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The mechanism of the opening of the blood–brain barrier by borneol: A pharmacodynamics and pharmacokinetics combination study

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ABSTRACT

Ethnopharmacological relevance: Borneol is widely used in traditional Chinese medicine to facilitate the distribution of central nervous system (CNS) drugs in brain due to its ability to open blood–brain barrier (BBB), however, the underlying mechanism is still unclear. In this study, the effect of borneol on different brain regions were investigated to explore the mechanism.

Materials and methods: After oral administration of borneol (0.1, 0.2 g/kg) for seven consecutive days, SD rats were injected with Rh123 (1.0 mg/kg). The concentrations of Rh123 were detected in four brain regions of cortex, hippocampus, hypothalamus and striatum by a small animal vivo imaging system and a fluorescence microplate reader respectively. The ultrastructures of BBB were examined. Moreover, the expressions of the four transporters of ATP-binding cassette (ABC) family, multidrug resistance 1a (Mdr1a), multidrug resistance 1b (Mdr1b), multidrug resistance protein 1 (Mrp1), Mrp4, Mrp5 and breast cancer resistance protein (Bcrp) in the four brain regions were analyzed. Finally, the deliveries of borneol in the plasma and the four brain regions were examined by a pharmacokinetics study.

Results: Administration of 0.2 g/kg borneol produced loose structure in the tight junction and void structure between the endothelial cell and mesangial cell. Borneol at 0.1 g/kg and 0.2 g/kg increased the delivery of Rh123 in hippocampus and hypothalamus obviously. Permeability index followed a similar trend. Protein expression assays showed that borneol decreased the expression of Mdr1 and Mrp1 in hippocampus and hypothalamus. Further RT-PCR study showed that borneol decreased the expressions of both Mdr1a and Mdr1b in hippocampus and hypothalamus. The pharmacokinetics study demonstrated that the delivery of borneol in cortex was the most and that in striatum the least, with the deliveries of borneol in hippocampus and hypothalamus in between.

Conclusions: Borneol showed tissue specific BBB-opening effect, which was associated with its regulation of the ultrastructure of brain tissues and the expressions of Mdr1a, Mdr1b and Mrp1. The present study indicated that borneol should be used in concert with drugs targeting hippocampus or hypothalamus to exert its synergistic effect to the maximum.

1. Introduction

Blood–brain barrier (BBB) plays vital role in controlling the exchange of endogenous and exogenous substances between blood and brain to maintain the homeostasis and normal function of central nervous system (CNS) (Von Wedel-Parlow et al., 2009). BBB is mainly formed by brain capillary endothelial cells, void of fenestration, of low pinocytosis and tight junctions, which inhibits transcellular passage of molecules across the barrier and restrict the paracellular diffusion of hydrophilic molecules through endothelial junctions (González-Mariscal et al., 2003; Golden and Pollack, 2003). ATP-binding cassette (ABC) proteins, including *P*-glycoprotein (*P*-gp), multidrug resistance-associated proteins (Mrps) and the breast cancer resistance proteins (Bcrp), have been verified to contribute to the function of the BBB in preventing the influx of agent from the blood into the brain and facilitate the efflux of compounds from the brain into the blood. BBB is beneficial for the stabilization of the internal environment of nerve cells, but against the permeation of drugs targeting the brain, affecting the theraputic efficacy of many CNS illness.

Borneol is a simple bicyclic monoterpene (Fig. 1) from resin of *Dryoblanops armarica Gaertn.f*, and according to the theory of traditional Chinese medicine, can direct drugs upward to head targeting the brain (Liu et al., 1994). It has been frequently found in many traditional chinese compound medicine in clinic for the treatment of CNS illness, such as Alzheimer disease, stroke,

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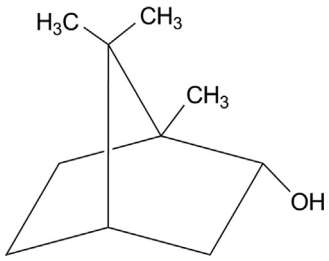

Fig. 1. Chemical structure of borneol.

cerebral ischemia, cerebritis and cerebral edema (Hong et al., 2011; Zheng et al., 2013; Zhang et al., 2012; Lin et al., 2006). Recent studies also demonstrated that borneol assisted the permeation of drugs across BBB and enhanced their distribution in the brain tissue (Yu et al., 2011; Chen et al., 2010; Cai et al., 2008).

Generally, BBB does not occur uniformly in all parts of the brain. Some parts around the ventricles are accessible to vital dyes and electron-dense tracers. These areas include the area postrema, median eminence, subcommissural organ, pineal gland, subfornical organ, supraoptic crest and neurohypophysis (Pritchard and Alloway, 1999; Gilgun-Sherki et al., 2001). Cortex, hippocampus, hypothalamus and striatum are always the brain-targeting areas of CNS disorder (Riceberg and Shapiro, 2012; Wimmer and Shohamy, 2012; Kokoeva et al., 2005; Dahlin et al., 2008). We had found that borneol increased the permeability of geniposide in hippocampus and hypothalamus regions while showed no change in cortex and striatum regions by a microdialysis-UPLC–MS technique (Yu et al., 2013). The result indicated that borneol had different effects in the four brain regions, and interestingly, that borneol, in a low dose range of 0.05–2.0 g/kg, increased the delivery of geniposide in the rat brain, but, in a high dose increasing from 2.0 g/kg to 4.0 g/kg, decreased the brain delivery of geniposide obviously (Dong et al., 2012). These results indicated the importance of brain borneol concentration in its BBB-opening effect. To explore the BBB-opening mechanism of borneol, the relationship between the BBB-opening effect of borneol in the four brain regions and its delivery diversity is investigated in this study by inspection of the ultrastructure of BBB and determination of the expression of ABC transporters in the above four regions.

2. Materials and methods

2.1. Materials

Healthy male Sprague Dawley rats (180–220 g) were purchased from Laboratory Animal Center of Nanjing University of Traditional Chinese Medicine (Nanjing, China). Rats were housed in SPF housing facility with the dust (>0.5 μm) less than $3.52\times10^5/m^3$ under the temperature of 25 °C and humidity at 55%. The study was performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Experimental Animal Management Committee of Jiangsu province.

Borneol was purchased from Nanjing Pharaceutical Co., Ltd (Nanjing, China). Verapamil tablet was purchased from Shanghai Sine Pharmaceutical Co., Ltd (Shanghai, China). Rh123 was purchased from Sigma-Aldrich company (St. Louis, Missouri, USA). The standard substance of borneol (purity > 98%) and naphthalene (internal substance, IS, purity > 98%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Saline solution and other HPLC-grade reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

2.2. Methods

2.2.1. Effects of borneol on ultrastructure and P-glycoprotein function in the cortex, hippocampus, hypothalamus and striatum

2.2.1.1. Animal grouping and drug treatment. SD rats were randomly divided into four groups of 10 rats each, i.e. control group, verapamil group (25 mg/kg, ig) and borneol groups (0.1, 0.2 g/kg, ig). Borneol and verapamil were given orally once a day and the control group was treated with physiological saline at the volume of 10 mL/kg.

2.2.1.2. Sample preparation. Seven days after drug treatment, the rats were injected with Rh123 (1.0 mg/kg) via jugular vein after they were anesthetized by chloral hydrate (0.3 g/kg, ip). Fifteen minutes after the injection, blood were drawn from retrobulbar venous plexus with a capillary glass tube before they were sacrificed by decapitation, and centrifuged at 4000 rpm for 10 min. The obtained plasma samples were stored in −80 °C before use. Rat brains were taken out immediately after their decapitation, washed by physiological saline and dried by a filter paper. The cortex, hippocampus, hypothalamus and striatum were separated by a brush and a surgical knife.

2.2.1.3. Ultrastructure examination. A small piece (< 1 cubic millimeter) of brain tissue was incised from the four regions, and fixed in 2% glutaraldehyde (0.1 M phosphate buffer, pH 7.4) for 3 h, and osmicated for 1 h at 4 °C with 1% OsO_4 and 0.8% potassium ferricyanide using the same buffer. The section was dehydrated in a graded series of acetone and finally embedded in Epon 812 epoxy resin and then was sliced into ultrathin sections. After stained with uranyl acetate and lead citrate, it was examined using a H7650 transmission electron microscope (Hitachi, Japan).

2.2.1.4. Determination of P-gp function. The fluorescence intensity of Rh123 in brain tissue was detected and photographed by a small animal vivo imaging system (λex/λem = 505 nm/533 nm) (Carestream DXS PRO, Rochester, USA). The fluorescence value was obtained by Carestream Molecular Imaging software.

After weighing, the brain tissue was homogenized with ultra-pure water (5 mL/g) using a XHF-D tissue homogenizer (Scientz Biotech Co., Ltd., Ningbo, China). The brain homogenate was centrifuged at 12000 rpm for 10 min at 4 °C after vortex-mixing for 1 min. The supernatant (200 μL) was collected to determine the fluorescence optical density (FOD) value of Rh123 by a Synergy HT UV-fluorescence microplate reader (λex/λem = 505 nm/533 nm) (BioTek Instruments, Winooski, Vermont, USA). The concentration of Rh123 was calculated according to the constructed concentration-FOD standard curve for Rh123. Similarly, the concentration of Rh123 in plasma was determined. The BBB permeability index (Kp) was calculated by the concentration ratio of Rh123 in each brain regions and that in plasma to evaluate the permeability of BBB (He and Ji, 2008).

The brain homogenate calibration curves were constructed by plotting the FOD of Rh123 against the Rh123 concentration in the blank supernatant from homogenized cortex, hippocampus, hypothalamus and striatum tissue, respectively. The concentrations of standard solution were ranging from 0.010276 to 0.5138 ng/g.

The plasma calibration curve was constructed by plotting the FOD of Rh123 against the Rh123 concentration (0.5138–51.38 ng/mL) in the blank plasma.

2.2.2. Effect of borneol on the expressions of Mdr1, Mrp1, Mrp4, Mrp5 and Bcrp in cortex, hippocampus, hypothalamus and striatum

2.2.2.1. Animal grouping and drug treatment. SD rats were randomly divided into three groups of 10 rats each, i.e. control group and borneol groups (0.1, 0.2 g/kg, ig). The administration method was the same as "2.2.1.1" above. Seven days later, the rats