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Suppression of beta-naphthoflavone induced CYP1A expression and lipid-peroxidation by berberine

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ABSTRACT

Impacts of berberine, a major isoquinoline alkaloid in herbal plants, on beta-naphthoflavone (BNF)-induced CYP1A expression were determined both in primary mouse hepatocytes and in vivo. Berberine concentration-dependently suppressed BNF-induced CYP1A expression in mouse hepatocyte and it significantly down-regulated BNF-induced CYP1A in mouse liver via suppression of mRNA and protein expression, and decreases of EROD and MROD activities. Moreover, berberine showed significant potential on suppression of BNF-induced lipid peroxidation in mouse hepatic microsome. Therefore, using berberine as a health supplement or an alternative medication might provide extra-benefit due to its inhibitory regulation on CYP1A expression and anti-lipid peroxidation activity.

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1. Introduction

CYP1A1 and CYP1A2 are the most important human CYP enzymes in the metabolic activation of polycyclic aromatic hydrocarbons (PAHs) [1-3]. In most cases, oxidation of PAHs by CYP1A enzyme is an initial step in the activation of carcinogenesis [4]. CYP1A1 variants and cancer risk have been investigated in several studies [5], while CYP1A2 is the key enzyme in the metabolic activation of heterocyclic amine to DNA binding form [6]. There was evidence that showed high activity of CYP1A2 increased risk of lung cancer [7]. CYP1A1 was also inducible by cigarette smoke condensate, subsequently increase risk of oral squamous cell carcinoma [8]. Therefore, the inducible and constitutive expression of CYP1A is considered to be an important factor of carcinogenesis.

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. Several free radicals lead to a majority of disease conditions such as atherosclerosis, hypertension, ischaemic diseases, Alzheimer's disease, Parkinsonism, cancer, diabetes mellitus, and inflammatory conditions [9]. Lipid peroxidation, a reaction caused by reactive oxygen species (ROS), is involved in the processes of several organ disorders [10]. The liver is an important organ which is at high risk for damage from lipid peroxidation, since the liver is a major organ responsible for the metabolism of drugs and toxic chemicals, and the liver is the primary target organ for nearly all toxic chemicals [11]. A natural antioxidant could be an alternative hepato-protective agent by scavenging free radicals and other ROS [12].

Berberine (Fig. 1), a natural isoquinoline alkaloid, is a major active constituent in several plants such as *Coscinium fenestratum*, *Berberis aristata*, *Coptis japonica*, and *Coptis chinensis* [13,14]. Berberine possesses a wide range of biochemical and pharmacological activities including anti-inflammation [15], anti-microorganism [16], and anti-cancer activities [17-19]. In addition, it has showed protective activities to acetaminophen or CCl_4-induced hepatotoxicity [20], to DNA cleavage-induced by hydrogen peroxide [21]. Berberine also inhibited cyclooxygenase-2 transcriptional activity in human colon cancer cells

Abbreviations: B, berberine chloride;BNF, beta-naphthoflavone;CYP, cytochrome P450;CYP1A, cytochrome P450 subfamily 1A;DPPH, α,α′-diphenyl-β-picrylhydrazyl radical;DOPC, dioleoyl phosphatidylcholine; MDA, malondialdehyde;PAHs, polycyclic aromatic hydrocarbons;ROS, reactive oxygen species;TBARS, thiobarbituric acid reactive substances.

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[22], and improved insulin sensitivity in insulin resistant animals [23]. Berberine inhibited cholesterol and triglyceride (TG) synthesis [24]. In humans, administration of berberine for 3 months significantly reduced serum cholesterol, TG, and low density lipoprotein (LDL) levels [25]. Recently, berberine was promoted as a novel cholesterol-lowering drug that up-regulated LDL receptor's stabilization via the extracellular signal-regulated kinase (ERK) signaling pathway [26]. Several pharmacological activities of berberine have been already well-established, but studies in the field of cytochrome P450 (CYP) are still less, especially cytochrome P450 family 1A (CYP1A). Administration of berberine has decreased CYP total contents and inhibited CYP2E1 activities in rat microsome [27]. Moreover, berberine was demonstrated to inhibit activities of CYP3A4, CYP2C and CYP2D in human microsomes [28]. There was only a report of the effect of berberine on CYP1A expression in some cell lines, but its effect on typical CYP1A inducer-related regulation was not established. Recently, berberine is very popularly used as a health supplement, and available in drug stores and on-line markets. It is claimed to support healthy intestinal environment, healthy blood pressure, and cholesterol health. From clinical trials, berberine was a potential compound to be as hypoglycemic and hypolipidemic drugs [25,29]. The study of Vrzal et al. [30] demonstrated berberine as a potent inhibitor of CYP1A1 in human HepG2 hepatoma cells. Berberine showed inhibitory activity on CYP1A2 in microsome from diethylnitrosamine plus phenobarbital treated rats [27] and possessed antioxidative activity in DPPH and DOPC liposomal membrane test [31], anti-lipid peroxidation in rat brain [9], and preventive effect of CCl_4-induced liver damage in rats [20].

In the present study, the protective effects of berberine against BNF-induced CYP1A expression and BNF-induced lipid peroxidation in the liver were investigated. Success of this study might lead to a useful indication or extra-benefit of using berberine as a health supplement or an alternative medication.

2. Materials and methods

2.1. Chemicals

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, Sweden) and Sigma Chemical (St. Louis, MO), respectively.

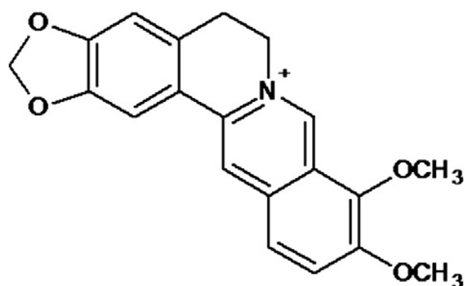

Fig. 1. Structure of berberine (B).

Berberine chloride (B), beta-naphthoflavone (BNF), malondialdehyde (MDA), thiobarbituric acid (TBA), ethoxyresorufin, and methoxyresorufin were supplied by Sigma-Aldrich Chemical (St. Louis, MO). ReverTraAce was a product of Toyobo (Osaka, Japan). SYBR® Green I and *Taq* DNA polymerase were products of Lonza (Basel, Switzerland) and Invitrogen™ (Carlsbad, CA), respectively. The TaqMan® Gene Expression Assays and TaqMan® Gene Expression Detection kit were products of Applied Biosystems (Foster city, CA). All other laboratory chemicals were of the highest purity available from commercial suppliers.

2.2. Preparation and treatment of primary cultures of mouse hepatocytes

The liver of male C57BL/6 mice (Sankyo Laboratories, Shizuoka, Japan) at 7 weeks of age was perfused with collagenase, and viable hepatocytes were isolated by Percoll isodensity centrifugation as described [32]. Standard culture conditions were as follows: the cells were dispersed in Waymouth MB752/1 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 at a density of 5×10^5 cells/1.5 ml/35-mm collagen-coated dish. The Waymouth medium did not contain phenol red, a pH indicator, to exclude the possibility of estrogen-like action. The hepatocytes anchored to the collagen-coated dishes within 3 h and subsequently formed a monolayer. The culture dishes were maintained at 37 °C in a 5% CO_2-humidified incubator. The medium was renewed 3 h after plating and then, 24 h later, 1, 5, and 10 μM berberine (B) and/or 10 μM beta-naphthoflavone (BNF) were added after a change of medium. Measurements of lactate dehydrogenase activity in medium indicated it to be non-toxic at these concentrations, compared to 0.2% DMSO as a control and 0.3% Tween 20 as a positive control (100% cytotoxicity). Total RNA was prepared from the cells at 24 h after treatments [33].

2.3. Animals

Male C57BL/6 mice were supplied by National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. All mice were housed in the Animal Unit of Faculty of Pharmaceutical Sciences, Khon Kaen University. Mice were treated according to the research protocol approved by Khon Kaen University's Animal Care and Use Committee (AEKKU 06/2553). At all times, the mice were housed on wood shaving bedding in stainless steel cages, with water and a commercial inbred mouse diet supplied ad libitum. The mice quarters were air conditioned (22–25 °C) and had a 12-h light/dark cycle. Seven week-old mice were orally fed daily with 7.5 mg/kg/day of berberine (B) for 7 days, and/or intraperitoneally given 30 mg/kg/day of beta-naphthoflavone (BNF) in the last 3 days. These compounds were dissolved in corn oil. The control group was orally given corn oil daily for 7 days to assure non-significant change of CYP1A expression. The mice were sacrificed 24 h after the last treatment and the livers were excised immediately for preparation of total RNA and hepatic microsomes [33].

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