CHAPTER I
INTRODUCTION

1.1 Rational and background of study

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 research has 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), and immunostimulatory supplement (Puri et al., 1993; Iruretagoneya et al., 2005). Andrographolide (3-[2-[decahydro-6-hydroxy-5-(hydroxymethyl)-5,8α-dimethyl-2-methylene-1-napthalenyl] ethylidene] dihydro-4-hydroxy-2(3H)-furanone) is a major diterpenoid constituent of the plant *A. paniculata*, which has been an herbal supplement for health promotion. Andrographolide has been reported to show hepatoprotective activity in mice against carbon tetrachloride and paracetamol intoxication (Handa & Sharma, 1990), and to possess several pharmacological activities, including inhibition of expression of inducible nitric oxide synthase and an essential integrin mediated in neutrophil adhesion and transmigration (CD11b/CD18, Mac-1) (Chiou et al., 2000; Chiou et al., 1998), and reactive oxygen species (ROS) production (Shen et al., 2000), anticancer (Rajagopal et al., 2003), and a protective effect against cytotoxicity (Kapil et al., 1993). In addition, andrographolide has been employed to prevent and treat the common cold (Caceres et al., 1997).

Several activities of *A. paniculata* and andrographolide have been already well-established. However, the studies in the aspect of toxicity are still less, especially drug-herb interaction via cytochrome P450 (CYP). CYP plays important roles in pharmacology of drugs and toxicology of xenobiotics. Understanding the way in which drugs or xenobiotics induce or inhibit CYP is biologically relevant and ultimately leads to better models for screening and predicting drug interactions. Cytochrome P450 1A1 isofrom (CYP1A1) is the most important human CYP enzymes in the metabolic activation of polycyclic aromatic hydrocarbons (PAHs)

(Kim et al., 1998; Shimada et al., 1996; Nebert et al., 2004). In most cases, oxidation of PAHs by CYP1A enzyme is an initial step in the activation of carcinogenesis (Roberts-Thompson et al., 1993). CYP1A1 variants and cancer risk have been investigated in several studies (Bartsch et al., 2000). Therefore, the inducible and constitutive expression of CYP1A is considered to be important factor of carcinogenesis.

Previous studies reported that *A. paniculata* induced mouse hepatic cytochrome P450 isoforms CYP1A1 and CYP2B via significant increases of ethoxy resorufin *O*-dealkylase and pentoxyresorufin *O*-dealkylase activities (Jarukamjorn et al., 2006). Moreover, andrographolide significantly induced the expression of CYP1A1 mRNA in a concentration-dependent manner in mouse hepatocytes in primary culture, as did typical CYP1A inducers. Interestingly, andrographolide plus a typical CYP1A inducer synergistically induced CYP1A1 expression, and the synergism was blocked by an arylhydrocarbon receptor (AhR)-antagonist, resveratrol (Jaruchotikamol et al., 2007). Recently, Pekthong et al. (2009) reported the effect of *A. paniculata* extract or andrographolide on the expression of a limited number of cytochrome P450 *in vivo* in rat liver and *in vitro* in human and rat hepatocyte cultures, indicating that the effect was diverse. For example, the treatment decreased expression of human CYP2C9 and CYP3A4 or rat CYP2C11 and CYP3A1, while CYP1A2 expression was enhanced in rats.

Recent advances in characterization of specific CYP isozymes involved in drug metabolism allow the identification of enzyme isoforms that are affected by sex (Harris et al., 1995). The majority of studies show that apparent CYP3A4 activity is higher in women than in men (Gleiter & Gundert-Remy, 1996). In mouse species, CYP2B9 is expressed in female higher than male mice (Jarukamjorn et al., 2002), and CYP3A41 and CYP3A44 are female-specific isoforms (Sakuma et al., 2002). The clinical significance of sex-different expression will be important in the administration of drugs that have a narrow therapeutic range of dosage. In addition, sexual dimorphism in drug metabolism may be involved in higher incidence of adverse reactions to drugs in women compared with men. Until now, sexual dimorphic expression of CYP1A1 is still unclear. Sexual dimorphism of lung CYP1A1 expression has been reported. Female smokers exhibited higher expression

levels of lung CYP1A1 mRNA than males in cancer patients (Mollerup et al., 1999). Larsen-su and Williams (2001) showed that indole-3-carbinol induced CYP1A1 protein in the male neonatal liver of rats, but not in the females. Therefore, it is of interest to investigate whether combination of andrographolide and a CYP1A typical inducer synergistically induce CYP1A1 expression both in the males and the females. If the synergistic induction of CYP1A1 expression by andrographolide plus a CYP1A typical inducer is regulated sex-dependently, sex-hormone is involved in the synergism or not.

Andrographolide has been reported to possess several pharmacological activities via its anti-oxidative property (Chen et al., 2004). Redox chemistry involves electron exchanges between molecules that display several possible oxidation states according to their own oxidation potential. Within the cellular context, the redox status depends on the relative amounts of the oxidized and reduced partners of major redox molecules, such as glutathione (Morel & Barouki, 1999). Glutathione (GSH) has been described for a long time just as a defensive compound against the action of toxic xenobiotics (drugs, pollutants, carcinogens). As a prototype antioxidant, it has been involved in cell protection from the noxious effect by excess oxidant stress (Pompella et al., 2003). The ratio of GSSG (oxidized form) to GSH (reduced form), reflects the redox status within the cell. The glutathione system acts as a homeostatic redox buffer. This is an important cellular parameter, since the intracellular redox status monitors the relative amounts of the oxidized and reduced species of each redox system within the cell, depending on its oxidation potential (Dalton et al., 1999). Reactive oxygen species (ROS) are produced by all aerobic cells and widely believed to play a pivotal role in aging as well as a number of degenerative diseases (Allen & Tresini, 2000). The mono-oxygenases are also widespread ROS production using both O_2 and electron transport (Morel & Barouki, 1999). Furthermore, ROS and antioxidants are known to influence the expression of a number of genes and signal transduction pathways (Sen & Packer, 1996). Extensive researches revealed that oxidative stress suppresses CYP1A1 expression (Morel & Barouki, 1998; Barouki & Morel, 2001). Induction of CYP1A1 and CYPlA2 mRNAs by beta-naphthoflavone (β-NF) were maximally reduced in the presence of hydrogen peroxide (Barker et al., 1994). Proposed mechanism of the synergism might be raised by anti-oxidation

activity of andrographolide because down-regulated expression of CYP1A1 by oxidative stress has been observed in several cell lines. Hence, the effect of ROS and GSH on CYP1A1 expression was observed in the present study.

In this study, we determined how andrographolide influenced the expression of analogous or other CYP genes in mouse livers, and investigated effect of glutathione on synergistic CYP1A1 expression by andrographolide with CYP1A inducers. Furthermore, the studies on impacts of sex-hormone and glutathione on the synergistic β-NF-induced CYP1A1 expression by andrographolide conveyed important information for elaborating factors associated the synergism of CYP1A1 induction by andrographolide and typical CYP1A1 inducers. Finally, success of the study will lead a useful guideline of safety and precaution for the use of andrographolide and *A. paniculata* as alternative medication or health supplement.

1.2 Anticipated results of the study

The effects of andrographolide on synergistic CYP1A1 expression will be explored based on the previous report, in which the crude extract of *A. paniculata* induced mouse hepatic cytochrome P450 1A1 and 2B (Jarukamjorn et al., 2006). However, the mechanism of CYP1A1 induction by andrographolide or *A. paniculata* is still unclear. The determination of the effects of andrographolide on all of metabolizing related genes expression in mouse hepatocytes using microarray analysis will be useful information for herb-drug interaction between andrographolide or *A. paniculata* and other drugs or food. Moreover, the specific study of the effects of andrographolide on CYP1A1 induction will confirm the safety or toxicity of andrographolide because we unavoidably expose to CYP1A1 inducers or environmental carcinogens such as cigarette smoke, vehicle smog, charred foods, and petroleum byproducts in our daily life. Therefore, these lead to questions whether use of andrographolide or *A. paniculata* as health supplement will gain benefit from its pharmacological activities or increase risk of cancer via its inducible potential on CYP1A1 pathway. Furthermore, the studies on effects of sex-hormone and glutathione on the synergistic enhancement of β-NF-induced CYP1A1 expression by andrographolide might be significant information for elaborating factors associated synergism of CYP1A1 by andrographolide. Understanding factors related to the

expression of CYP1A1 by andrographolide will advise consumers to avoid risk of using andrographolide or *A. paniculata* in unusual conditions. The results from this study might lead a useful guideline of safety and precaution for using of andrographolide and *A. paniculata* as alternative medication or health supplement.

1.3 Objectives

The objectives of the present study were to investigate impacts of andrographolide on synergistic CYP1A1 expression in primary cultures in mouse hepatocytes and in mouse livers as following:

1.3.1 Determination of the effects of andrographolide on gene expression of CYPs and other metabolizing enzymes using a microarray analysis to screen the effects of andrographolide and/or a typical CYP1A inducer, beta-naphthoflavone, on mouse genes.

1.3.2 Determination of the sexual dimorphism and the effects of a male sex-hormone, testosterone, on synergistic CYP1A expression by andrographolide plus a typical CYP1A inducer, 3-methylchloranthrene.

1.3.3 Determination of the effects of glutathione and redox status on synergistic CYP1A expression by andrographolide plus a typical CYP1A inducer, beta-naphthoflavone.