AhR in HepG2 cells. Our findings suggest that rational administration of tanshinones should be considered with respect to their effect on AhR and CYP1A1/2 expression.

Introduction

Human cytochrome P450 (CYP) enzymes play a key role in the metabolism of drugs and environmental chemicals. CYP1A1 and CYP1A2 are the major isoenzymes for metabolism of toxic and carcinogenic chemicals, such as polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HAAs), to form reactive intermediates, which can attack cellular DNA, and initiate the cancer process (Guengerich and Shimada, 1998). Additionally, various clinical drugs including phenacetin and theophylline are metabolized by these isoenzymes (McFadyen et al., 2004; Zhou et al., 2009). Therefore, the modulation of CYP1A1/2 by xenobiotics might raise significant concerns in risk assessment and potential drug–drug interactions (DDI) in the clinic. Whereas the hepatic expression of CYP1A1 is insubstantial, CYP1A1/2 is highly inducible by a range of chemicals mediated through the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor (Ma and Lu, 2007). Besides the harmful environmental toxicants, such as 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (Whitlock, 1999) and B[a]P (Vakharia et al., 2001), a lot of phytochemicals (Ciolino et al., 1999; Wang et al., 2001; Zhang et al., 2003; Wang et al., 2008) have also been identified as AhR agonists and exhibited a CYP1A1/2 induction effect. Given the pharmacotoxicological significance of CYP1A1/2 induction (Ma and Lu, 2007), and the fact that increasing numbers of people are consuming diverse herbal preparations that contain many constituents with potential for CYP modulation, it is of clear interest to examine the regulation of CYP1A1/2 by herb components.

Danshen, the dried root of salvia miltiorrhiza, is widely used in both China and many other countries as a therapeutic agent for a variety of ailments, especially for cardiovascular diseases (Zhou et

Abbreviations: CYP, cytochrome P450; AhR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; DRE, dioxin response element; EROD, 7-ethoxyresorufin O-deethylation; B[a]P, benzo[a]pyrene; BNF, β-naphthoflavone; TIIA, tanshinone IIA; CTS, cryptotanshinone; TI, tanshinone I; DHTI, dihydrotanshinone I.

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al., 2005; Cheng, 2007). Tanshinones, a group of abietane-type diterpenes, have been demonstrated to be the main active components in danshen. It has been actively investigated for its powerful and wide pharmacological activities. The major tanshinones isolated from danshen, including tanshinone IIA (TIIA), cryptotanshinone (CTS), tanshinone I (TI) and dihydrotanshinone I (DHTI), have been shown to possess pharmacological activities such as antioxidant (Cao et al., 1996), antiinflammatory (Kang et al., 2000; Fan et al., 2009), and antiangiogenic (Hur et al., 2005). Recently, accumulating *in vitro* and *in vivo* evidences suggested that they have diverse anti-tumor potency (Wang et al., 2005; Tian et al., 2007; Tsai et al., 2007; Lee et al., 2008; Lu et al., 2009; Shin et al., 2009; Chen et al., 2010; Gong et al., 2010). Thus, tanshinones are regarded as agents with considerable promise for treatment of cancer and cardiovascular disease.

Our previous studies have demonstrated that TIIA, CTS and TI exhibited a potent inhibitory effect towards CYP1A2 in human liver microsomes (Qiu et al., 2008b). On the other hand, many reports have indicated that tanshinones could induce CYP1A in the liver. Kuo et al. (2006) found that there were mouse CYP1A-inducing agents present in the lipophilic components from danshen extract. A study in rats demonstrated that extract of tanshinone significantly increased liver CYP1A activity after 10 days of administration (Yang et al., 2003). Ueng et al. (2004) reported that tanshinone IIA could induce CYP1A2 expression in the AhR-responsive C57BL/6J mice. Although previous research has shed a little light on the effect of tanshinones on regulating CYP1A, there are several critical issues that still need to be clarified. Firstly, the ability of tanshinone to induce CYP1A1/2 was not conclusive, since danshen extract/preparation appeared to be devoid of any effect in some reports (Qiu et al., 2008a; Wang et al., 2009). Secondly, although CYP1A1/2 showed strong conservation across species, appreciable interspecies differences of some inducers have also been observed (Shih et al., 1999; Xu et al., 2000; Martignoni et al., 2006). So, these induction effects mediated by tanshinones cannot always be extrapolated to human since previous studies have largely been limited to experimental animals. It is important to use other alternative procedures, such as liver cell lines, to validate these induction effects. Furthermore, most of those studies were conducted with an extract of danshen/tanshinones containing different abundance of tanshinones and other components of danshen, and it was not determined which individual constituents are responsible for the observed effect of the extract. Thus, the direct effects of individual tanshinones on the gene expression of CYP1A1/2 are unknown. Finally, the mechanism underlying the induction effect, if any, is still unclear.

The goal of our present study was to investigate whether tanshinones could modulate the expression of CYP1A1 and CYP1A2, the major isoenzymes in the bioactivation of carcinogens. Main components belonging to the tanshinones, such as tanshinone IIA (TIIA), cryptotanshinone (CTS), tanshinone I (TI) and dihydrotanshinone I (DHTI), were comparatively studied for their CYP1A1/2 induction capability in HepG2 human hepatoma cell line, a well-validated alternative system of the human hepatocyte for CYP1A cell signaling (Takahashi et al., 1994; Wilkening et al., 2003; Westerink and Schoonen, 2007). The mechanism of the induction was also investigated. Our findings provide a systematic insight into the effects of individual tanshinones on regulating CYP1A1/2 expression *in vitro*. Such information may be important for further development of these potentially promising agents.

Materials and methods

Chemicals and reagents. Tanshinone IIA (TIIA), cryptotanshinone (CTS), tanshinone I (TI) and dihydrotanshinone I (DHTI) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purity of these chemicals was above 98% and the structures are shown in Fig. 1. Benzo[a]pyrene (B[a]P), β-naphthoflavone (BNF), resorufin, 7-

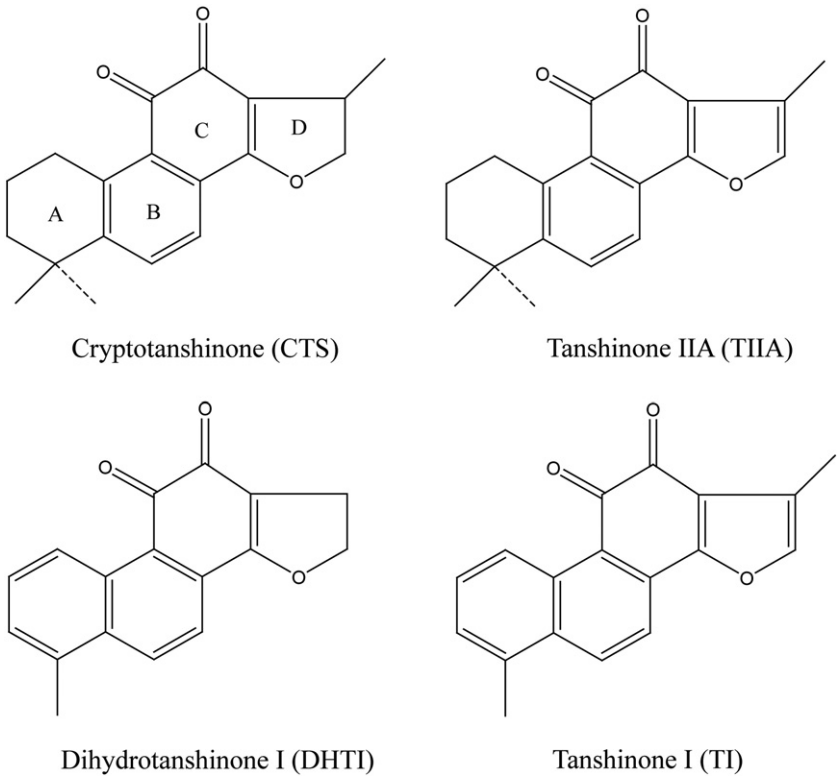

Fig. 1. Chemical structures of tanshinone components used in the present study.