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

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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 was 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 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.

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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 therapeutic efficacity 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,
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 neurohypophys (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 × 10^4/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 Pharmaceutical Co., Ltd (Nanjing, China). Verapamil tablet was purchased from Shanghai Sine Pharmaceutical Co., Ltd (Shanghai, China). Rh123 was purchased 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% OsO4 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 (Lexi/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 (Sientz 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

Fig. 1. Chemical structure of borneol.
were killed by cervical dislocation and their cortex, hippocampus, hypothalamus and striatum were collected immediately. The expressions of Mdr1, Mrp1, Mrp4, Mrp5 and Bcrp in these tissues were assayed by the following methods.

2.2.2.2. Immunofluorescence histochemistry assay. The brain tissues were fixed overnight at 4 °C in 3% paraformaldehyde solution in PBS and then equilibrated in 30% sucrose for 72 h and were cryoprotected. After freezing at −80 °C for 24 h, the tissues were cut into slices of 4–8 μm thickness and mounted on a glass slices. The sections were rinsed three times with PBS, immersed in 5% normal goat serum PBS for 1 h to block the potential nonspecific binding sites and incubated overnight at 4 °C with antibodies, i.e. rabbit anti-Mdr1 (dilution 1:200, Wuhan Boster Bio-engineering Co., Ltd., Wuhan, China), rabbit anti-Mrp1 (dilution 1:200, Wuhan Boster Bio-engineering Co., Ltd., Wuhan, China), rabbit anti-Mrp4 (dilution 1:600, Abcam PLC, Cambridge, England).

Fig. 2. Ultrastructures of brain regions (A)–(D) represented cortex, striatum, hippocampus and hypothalamus respectively; 1–4 represented control, borneol 0.1 g/kg, borneol 0.2 g/kg and verapamil 25 mg/kg, respectively; The black arrows showed the tight junctions and the red stars showed the void structures appeared between the endothelial cell and mesangial cell).
UK), goat anti-Mrp5 (dilution 1:200, Santa Cruz Biotechnology, Santa Cruz, USA) and rabbit anti-Bcrp (dilution 1:200, Wuhan Boster Bio-engineering Co., Ltd., Wuhan, China).

The sections were rinsed with PBS for 10 min, and incubated at 37 °C for 2 h with FITC-conjugated goat anti-rabbit (dilution 1:200, Jackson ImmunoRes. PA, USA) or rabbit anti-goat (dilution 1:50, Wuhan Boster Bio-engineering Co., Ltd., Wuhan, China) antibodies. Finally, the sections were washed with PBS, air-dried, and observed under an IX71 fluorescence microscope (Olympus, Tokyo, Japan). The fluorescence expression data of Mdr1, Mrp1, Mrp4, Mrp5 and Bcrp were analyzed by an Image pro plus 7.0 software (Media Cybernetics, Inc., Warrendale, PA, USA).

2.2.2.3. Western blot assay. After the homogenization of brain tissues at 4 °C with 1 mM EDTA and 2.5 mL cell lysate, the proteins (70 μg) were separated by SDA-PAGE and transferred to positively charged nylon membranes (Pall corporation, NY, USA). The blots were probed overnight at 4 °C with the antibodies of rabbit anti-Mdr1, rabbit anti-Mrp1, rabbit anti-Mrp4, goat anti-Mrp5 and rabbit anti-Bcrp, respectively. Goat anti-rabbit (for Mdr1, Mrp1, Mrp4 and Bcrp) or rabbit anti-goat (for Mrp5) IgG-HRPs were used as secondary antibodies (1:5000; KeyGEN Biotech, Nanjing, China) and the blots were washed with TBST. Signals were detected by an enhanced chemiluminescence detection system (Millipore, St. Louis, MO, USA) and analyzed by Image pro plus 7.0 software. Targeted bands were normalized to GAPDH (1:3000; KeyGEN Biotech, Nanjing, China) to ensure equal protein loading.

2.2.2.4. RT-PCR assay. Total RNA of each brain region tissue (20 mg) was isolated using a TRIzol reagent according to the manufacturer’s instruction (Invitrogen, Carlsbad, CA, USA). Total RNA (4 μg) from each sample was reverse-transcribed into cDNA with a RevertAid first strand cDNA synthesis kit (Fermentas, Lithuania) based on the manufacturer’s protocol. The following primers were used: Mdr1a (321 bp), forward primer: 5′-AACTATCCCACCCGACCCAA-3′, reverse primer: 5′-GGCTGCCTTCACGATCTCCT-3′; Mdr1b (316 bp), forward primer: 5′-CAGCCGTGTCGTGTCTCAT-3′, reverse primer: 5′-GTCTGCGTTCTGGATGGTG-3′; Mrp1 (243 bp), forward primer: 5′-AGGTGCTATTGTGGCTCCAT-3′, reverse primer: 5′-GACCCATCCAGCTTCTCATC-3′; Mrp4 (214 bp), forward primer: 5′-GAGTCATTCGCCGACCTCAT-3′, reverse primer: 5′-CAACCAGACAGCATTGCT-3′; Mrp5 (313 bp), forward primer: 5′-GGGAAACGAGTGTATGC-3′, reverse primer: 5′-CCCTGACGAGCCACCTTGTG-3′; Bcrp (285 bp), forward primer: 5′-TTGGTGCCCTTTACTTTGTGTC-3′, reverse primer: 5′-CCACCGTCCTCTCATACTGTA-3′; Gapdh

Fig. 3. Effect of borneol on the P-gp functions in the four brain regions by a small animal vivo imaging system assay (mean ± SD, n = 10). The content of Rh123, a fluorescent substance, was inversely correlated with the function of P-gp. *P < 0.05 and **P < 0.01, compared to the control group.
(352 bp): forward primer 5′-AAGGTCGGTGAGAAGGATT-3′; reverse primer 5′-AGATGACCCCTTTGCCC-3′. The PCR reaction was carried out as followed: 5 min at 95 °C, 40 s at 95 °C, 35 s at 55 °C, 45 s at 72 °C and 10 min at 72 °C. The samples were amplified 32 cycles. PCR products were electrophoresed on a 1% agarose gel and the bands were analyzed by BandScan 4.3 software (Glyko, Novato, USA) to achieve their gray values. Then, the ratios of Mdr1a/Gapdh, Mdr1b/Gapdh, Mrp1/Gapdh, Mrp4/Gapdh and Mrp5/Gapdh

Fig. 4. Effects of borneol on the concentration of Rh123 in the four brain regions by a fluorescence microplate reader assay (mean ± SD, n = 10). *P < 0.05 and **P < 0.01, compared to the control group.
for each group and each brain region were calculated to represent the expressions of the ABC transporters.

2.2.3. Research on the delivery of borneol in the rat brain regions of cortex, hippocampus, hypothalamus and striatum

2.2.3.1. Instrument and chromatographic conditions. GC assay was performed on a 4890D GC-FID system (Agilent Technologies, USA) consisted of a gas chromatographic system, a FID and an Agilent cerity software for data analysis. Separation of borneol and IS was achieved on an HP-INNOWax capillary column (30 m x 0.53 mm, 1.0 μm). Nitrogen was used as the carrier gas with pre-column pressure at 10 psi. The oven temperature was maintained at 140 °C. The injection port temperature was 220 °C. The detector temperature was 250 °C. Samples of 2 μL were injected manually with split mode.

2.2.3.2. Method validation. Method validation was done by evaluating a series of method-performance characteristics, such as selectivity, linearity, limit of quantification (LOQ), precision, accuracy, stability and recovery according to the criteria suggested by the USFDA (2001).

2.2.3.3. Pharmacokinetics study of borneol in rat plasma and four brain regions. Fifty five male SD rats (180–220 g) were divided randomly into eleven groups of five rats each. After oral administration with 0.2 g/kg borneol, the rats were killed by decapitation and blood samples (1 mL) were collected into a heparinized centrifuge tube by picking off eyeballs at time intervals of 1, 3, 5, 10, 15, 30, 45, 60, 90, 120, 180 min. Simultaneously, the brain was removed and rinsed by pure water. After the brain was dried with filter paper, tissues of cortex, hippocampus, hypothalamus and striatum were separated from the whole brain by a brush and a surgical knife.

The blood was added into 20 μL of IS solution (0.1 mg/mL of naphthalene dissolved in dichloromethane) and centrifuged at 5000 rpm for 10 min. The supernatant was added with 1 mL n-hexane, vortex-mixing for 1 min, then the mixture was centrifuged at 12000 rpm for 10 min at 4 °C. The supernatant was collected and evaporated to 50 μL under nitrogen flow, stored at −80 °C before use.

The brain tissue was homogenized with pure water and then mixed with 20 μL of IS (0.1 mg/mL). After centrifugation at 5000 rpm for 10 min, the supernatant was added with 1 mL n-hexane and

![Fig. 5. Effects of borneol on the Kp of Rh123 in the four brain regions by a fluorescence microplate reader assay (mean ± SD, n = 10). *P < 0.05 and **P < 0.01, compared to the control group.](image-url)
vortex-mixed for 1 min, the mixture was centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was collected and evaporated to 50 μL under nitrogen flow, stored at −80 °C before use.

2.2.4. Statistical analysis
The data were presented as mean ± SD. A two-tailed, two sample t-test was used for difference assessment among groups which assumed equal variance, and p < 0.05 was considered statistically significant.

3. Results

3.1. Effects of borneol on the ultrastructure of BBB
In the four brain regions of the control group, the tight junction between capillary endothelial cells was clearly visible (Fig. 2A1–D1). With the administration of borneol, the tight structure was not obviously influenced except for hippocampus where the tight junction was enlarged by 0.2 g/kg of borneol (Fig. 2C3). Moreover, some void structures appeared between the endothelial cell and mesangial cell, in hypothalamus region treated with 0.2 g/kg of borneol (Fig. 2D3).

The number of pinocytotic vesicle did not show obvious increase in each brain region of the two borneol groups. Verapamil treatment showed no obvious effect on the ultrastructure of BBB (Fig. 2A4–D4).

3.2. The effect of borneol on the P-gp function in the four brain regions
Fig. 3 showed the assay results of small animal vivo imaging system. After oral administration of borneol (0.1 g/kg and 0.2 g/kg), the delivery of Rh123 in hippocampus and hypothalamus increased obviously (vs control group, P < 0.05, 0.01). Cortex and striatum regions showed no change with the interference of borneol. In addition, verapamil, as a positive control, enhanced the content of Rh123 significantly in cortex, hippocampus and hypothalamus regions (vs control group, P < 0.01).

Fig. 4 showed the result of fluorescence microplate reader assay. For brain homogenate, the calibration curves were expressed as $y = 12480x + 1071.3$ ($r^2 = 0.9909$), $y = 10653x + 1273.4$ ($r^2 = 0.9946$), $y = 11286x + 1040.6$ ($r^2 = 0.9915$) and $y = 11797x + 1014.6$ ($r^2 = 0.9986$) in cortex, hippocampus, hypothalamus and striatum, respectively. For plasma, the calibration curve was expressed as $y = 1911.4x + 3253.9$.
The results above indicated that FOD value showed a line relationship with the concentration of Rh123 in the matrix above. The result of this assay was similar to that of small animal vivo imaging system. Borneol (0.1 g/kg and 0.2 g/kg) obviously increased the delivery of Rh123 in hippocampus and hypothalamus (vs control group, $P < 0.05, 0.01$). Verapamil enhanced the content of Rh123 significantly in cortex, hippocampus and hypothalamus regions (vs control group, $P < 0.01$). In addition, Kp showed the similar trends to the concentration of Rh123, as show in Fig. 5.

The content of Rh123, a fluorescent substance, was inversely correlated with the function of P-gp because Rh123 was the substrate of the latter. Borneol (0.1 g/kg, 0.2 g/kg) showed obvious inhibition on the function of P-gp located in hippocampus and hypothalamus rather than that in cortex and striatum.

### 3.3. Effect of borneol on the expressions of Mdr1, Mrp1, Mrp4, Mrp5 and Bcrp in the four brain regions

3.3.1. Results of immunofluorescence histochemistry assay

Fig. 6 showed the results of immunofluorescence histochemistry assay. With the interference of borneol, the expressions of Mdr1 and Mrp1 decreased significantly in the two brain regions of hippocampus and hypothalamus (vs control group, $P < 0.01, 0.05$). Borneol showed no obvious influence on the expressions of Mrp4, Mrp5 and Bcrp in the two regions (vs control group, $P > 0.05$). In addition, there was no obvious change on the expressions of the five proteins in cortex and striatum (vs control group, $P > 0.05$).

3.3.2. Results of Western blot assay

Fig. 7 showed the results of western blot assay which were similar to those of immunofluorescence histochemistry assay. Borneol significantly decreased the expression of Mdr1 and Mrp1 in the hippocampus and hypothalamus regions (vs control group, $P < 0.01, 0.05$). Borneol showed no obvious influence on the expression of Mrp4, Mrp5 and Bcrp in the two regions (vs control group, $P > 0.05$). Furthermore, there was no obvious change on the expressions of the five proteins in cortex and striatum (vs control group, $P > 0.05$). The images of western blot were showed in Fig. 8.
3.3.3. Results of RT-PCR assay

Fig. 9 showed the results of RT-PCR assay which were similar to those of western blot assay. Borneol significantly decreased the expressions of Mdr1a, Mdr1b and Mrp1 in the hippocampus and hypothalamus regions (vs control group, \( P < 0.01, 0.05 \)), but showed no obvious influence on the expressions of Mrp4, Mrp5 and Bcrp in the two regions (vs control group, \( P > 0.05 \)). Moreover, there was no obvious change on the expressions of the five proteins in cortex and striatum (vs control group, \( P > 0.05 \)). The images of RT-PCR were showed in Fig. 10.

![Fig. 9. Images of the expressions of Mdr1, Mrp1, Mrp4, Mrp5 and Bcrp in the four brain regions by western blot assay (A)-(D) represented the tissue of cortex, striatum, hippocampus and hypothalamus respectively; 1–3 represented control group, borneol 0.1 g/kg group and borneol 0.2 g/kg group, respectively.](image)

![Fig. 10. Images of the expressions of Mdr1a, Mdr1b, Mrp1, Mrp4, Mrp5 and Bcrp in the four brain regions by RT-PCR assay (mean ± SD, \( n = 10 \)). *P < 0.05 and **P < 0.01, compared to the control group.](image)
Fig. 11. Typical chromatograms of borneol and IS ((A)–(E) represented plasma, cortex, striatum, hippocampus and hypothalamus, respectively; 1–3 represented blank samples, standard solutions and samples).
3.4. Delivery of borneol in the rat brain regions of cortex, hippocampus, hypothalamus and striatum

3.4.1. Validation of assay method

Typical chromatograms of blank samples, standard solutions and samples were shown in Fig. 11. The retention times of borneol and IS were 6.94 and 8.52 min, respectively. There was no interference in the detection of the two compounds in the analytes. The calibration curves of borneol in the plasma and four brain regions displayed good linear relationships (Table 1). The LOQ of borneol was 50 ng/mL. The intra- and inter-accuracies were within 8.7%. The intra- and inter-day precisions were within 9.89%, as shown in Table 2. Both precision and accuracy were acceptable for quantification. The stability of QC samples was assayed in three conditions, i.e. freeze-thaw for three cycles, room temperature for 24 h and –70 °C for 30 days. The recoveries ranged between 93.0% and 106.8% and the result suggested that borneol was stable throughout the stability study. The average extraction recoveries of borneol were between 89.6% and 96.3%, and the method recoveries were between 91.6% and 102.3%, in the five regions, all were acceptable in the present study.

Tables 3 and 4 showed the pharmacokinetics parameters of borneol in plasma and the four brain regions respectively. In hippocampus and hypothalamus, the deliveries of borneol were similar. The delivery of borneol was the least in striatum comparing with those in the other regions. The delivery of borneol in cortex was much more than those in the other brain regions which might due to the large Cmax and small CL/F, especially the former. In addition, T1/2 in cortex was much less than those in the other regions, suggesting an efficient elimination in this region. The concentration-time curves of borneol were shown in Fig. 12.

4. Discussion

Blood–brain barrier (BBB) plays a central role in maintaining brain homeostasis and low permeability, but also against the permeation of drugs targeting the brain, affecting the therapeutic effect of many CNS illnesses. Understanding and circumventing the blood–brain barrier is crucial for easy penetration and high efficacy of CNS drugs into the brain (Pardridge, 2005; Misra et al., 2003; Zhang and Pardridge, 2005). Previous studies had demonstrated that borneol enhanced the delivery of some CNS agents in the brain (Yu et al., 2009; Lu et al., 2012; Cao et al., 2010).

The endothelial cells in the lumen of brain capillaries form tight junctions which limit the penetration of large, hydrophilic compounds. These cells also have efflux transporters (e.g. ABC proteins) for cationic compounds and organic anions (Liu et al., 2013; Zhu et al., 2012). In the present study, borneol increased the content of Rh123, a substrate of P-gp, in hippocampus and hypothalamus, indicating an inhibitory effect of borneol on P-gp in these two regions, which was further confirmed by a small animal vivo imaging system and a fluorescence microplate reader assays. The effects of borneol on the expressions of relevant ABC transporters (Mdr1, Mrp1, Mrp4, Mrp5 and Bcrp) were further investigated. Borneol obviously increased the expression of Mdr1 and Mrp1 as evidenced by immunofluorescence histochemistry, western blot and RT-PCR assays. Mdr1, Mrp5 and Bcrp are localized to the luminal membrane of endothelial cells (Baulieu et al., 1997; Orion et al., 1997; Abbott et al., 2010; Cooray et al., 2002). Sane reported that the brain-to-plasma partition coefficient of elacridar, a anti-brain tumor drug, was 0.82 in wild-type mice.

Table 1
The linear regression equations of borneol in the plasma and the four brain regions.

<table>
<thead>
<tr>
<th>Regions</th>
<th>Linear equation</th>
<th>Linear range (μg/mL)</th>
<th>r²</th>
</tr>
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<tbody>
<tr>
<td>Plasma</td>
<td>Y=0.4724X−0.3122</td>
<td>0.5−50</td>
<td>0.9998</td>
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<tr>
<td>Cortex</td>
<td>Y=0.5078X−0.0481</td>
<td>0.2−25</td>
<td>0.9997</td>
</tr>
<tr>
<td>Striatium</td>
<td>Y=0.9278X−0.086</td>
<td>0.2−25</td>
<td>0.9999</td>
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<td>Hippocampus</td>
<td>Y=0.7619X+0.0881</td>
<td>0.2−25</td>
<td>0.9989</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>Y=1.0882X−0.2851</td>
<td>0.2−25</td>
<td>0.9995</td>
</tr>
</tbody>
</table>

Y represented the peak area ratio of borneol and IS. X represented the concentration of borneol.

Table 2
The intra- and inter-assay accuracy and precision of measurement of borneol (n=5).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration added (μg/mL)</th>
<th>Accuracy (RE %)</th>
<th>Precision (RSD %)</th>
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</thead>
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<tr>
<td></td>
<td>Intra-day</td>
<td>Inter-day</td>
<td>Intra-day</td>
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<td>4.97</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>8.70</td>
<td>5.37</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>5</td>
<td>2.16</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5.24</td>
<td>4.18</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>7.40</td>
<td>8.22</td>
</tr>
</tbody>
</table>

Table 3
Pharmacokinetics parameters of borneol in rat plasma (mean ± SD, n=5).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0−t (min/mg/L)</td>
<td>13.99 ± 1.68</td>
</tr>
<tr>
<td>MRT0−t (min)</td>
<td>55.01 ± 2.61</td>
</tr>
<tr>
<td>T1/2 (min)</td>
<td>139.83 ± 87.52</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>11.00 ± 2.24</td>
</tr>
<tr>
<td>CLz/F (L/min/kg)</td>
<td>10.85 ± 3.22</td>
</tr>
<tr>
<td>Cmax (mg/L)</td>
<td>0.27 ± 0.06</td>
</tr>
</tbody>
</table>

Table 4
Pharmacokinetics parameters of borneol in rat four brain regions (mean ± SD, n=5).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cortex</th>
<th>Hippocampus</th>
<th>Hypothalamus</th>
<th>Striatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0−t (min/μg/g)</td>
<td>11.89 ± 3.55</td>
<td>4.00 ± 1.04</td>
<td>4.21 ± 1.04</td>
<td>2.86 ± 0.46</td>
</tr>
<tr>
<td>MRT0−t (min)</td>
<td>63.34 ± 7.33</td>
<td>63.00 ± 2.70</td>
<td>63.98 ± 8.24</td>
<td>70.82 ± 3.12</td>
</tr>
<tr>
<td>T1/2 (min)</td>
<td>80.02 ± 48.02</td>
<td>24.34 ± 44.11</td>
<td>530.63 ± 568.27</td>
<td>170.00 ± 5.78</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>30.00 ± 10.61</td>
<td>27.00 ± 6.71</td>
<td>18.00 ± 6.71</td>
<td>10.00 ± 0.73</td>
</tr>
<tr>
<td>CLz/F (g/min/g)</td>
<td>14.82 ± 4.40</td>
<td>33.82 ± 11.60</td>
<td>22.22 ± 13.30</td>
<td>43.42 ± 14.30</td>
</tr>
<tr>
<td>Cmax (μg/g)</td>
<td>0.18 ± 0.07</td>
<td>0.06 ± 0.01</td>
<td>0.07 ± 0.03</td>
<td>0.04 ± 0.01</td>
</tr>
</tbody>
</table>
while 3.5 in Mdr1a/b(−/−) mice, 6.6 in Bcrp1(−/−) mice, and 15 in Mdr1a/b(−/−)/Bcrp1(−/−) mice, indicating that both Mdr1 and Bcrp limit the brain distribution of elacridar (Sane et al., 2013). Mrp1 was reported to locate at the BBB more likely in astrocyte foot processes rather than in endothelial cells (Marie-Claire et al., 2004) and it efflux its substrates from the brain to the blood (Sugiyama et al., 2003), but did not inhibit drug transporting from the blood to the brain (Cistermino et al., 2003). Mrp4 dually located in the basolateral membrane of the choroid plexus epithelium and in the brain capillary endothelium. Mrp4-deficient mice showed enhanced accumulation of an anticancer agent, topotecan, in brain tissue and cerebrospinal fluid, and Mrp4 at the choroid epithelium was integral to its function in limiting drug penetration into the CSF (Leggas et al., 2004; Urquhart and Kim, 2009).

In the present study, borneol inhibited the expressions of Mdr1a, Mdr1b, Mrp1 only in the hippocampus and hypothalamus regions, but fails to do so for the other transporters or in the other two brain regions. Why did borneol show different effects in the regions? Our previous research demonstrated that the BBB-opening effect of borneol was closely relevant to its dose. Its opening effect decreased when its intragastric dose was more than 4.0 g/kg for rats (Dong et al., 2012). So, the pharmacokinetics study showed that both too much delivery of borneol (such as in cortex) and too little delivery (such as in striatum) are unfavorable for its BBB-opening activity.

5. Conclusions

Borneol showed region specific BBB-opening activity. The expressions of Mdr1a, Mdr1b and Mrp1 were implicated in its opening effect in hippocampus and hypothalamus. The pharmacokinetics study showed that both too much delivery of borneol (such as in cortex) and too little delivery (such as in striatum) are unfavorable for its BBB-opening activity.

Acknowledgments

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References


