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Strong synergistic induction of CYP1A1 expression by andrographolide plus typical CYP1A inducers in mouse hepatocytes

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Abstract

The effects of andrographolide, the major diterpenoid constituent of *Andrographis paniculata*, on the expression of cytochrome P450 superfamily 1 members, including CYP1A1, CYP1A2, and CYP1B1, as well as on aryl hydrocarbon receptor (AhR) expression in primary cultures of mouse hepatocytes were investigated in comparison with the effects of typical CYP1A inducers, including benz[*a*]anthracene, β-naphthoflavone, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Andrographolide significantly induced the expression of CYP1A1 and CYP1A2 mRNAs in a concentration-dependent manner, as did the typical CYP1A inducers, but did not induce that of CYP1B1 or AhR. Interestingly, andrographolide plus the typical CYP1A inducers synergistically induced CYP1A1 expression, and the synergism was blocked by an AhR antagonist, resveratrol. The CYP1A1 enzyme activity showed a similar pattern of induction. This is the first report that shows that andrographolide has a potency to induce CYP1A1 enzyme and indicates that andrographolide could be a very useful compound for investigating the regulatory mechanism of the CYP1A1 induction pathway. In addition, our findings suggest preparing advice for rational administration of *A. paniculata*, according to its ability to induce CYP1A1 expression.

Keywords: Andrographolide; CYP1A1; CYP1A2; CYP1B1; AhR; Mouse hepatocyte

Introduction

Cytochrome P450s (P450) constitute a superfamily of heme-proteins that play an important role in the metabolism of xenobiotics, including drugs, toxins, and chemical carcinogens (Guengerich, 2000; Guengerich and Shimada, 1998). Of these, P450s, CYP1A1 and CYP1A2 have been shown to be the major enzymes in the metabolism of potential procarcinogens such as polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs, and aryl and heterocyclic arylamines. In addition to being substrates, PAHs are also inducers of *CYP1A1* and *CYP1A2* genes. The mechanisms of transcriptional regulation of the two genes are not the same. CYP1A1 is expressed constitutively in several extrahepatic tissues, but not in the liver. However, while CYP1A1 expression has been demonstrated in the liver after inducer treatment, CYP1A2 is constitutively and inducibly expressed only in the liver (Kimura et al., 1986; Iwanari et al., 2002). Aryl hydrocarbon receptor (AhR) has been shown to play central roles in the regulation and induction of CYP1A1 and CYP1A2 by a prototype inducer, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; Whitlock, 1999). In addition to the two members of the CYP1A subfamily, CYP1B1, a relatively new member of the superfamily 1 (Brake et al., 1999; Ryu and Hodgson, 1999; Savas et al., 1994), has been postulated to be involved in the metabolism of PAHs such as TCDD through

Abbreviations: AhR, aryl hydrocarbon receptor; ARNT, AhR nuclear translocator; B[*a*]A, benz[*a*]anthracene; β-NF, β-naphthoflavone; DMSO, dimethylsulfoxide; DRE, Dioxin Responsive Element; EROD, ethoxyresorufin *O*-deethylase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; iNOS, inducible nitric oxide synthase; Mac, membrane-activated complex; P450, cytochrome P450; ROS, reactive oxygen species; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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AhR and the AhR nuclear translocator (ARNT)-mediated pathway (Ryu and Hodgson, 1999; Savas et al., 1994). Constitutive expression of CYP1B1 was detected in steroidogenic tissues such as adrenal glands, ovaries, and testes, but it was not detected in xenobiotic-metabolizing organs such as liver, kidney, and lung (Iwanari et al., 2002; Savas et al., 1994). Since CYP1 is responsible for activating carcinogenic aromatic amines and heterocyclic amines, to which we are exposed to every day via smoking, diet, and the environment, its regulation is of clear interest.

Andrographolide (3-[2-[decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylene-1-napthalenyl]ethylidene] dihydro-4-hydroxy-2(3H)-furanone) is the major diterpenoid constituent of the plant *Andrographis paniculata* Nees (Family Acanthaceae), which has been traditionally employed for centuries in Asia and Europe as a folk remedy for a wide spectrum of ailments or a herbal supplement for health promotion, and is nowadays incorporated in a number of herbal medicinal preparations. It is found in the *Indian Pharmacopoeias* and is a prominent component in at least 26 Ayurvedic formulas (Madav et al., 1995). In traditional Chinese medicine, it is an important "cold property" herb used to rid the body of heat, as in fevers, and to dispel toxins from the body (Deng, 1978). In Scandinavian countries, it is commonly used to prevent and treat the common cold (Caceres et al., 1997). *A. paniculata* is one of the top 10 herbal medicines which the Thai FDA has promoted as an alternative medicinal therapy for fever and inflammation. Extensive research has revealed that the whole-plant extract is useful for anti-inflammatory (Shen et al., 2002), antiviral (Calabrese et al., 2000), anticancer (Kumar et al., 2004), and immunostimulatory (Puri et al., 1993; Iruretagoneya et al., 2005) treatments. On the other hand, male reproductive toxicity (Akbarsha and Murugaian, 2000) and cytotoxicity (Nanduri et al., 2004) of this plant have been reported as well. Andrographolide has been reported to show hepatoprotective activity in mice against carbon tetrachloride and paracetamol intoxication (Handa and Sharma, 1990a; Handa and Sharma, 1990b), and to possess several pharmacological activities, including inhibition of iNOS expression (Chiou et al., 2000, 1998), Mac-1 expression, and ROS production (Shen et al., 2002, 2000), and a protective effect against cytotoxicity (Kapil et al., 1993). This compound has recently been shown to work as an anti-inflammatory agent by reducing the generation of ROS in human neutrophils (Shen et al., 2002), as well as preventing microglia activation (Wang et al., 2004) and interfering with T cell activation (Iruretagoneya et al., 2005). Recently, we reported that a crude extract of *A. paniculata* might induce mouse hepatic cytochrome P450 isoforms CYP1A1 and CYP2B via significant increases in ethoxyresorufin *O*-dealkylase (EROD) and pentoxyresorufin *O*-dealkylase activities (Jarukamjorn et al., 2006). However, there have been no studies on the impact of andrographolide on the expression of hepatic P450 enzymes. Therefore, it was of interest to examine how andrographolide influences the expression of hepatic P450s. The results of such an inquiry might provide invaluable guidelines for the rational administration and precautions for the use of the herbal plant.

In the present study, the ability of andrographolide to elevate the hepatic expression of CYP1A1, CYP1A2, CYP1B1, and AhR was examined in mouse hepatocytes and compared with that of typical CYP1A inducers, including benz[*a*]anthrazene (B[*a*]A), β-naphthoflavone (β-NF), and TCDD. Subsequently, the synergistic effects of concomitant treatment with andrographolide and the typical CYP1A inducers on the induction of CYP1A1 mRNA, protein, and enzyme activity were examined. The findings revealed that andrographolide has a potency to induce CYP1A1 enzyme.

Materials and methods

Materials. Materials for culturing hepatocytes were purchased from Gibco® Invitrogen Cell Culture (Carlsbad, CA), BioWhittaker™ Cambrex Bio-Sciences (Walkerville, MD), and Wako Pure Chemical (Osaka, Japan). Percoll and collagenase (Type I) were products of GE Healthcare Bio-Sciences AB (Uppsala) and the Sigma Chemical Co. (St. Louis, MO), respectively. Andrographolide, resveratrol, and the LDH-cytotoxic test were supplied by Wako Pure Chemical. B[*a*]A, β-NF, TCDD, ethoxyresorufin, and resorufin were obtained from Sigma Chemical Co. The TaKaRa RT-PCR kit (Perfect Real Time) and SYBR® Green *Premix Ex Taq*™ (Perfect Real Time) were products of TaKaRa Biomedicals Inc. (Shiga, Japan). The TaqMan® Gene Expression Assays were products of Applied Biosystems (Branchburg, NJ). The antibody against rat CYP1A1 was a generous gift from Dr. Y. Funae (Osaka City University, Osaka, Japan). The Amersham Pharmacia Biotech Co. supplied Hybond-C membranes for blotting. The Dual-Luciferase Reporter Assay System was a product of Promega® (Madison, WI). All other laboratory chemicals were of the highest available purity from commercial suppliers.

Preparation of primary hepatocyte cultures. The liver of a ddY male mouse (Sankyo Laboratories, Shizuoka, Japan) at 8 weeks of age was perfused with collagenase, and viable hepatocytes were isolated by means of Percoll isodensity centrifugation as described (Nemoto and Sakurai, 1995). Standard culture conditions were used as follows: the cells were dispersed in Waymouth MB 752/1 medium containing bovine serum albumin (2 g/l), insulin (0.5 mg/l), transferrin (0.5 mg/l), and selenium (0.5 μg/l), and seeded in dishes at a density of 5×10^6 cells/10 ml/100-mm collagen-coated dish. The Waymouth medium did not contain phenol red, a pH indicator, to exclude the possibility of estrogen-like action. Depending on the presence of cell attachment factors, the hepatocytes anchor to the dishes within 3 h and subsequently form a monolayer. The culture dishes were maintained at 37 °C in a CO_2-humidified incubator. The medium was renewed 3 h after plating and then, after 24 h, the treatment with andrographolide and/or typical CYP1A inducers was performed immediately following the medium change, unless otherwise indicated. The inducers were dissolved in DMSO, which itself had no influence on enzyme activity at the routinely employed maximum concentration of 0.1% (Nemoto and Sakurai, 1992). The cells were harvested after another 24 h to prepare total RNA or microsomal proteins as described elsewhere (Jarukamjorn et al., 1999). These culture conditions were convenient for maintaining CYP1A1 and CYP1A2 expression (Nemoto and Sakurai, 1992) and the concentrations of added compounds were proved to be non-cytotoxic by the methods of the LDH-cytotoxic test and the normalized level of GAPDH.

Real-time RT-PCR. Mouse CYP1A1, CYP1A2, CYP1B1, AhR, and GAPDH mRNAs were quantified by real-time RT-PCR. Hepatic total RNA was reverse-transcribed and cDNA was amplified under the conditions recommended by the supplier (TaKaRa Biomedicals Inc., Shiga, Japan) of the TaKaRa® RT-PCR kit (Perfect Real Time) using specific TaqMan® Gene Expression Assays (Inventoried) for *Cyp1a1* (assay ID, Mm00487218_m1), *Cyp1a2* (Mm00487224_m1), *Cyp1b1* (Mm00487229_m1), and AhR (Mm00478932_m1), as well as the SYBR® *Premix Ex Taq*™ (Perfect Real Time) for GAPDH, in which the forward and reverse primers were 5′-TCC ACT CAC GGC AAA TTC AAC G-3′ and 5′-TAG ACT CCA CGA CAT ACT CAG C-3′, respectively. The specificity of amplification of GAPDH cDNA was confirmed by both polyacrylamide gel electrophoresis and the dissociation curve of the product. Real-time PCR was