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In vivo effect of borneol on rat hepatic CYP2B expression and activity

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ABSTRACT

CYP2B subfamily accounts for 2–10% of total hepatic CYP450 enzymes and participate in the metabolism of around 8% of clinical drugs. Borneol has been widely used in traditional Chinese medicine for thousands of years. There are many studies about borneol-induced promoting penetration role for a number of drugs through various physiologic barriers, whereas there is no report involved the effect of borneol on hepatic CYP2B. The present work studied the *in vivo* effect of borneol on the expression and activity of rat hepatic CYP2B. The results indicated that the oral administration of borneol (33, 100 and 300 mg/kg/d) to rats for consecutive 7 days increased the hepatic CYP2B1/2 activity by 1.4-, 1.7- and 2.8-fold, hepatic CYP2B1 mRNA expression by 6.3-, 8.7- and 18.1-fold, and hepatic CYP2B1/2 protein expression by 1.2-, 1.9- and 2.6-fold, respectively compared to the control. Additionally, in the borneol pre-dosing (300 mg/kg/d for consecutive 7 days) rats, the increased Cl_{int} and decreased AUC_{0-24} of bupropion were observed as compared to the control. Moreover, there were no obvious effects on CAR protein level in rat liver microsome and nucleus following the borneol treatment. Taken together, our observations indicate that borneol is an *in vivo* inducer of rat hepatic CYP2B with different regulatory mechanism from phenobarbital-like inducers which caused CYP2B induction with CAR activation.

1. Introduction

The CYP2B subfamily is expressed primarily in the liver and regulated by the xenobiotic receptors constitutive androstane receptor (CAR) and pregnane X receptor (PXR) [11,24,36]. CYP2B6 metabolizes as much as 8% of clinical drugs including the inactivation of methadone, bupropion, meperidine, propofol, efavirenz, ketamine and tamoxifen, and the bioactivation of cyclophosphamide [27,43]. It also metabolizes drugs of abuse (e.g. nicotine, cocaine, ecstasy and phencyclidine), as well as some important endogenous neuro-substrates serotonin and testosterone, and neurotoxins chlorpyrifos [34]. The CYP2B subfamily is susceptible to induction and inhibition after exposure to particular chemicals [9,35]. The induction of hepatic CYP2B has been shown to be associated with liver tumor formation in rodents [7,13]. These findings make great interest for toxicologists and pharmacologists to study CYP2B inducer and the underlying mechanisms [3,30–32,37].

The natural borneol (D-borneol) has been widely used in traditional Chinese medicine (TCM) for thousands of years. Nowadays, the synthetic borneol (including D-borneol and isoborneol) is predominately used in TCM and cosmetics to replace natural borneol. There are many Chinese patent medicine (CPM) containing borneol recorded in Chinese Pharmacopoeia, such as Compound Danshen dripping pills and Suxiaojiuxin pills (both mainly used in the treatment of coronary heart disease and angina in TCM), Xingnaojing injection (mainly used in the treatment of epidemic encephalitis b and hepatic coma in TCM), etc. As reported, borneol can obviously loosen the intercellular tight junction, inhibit the function of *P*-glycoprotein (*P*-gp) on cell membrane, increase the fluidity of membrane and influence the permeability of bilayer lipid membrane [10,12,41]. Many studies show that borneol can act as a penetration enhancer for a number of drugs through various physiologic barriers such as skin [5], brain [2,38], mucous membrane [23,44], intestinal epithelial membrane [6,19], ocular–blood barrier [22,33,40], blood–optic nerve barrier [16]. However, few studies examined the effect of borneol on the activity of hepatic cytochrome P450 (CYP) enzymes [4,42]. Nowadays, the co-administration of CPM containing borneol with other drugs is very common in clinical application of TCM. Therefore, the borneol-induced drug interaction should be an important issue to avoid the undesired adverse effects in patients. The purpose of the present

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work was to study the *in vivo* effect of borneol administered intragastically on the expression and activity of CYP2B in rat liver.

2. Materials and methods

2.1. Chemicals and reagents

Synthetic borneol (Batch number 10158762, purity > 98%) was purchased from Alfa Aesar (Tianjin, China). Bupropion was purchased from Cayman Chemical Company (Michigan, USA). Hydroxybupropion was supplied by Cerilliant (Texas, USA). Heparin sodium salt, acrylamide and Bis-acrylamide were supplied by Biosharp (Hefei, China). Reduced nicotinamide adenine dinucleotide phosphate (NADPH), cOmplete Tablets EDTA-free and EASYpack were obtained from Roche Co. (Basel, Switzerland). BCA protein assay kit and NE-PER nuclear and cytoplamic extraction reagents were purchased from Thermo Scientific (Rockford, IL, USA). HPLC-grade ethyl acetate, methanol and acetonitrile were the products of TEDIA (Ohio, USA). TRIzol reagent was obtained from Invitrogen (California, USA). THUNDERBIRD™ SYBR® qPCR Mix and ReverTra Ace qPCR RT Kit were obtained from TOYOBO (Osaka, JPN). CFX Connect™ real-time system was purchased from BIO-RAD (California, USA). Mouse anti-β-Actin monoclonal antibody and rabbit anti-CAR polyclonal antibody were supplied by Santa Cruz (Texas, USA). Mouse anti-CYP2B1/2 monoclonal antibody was supplied by Abcam (Massachusetts, USA). Rabbit anti-Lamin B polyclonal antibody was perchased from Boster (Wuhan, China). Goat anti-rabbit/mouse IgG conjugated with horseradish peroxidase were the products of KPL Inc. (Maryland, USA).

2.2. Animals

Male Wistar rats (200–250 g) were purchased from the disease prevention and control center of Hubei province and were maintained in SPF animal room at temperature 22 ± 2 °C, 60 ± 5% humidity and 12/12 h day/night cycle for 7 days to acclimate laboratory conditions. Prior to dosing, rats were fasted for at least 12 h and had free access to water and regular rat chow. All procedures were approved by Ethic Committee of Hubei University, and complied with health guidelines for the care and use of laboratory animals.

2.3. Drug treatment for the expression of hepatic CYP2B and CAR

Rats were randomly divided into 4 groups with six rats in each group to receive various administrations. In Group 1–3, rats were orally administered with 33, 100 and 300 mg/kg/d borneol (suspended in 0.5% CMC-Na solution), respectively. In Group 4 (control group), rats were orally administered with isopyknic 0.5% CMC-Na solution. The oral dosage of borneol used in this work was selected according to the dosage of borneol (240mg/60 kg^{-1} d^{-1}) for human [26] and the literature [41]. After administration of borneol for consecutive 7 days, rats were killed by decapitation 1 h after the last treatment. The liver was immediately removed and stored in liquid nitrogen until use.

The real-time PCR analysis was performed as follows: total RNA was extracted from each liver sample (100 mg) using TRIzol reagent according to the manufacturer's protocol. RNA (2 μg) sample from each liver sample was reverse transcribed to cDNA using ReverTra Ace qPCR RT Kit. cDNA sample was amplified by real-time fluorescence quantitative PCR for 38 cycles (denaturation at 94 °C for 15s, annealing at 58.3 °C for 30s and extension at 72 °C for 30s). The PCR primers for rat *GAPDH* were 5′-AGGGCTGCCTTCTCTTGTGAC-3' (forward) and 5′- TGGGTAGAATCATACTGGAACATGTAG-3' (reverse), for rat *CYP2B1* were 5′-AAGCACAGGGCCACCTTAGAC-3' (forward) and 5′- CATCAGCAGGAAACCATAGCG-3' (reverse). The relative mRNA expression levels of the target cDNA were normalized to the *GAPDH* expression by the $2^{-\Delta\Delta Ct}$ method. All experiments were repeated in triplicate.

The Western blotting analysis was performed as follows: the microsomal protein of rat liver was extracted from 100 mg of each liver sample using RIPA, and the nuclear protein of rat liver was extracted from 20 mg of each liver sample using NE-PER Nuclear and Cytoplamic Extraction Reagents. Protein (70 μg) was separated by SDS-PAGE and transferred onto polyvinylidene fluoride membranes. The membranes were incubated with the primary antibody (mouse anti-β-actin monoclonal antibody, mouse anti-CYP2B1/2 monoclonal antibody, rabbit anti-CAR polyclonal antibody and rabbit anti-Lamin B polyclonal antibody, respectively) over night. The immunoblot signals were examined using goat anti-rabbit/mouse IgG conjugated with horseradish peroxidase and visualized using an enhanced chemiluminescence detection kit.

The CYP2B1/2 activity of rat liver was determined as follows: rat liver microsome (RLM) was prepared by $CaCl_2$ precipitation method. Bupropion was used as CYP2B1/2 probe substrate and the production of its metabolite hydroxybupropion in RLM was used to evaluate CYP2B1/2 activity. Incubations were performed at 37 °C for 20min in 200 μL potassium phosphate buffer (0.1 M, pH 7.4) containing 5 mM $MgCl_2$, 0.5 mg/mL RLM, 1 mM NADPH and 75 μM bupropion. After pre-incubation (without NADPH in incubation medium) at 37 °C for 3 min, the incubation reaction was started by the addition of NADPH, and terminated by the addition of 2-fold volume ice-cold acetonitrile. The sample was then vortex-mixed and centrifuged at 12, 000 g for 10min. The supernatant was evaporated by freeze drying. The residue was dissolved in 500 μL HPLC mobile phase and the content of hydroxybupropion was determined by LC-MS/MS method.

2.4. Drug treatment for the in vivo pharmacokinetics of bupropion

Rats were randomly divided into 2 groups with six rats in each group. In Group 1 (borneol pretreatment group), rats were orally administered with 300 mg/kg/d borneol (suspended in 0.5% CMC-Na solution) for consecutive 7 days. In Group 2 (control group), rats were orally administered with isopyknic 0.5% CMC-Na solution for consecutive 7 days. After 1 h of the last treatment, rats in both groups were orally administered with a single dose of bupropion (15 mg/kg). The blood samples (0.3–0.4 mL) were obtained *via* the oculi chorioideae vein at the indicated times and centrifuged at 4000 rpm for 10min. 100 μL serum was vortex-mixed with 200 μL acetic ether for 3min and centrifuged at 12,000 rpm for 10 min. An aliquot of 100 μL of the supernatant was evaporated by freeze drying. The residue was dissolved in 500 μL HPLC mobile phase and hydroxybupropion was determined by LC-MS/MS method. Pharmacokinetic parameters were calculated with DAS 3.0 software (Chinese Mathematical Pharmacology Society) using non-compartmental model.

2.5. Quantification for hydroxybupropion and bupropion

The LC-MS/MS system included LC-20AD parallel pump, SIL-20A autosampler, DGU-20A3R degssing unit, CTO-20A column oven and MS-8040 spectrometer (Shimadzu), was used in the present work. Chromatographic separation was carried out on a Shimadzu VP-ODS column (2.0 mm × 150 mm, 4.6 μm) fitted with a Shimpack guard column (2 mm × 5 mm, 4. 6 μm. The mobile phase was consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile) at the gradient program as follows: 20% B as initial mobile phase; 0–5.5 min, linear gradient from 20 to 68% B; 5.5–6.0 min, isocratic elution with 68% B; 6.0–11.0 min, isocratic