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Different AhR binding sites of diterpenoid ligands from *Andrographis paniculata* caused differential CYP1A1 induction in primary culture in mouse hepatocytes

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ABSTRACT

Andrographis paniculata has been employed as a folklore remedy. Andrographolide (Andro), 14-deoxy-11,12-didehydroandrographolide (DHA), andrographiside (AS), and neoandrographolide (Neo), are major diterpenoids isolated from this plant. In the present study, influence of the four diterpenoids on CYP1A1 mRNA expression was investigated in primary cultured mouse hepatocytes. Additionally, binding of these compounds to aryl hydrocarbon receptor (AhR) was examined using molecular docking analysis to clarify mechanism of CYP1A1 induction. Andro and DHA induced CYP1A1 expression by itself, and co-treatment with a CYP1A1 inducer (BNF, beta-naphthoflavone) showed a synergistic increase of CYP1A1 expression. Andro demonstrated higher enhancing activity than DHA at every similar concentration. On the other hand, Neo suppressed BNF-induced CYP1A1 expression, but AS did not modify the induction. Results from molecular docking analysis of BNF and four diterpenoids on ligand binding domain of AhR were consistent with levels of CYP1A1 mRNA expressions. Furthermore, difference of binding sites of BNF in the presence of diterpenoids might affect the synergism or inhibition of CYP1A1 expression. These results suggest that use of *A. paniculata* as a health supplement should be concerned in term of herb–drugs interactions or risk of carcinogenesis, according to its ability to influence CYP1A1 expression.

1. Introduction

CYP1A1 is an important cytochrome P450 in the metabolism of polycyclic aromatic hydrocarbons (PAHs) (Kim et al., 1998; Shimada et al., 1999; Nebert et al., 2004). Oxidation of PAHs by CYP1A1 enzyme is an initial step in the activation of carcinogenesis (Roberts-Thompson et al., 1993). Relationship between CYP1A1 polymorphism and cancer risk has been extensively investigated (Bartsch et al., 2000; London et al., 1995; Xu et al., 1996). Expression of CYP1A1 is inducible by PAHs, which are found in cigarette smoke condensate as well as environmental pollutants. For that reason, an increasing risk of oral squamous cell carcinoma in smokers is recognized by epidemiological studies (Tanimoto et al., 1999; Sato et al., 2000; Nagaraj et al., 2006). Therefore, alteration of CYP1A1 expression is considered to be an important factor of carcinogenesis. Aryl hydrocarbon receptor (AhR) is a member of the Per-Arnt-Sim family of nuclear regulatory basic helix loop–helix proteins (Burbach et al., 1992; Swanson and Bradfield, 1993; Hahn, 1998). The AhR binds to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or other structurally similar PAHs in the cytosol, which is the initial step of CYP1A1 induction (Denison and Whitlock, 1995; Swanson and Bradfield, 1993; Hahn, 1998). The ligand bounded AhR translocates from the cytosol to the nuclei, and then heterodimerizes with AhR nuclear translocator (Arnt). The heterodimer binds to the promoter region of *CYP1A1* gene and subsequently activates transcription (Whitlock, 1999). Hence, the binding of ligands to AhR is the essential step to elicit CYP1A1 induction.

Andrographis paniculata Nees (Family Acanthaceae), traditionally employed for centuries in Asia as a folklore remedy for a wide spectrum of ailments, is nowadays incorporated into a number of herbal medicinal preparations. Extensive researches have revealed that this herbal extract is useful as an anti-inflammatory (Shen et al., 2002), antiviral (Calabrese et al., 2000), anticancer (Kumar et al., 2004; Rajagopal et al., 2003), and immunostimulatory medicine (Puri et al., 1993; Iruretagoneya et al., 2005). Andrographolide (Andro; Fig. 1) is a major diterpenoid constituent of the plant *A. paniculata*, Andro also possesses several pharmacological activities, including inhibition of iNOS expression (Chiou et al., 1998), Mac-1 expression and ROS production (Shen et al., 2000), anticancer (Kumar et al., 2004), and a protective effect against

Abbreviations: Andro, andrographolide; AhR, aryl hydrocarbon receptor; AS, andrographiside; BNF, beta-naphthoflavone; CYP1A1, cytochrome P450 subfamily 1A1; DHA, 14-deoxy-11,12-didehydroandrographolide; LBD, ligand binding domain; Neo, neoandrographolide; PAHs, polycyclic aromatic hydrocarbons; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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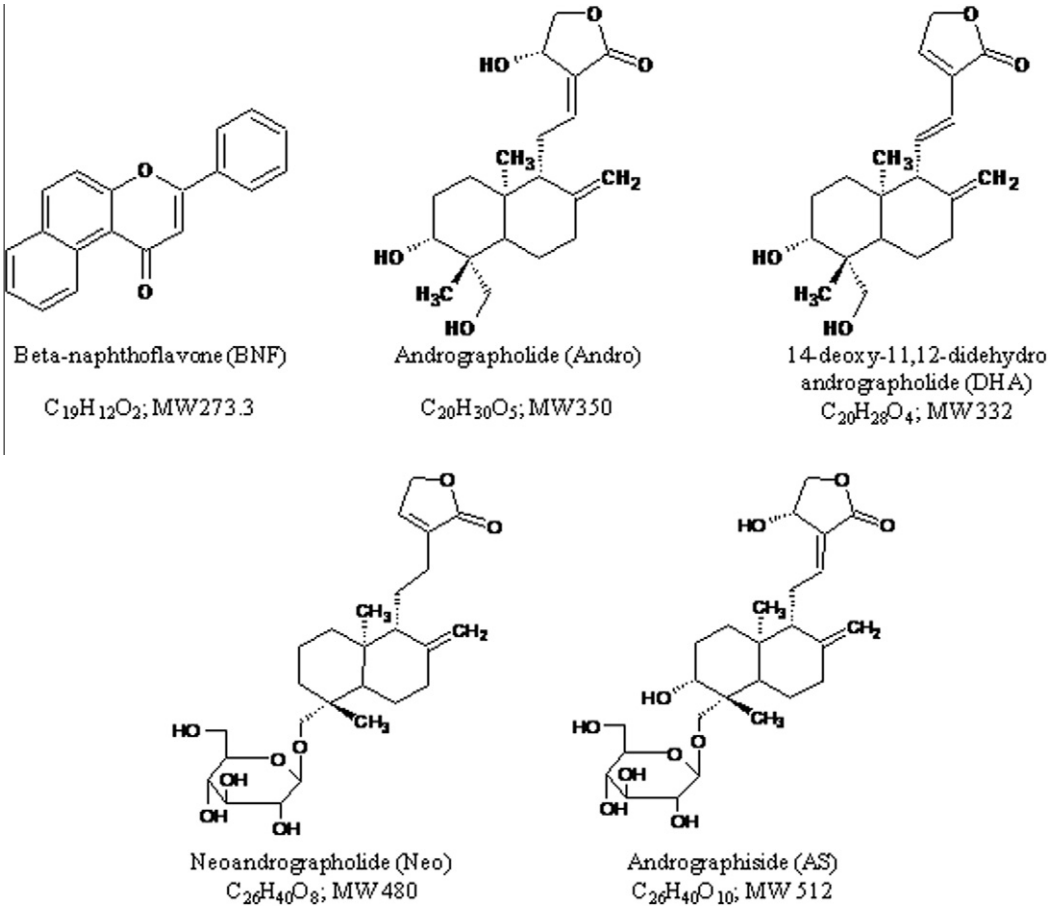

Fig. 1. Structures of BNF and diterpenoids isolated from *Andrographis paniculata.*

cytotoxicity (Kapil et al., 1993). In addition, Andro has been employed to prevent and treat the common cold (Caceres et al., 1997). Beside Andro, there are several diterpenoids (Fig. 1) contained in *A. paniculata* including 14-deoxy-11,12-didehydroandrographolide (DHA), neoandrographolide (Neo), and andrographiside (AS). Recently, numbers of publications on pharmacological activities of these diterpenoids have increased (Pinthong et al., 1991; Kamdem et al., 2002; Pholphana et al., 2004), while the reports from toxicity or drug metabolism related-field are still limited.

Previously, we observed induction of CYP1A1 enzyme by crude extract of *A. paniculata* (Jarukamjorn et al., 2006). Interestingly, Andro plus the typical CYP1A inducers synergistically induced CYP1A1 expression, and the synergism was blocked by an AhR antagonist (Jaruchotikamol et al., 2007). The effects of other diterpenoids isolated from *A. paniculata* on CYP1A1 expression have not been investigated to date.

In the present study, activations of CYP1A1 by the four diterpenoids from *A. paniculata* were compared in primary cultured mouse hepatocytes. Moreover, binding of a ligand with AhR in the presence of these diterpenoids was examined *in silico* using molecular docking analysis to clarify the mechanism of CYP1A1 induction.

2. Material and methods

2.1. Chemicals

Materials for culturing hepatocytes were purchased from Gibco Invitrogen Corporation (Grand Island, NY), Wako Pure Chemical (Osaka, Japan), and Sigma Chemicals (St. Louis, MO). Percoll was obtained from GE Healthcare Bio-Sciences (Uppsala, Sweden). Andro and LDH-cytotoxic Test Wako were from Wako Pure Chemical. Beta-naphthoflavone (BNF) was obtained from Sigma Chemicals. ReverTraAce and G-Taq DNA polymerase were purchased from TOYOBO (Osaka, Japan) and Hokkaido System Science (Sapporo, Japan), respectively. All other laboratory chemicals were of the highest purity available from commercial suppliers.

2.2. Isolation of diterpenoids from A. paniculata

Leaves of *A. paniculata* were collected in Khon Kaen, Thailand in 2008 and dried in oven at temperature 50 °C. The 700 g of dried leaves were macerated in 3 l of methanol and kept at room temperature for 3 days. After filtration, the methanol solution was evaporated under reduced pressure to give the methanolic extract (Batkhuu et al., 2002). The methanolic crude extract (31.9 g) was chromatographed on a silica gel 60 (Merck, Germany). Elution was carried out with dichloromethane followed by a dichloromethane–methanol mixture with increasing polarity. Fractions (250 ml each) were collected and monitored by TLC, compared to the standards. Some fractions were re-separated using hexane–ethyl acetate mixture (5:5), or Sephadex LH-20 with methanol (Jain et al., 2000; Kamdem et al., 2002; Patarapanich et al., 2007; Chao and Lin, 2010). The identifications of DHA, AS, and Neo were carried out by comparison of their NMR-spectral and high resolution mass-spectral data with those from the literatures (Fujita et al., 1984; Kamdem et al., 2002; Du et al., 2003).