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# *In situ* and *in vivo* study of nasal absorption of borneol in rats

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The aim of this work was to study the *in situ* and *in vivo* nasal absorption of borneol. A novel single pass *in situ* nasal perfusion technique was applied to examine the rate and extent of nasal absorption of borneol by rats. Experimental conditions, such as perfusion rate, pH and drug concentration, were investigated. The *in situ* experiments showed that the nasal absorption of borneol was not dependent on drug concentration, and fitted a first order process. The absorption rate constant, Ka, influenced with an increase in perfusion speed. The borneol was well absorbed in the conditions of the nasal cavity within the pH range and pH value of physiological conditions. *In vivo* studies of borneol absorption were carried out in rats and the pharmacokinetics parameters of intranasal (i.n.) was compared with intravenous (i.v.) administration. The bioavailabilities of borneol was 90.82 % for i.n. while $T_{max}$ values were 10 min. MRT (Mean Residence Time) were $262.55 \pm 67.35$ min and $204.22 \pm 14.50$ min for i.n. and i.v. methods, respectively. The results demonstrate that borneol could be absorbed promptly and thoroughly by i.n. administration in rats.

## 1. Introduction

Borneol is an ingredient commonly employed in medicinal preparations as an adjuvant. It is a bioactive substances derived from Chinese herbal medicines. It has been considered to have a therapeutic effect for waking up a patient from unconsciousness, expelling heat to alleviate pain and promoting granulation. Some studies showed that borneol could improve some drug's oral bioavailability, accelerate the passage through the blood–brain barriers (BBB), and enhance the distribution of drugs in brain tissue (Xioa et al. 2007; Cai et al. 2008). Studies also indicated that borneol has some antibacterial activity, anti-inflammatory action, analgesic effect, and may help to protect heart and brain, and to regulate the nervous system (Wei and Gu 2010). Borneol can be quickly absorbed after oral administration, but the bioavailability is relatively low compared with injection.

In the last decade, intranasal (i.n.) administration has drawn considerable interest since it provides a non-invasive method for bypassing the first-pass effect and possibly the blood brain barrier (Sakane et al. 1991, 1994; Wang et al. 1998). The i.n. administration could be a substitution for injection, as drugs can be absorbed sufficiently and rapidly into the blood for systemic administration (Duchene and Ponchel) and transported from the nasal cavity to the central nervous system (Ilium 2000). About 40 substances have been reported to reach the brain via the direct nose-to-brain pathway (Chen et al. 2008). Moreover, the i.n. route is safer than the intravenous (i.v.) one due to the barrier effect of the nasal mucosa. Based on these facts, research of the nasal absorption of borneol is important.

In this study, a novel single pass *in situ* nasal perfusion technique was applied to examine the rate and extent of nasal absorption of borneol in rats. *In vivo* studies were carried out in rats as well and the pharmacokinetics parameters after i.n. administration were compared with those after i.v. administration.

## 2. Investigations, results and discussion

### *2.1. In situ nasal perfusion experiments*

Circulatory perfusion technology has been successfully applied to research the nasal absorption of drugs *in situ* (Shi and Jiang 2004; Song et al. 2011). However, in our preliminary experiments, borneol was found to be absorbed by circulatory tubes made of common materials, such as PVA, rubber, silicone and low absorption silicone (Sani-Tech LA-60). The nasal absorption of borneol cannot be researched by traditional circulation equipment. Therefore, a single pass perfusion device without soft tubes of a peristaltic pump was invented to study the nasal absorption of borneol. This novel equipment and methods are also suited for the research of nasal absorption of drugs that are evaporable or absorbable by the tubes. Furthermore, it was found that water in the perfusate could be absorbed transnasally by rats. Hence, Ka was calculated through gravimetry adjustment to get more precise and reliable results.

#### *2.1.1. Impact of perfusion speed on nasal absorption of borneol*

Borneol nasal solutions (98.56 $\mu g \cdot mL^{-1}$ of borneol) were prepared for *in situ* nasal perfusion (see Experimental section) at the speed of 0.1 $mL \cdot min^{-1}$, 0.2 $mL \cdot min^{-1}$ and 0.3 $mL \cdot min^{-1}$, respectively. The results in Table 1 show that the absorption of borneol could be affected by the perfusion speed. The absorption at the speed of 0.1 $mL \cdot min^{-1}$ was significantly different from those at 0.2 $mL \cdot min^{-1}$ and 0.3 $mL \cdot min^{-1}$.

#### *2.1.2. Impact of pH value on nasal absorption of borneol*

Borneol solutions of pH 5.5, pH 6.0 and pH 7.0, pH 7.4 (98.56 $\mu g \cdot mL^{-1}$ of borneol; the pH was adjusted by buffer solu-

**Table 1: Ka of borneol nasal absorption with different perfusion speeds (n = 6, mean ± S.D.)**

| Perfusion speed ($min \cdot L^{-1}$) | Ka ± SD ($min^{-1}$) |
|---|---|
| 0.1 | $0.30 \pm 0.01^{**}$ |
| 0.2 | $0.61 \pm 0.01$ |
| 0.3 | $0.60 \pm 0.01$ |

** $P < 0.01$ vs. 0.2, 0.3

**Table 2: Ka of borneol nasal absorption with different pH (n = 6, mean ± S.D.)**

| pH Value | Ka ± SD ($min^{-1}$) |
|---|---|
| 5.5 | $0.61 \pm 0.01$ |
| 6.0 | $0.60 \pm 0.01$ |
| 7.0 | $0.61 \pm 0.02$ |
| 7.4 | $0.60 \pm 0.03$ |

$P > 0.05$

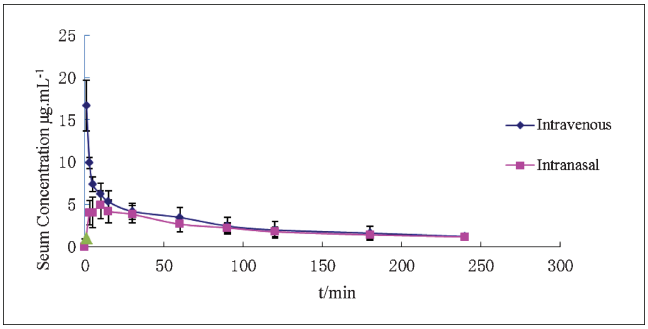


Fig. 1: Concentration of borneol in plasma (n = 5, mean ± S.D.) following a single i.v. (♦), i.n. (■), administration; calculated dosage of 12 $mg \cdot kg^{-1}$ of borneol in rats

tion) were prepared for in situ nasal perfusion. It was shown that borneol was well absorbed under the conditions of the nasal cavity within the pH range and pH value of physiological conditions (as shown in Table 2).

### *2.1.3. Impact of concentration on nasal absorption of borneol*

Borneol solutions of 98.56, 492.8 and 985.6 $\mu g \cdot mL^{-1}$ were prepared with NS (pH 7). Table 3 shows that absorption of borneol did not depend on concentration. The absorption pattern appeared to follow first order kinetics, indicating that borneol was transported across the nasal mucosa by passive diffusion.

## *2.2. In vivo studies*

The concentration-time curves resulting from administration of a single dose of borneol by i.v., i.n. in rats is shown in Fig. 1. In

**Table 3: Ka of borneol nasal absorption with different drug concentrations (n = 6, mean ± S.D.)**

| Drug concentration ($\mu g \cdot mL^{-1}$) | Ka ± SD ($min^{-1}$) |
|---|---|
| 98.56 | $0.60 \pm 0.010$ |
| 492.8 | $0.59 \pm 0.011$ |
| 985.6 | $0.61 \pm 0.013$ |

$P > 0.05$

**Table 4: Main pharmacokinetic parameters of plasma after i.v. and i.n. and administration of borneol in rats (n = 5, mean ± S.D.)**

| Parameter | Unit | i.v. | i.n. |
|---|---|---|---|
| $C_{max}$ | $\mu g \cdot mL^{-1}$ | $16.685 \pm 3.008$ | $4.933 \pm 1.811$ |
| $T_{max}$ | min | 1 | 10 |
| AUC | $\mu g \cdot mL^{-1} \cdot min$ | $939.614 \pm 259.105$ | $853.372 \pm 225.896$ |
| MRT | min | $204.219 \pm 14.495$ | $262.551 \pm 67.345$ |
| *F%* | – | – | 90.82 |

the case of i.n. administration, the drug was absorbed rapidly. The $C_{max}$ of borneol reached about 4.93 $\mu g \cdot mL^{-1}$ at the point of 10 min. The main pharmacokinetic parameters of the plasma after i.v., i.n. administration are shown in Table 4. MRT for injection, nasal solutions were 204.22 and 262.55 min, respectively while AUC (Area Under the Concentration-Time Curve) values were 939.61, and 853.37 $\mu g \cdot mL^{-1} \cdot min$ for i.v. and i.n., respectively. The absolute bioavailability (F%) of i.n. was 90.82%. Our present results demonstrate that after the i.n. administration, borneol could be absorbed rapidly and the bioavailability of borneol was relatively high.

## 3. Discussion

In both modern and traditional pharmaceutics, nasal drug delivery (NDD) is considered to be an effective and promising method for drug administration. This route is beneficial for drugs which can be absorbed sufficiently and rapidly into the systemic circulation and can be transported adequately to the brain as mentioned above. Borneol is often administered by injection. However, the administration by the i.v. route is inconvenient. Therefore, our research on the nasal absorption of borneol is of great value. Circulatory perfusion technology has been successfully applied to research the in situ nasal absorption of drugs. However, borneol was found to be absorbed by the circulatory tubes. We took a novel single pass perfusion device to study the nasal absorption of borneol. From investigations of the effects of perfusion speed, pH value and drug concentration on the *in situ* nasal absorption of borneol, it was shown that was similarly absorbed at 0.2 $min \cdot L^{-1}$ and 0.3 $min \cdot L^{-1}$ perfusion speed. However, a high perfusion speed may cause injury of the nasal mucosa so that the drug absorption is accelerated and the measured value may differ from reality. Therefore, a perfusion speed of 0.2 $min \cdot L^{-1}$ was adopted in subsequent experiments. Further research indicated that borneol absorption is not influenced by the physiological conditions in the nasal cavity. Since drug concentration had no effect on Ka, the absorption of borneol could be deemed as fitting the first order process. Pharmacokinetic studies of borneol after i.n. administration in rats revealed that borneol was rapidly absorbed. The $C_{max}$ of borneol after i.n. administration was achieved in 10 min. In addition, the absolute bioavailability of borneol by i.n. administration was 90.82%. The results of our study, both *in situ* and *in vivo*, indicated that borneol (Lu et al. 2010) can be absorbed by the nasal route. Owing to the safety, convenience and cheapness of nasal administration compared with injection treatment, developing new quick-action preparations of borneol based on NDD technologies is valuable, encouraging and worth to be further studied.

## 4. Experimental

### *4.1. Chemicals and reagents*

Borneol was obtained from the Natioanl Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, P.R. China).

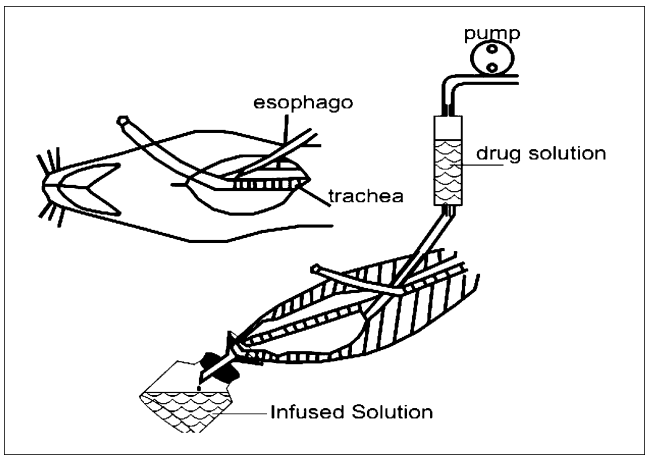


Fig. 2: Diagram of *in situ* nasal single pass perfusion experiments

Octadecane, (purity:99%) an internal standard (i.s.), was purchased from Alfa Aesar China (Tianjin, China) Co., Ltd. Ethylacetate was of HPLC grade (Fisher Scientific, America) and all other reagents were of analytical grade.

### 4.2. Animals

Male Sprague Dawley (SD) rats (250–280 g) were obtained from WeiTong Biotech-nology Inc. (Beijing, China), and were kept in a controlled-environment breeding room (temperature: $22 \pm 1\,^{\circ}C$, humidity: $50 \pm 5\%$, 12-h dark/light), with free access to common food and water in the first week. All experimental procedures were conducted in accordance with the European Union guidelines for the use of experimental animals and approved by the Beijing University of Chinese Medicine Committee on Animal Care and Use.

### 4.3. Chromatographic conditions

GC analysis was performed with an Agilent 8900 Gas Chromatography equipped with FID. Compounds were separated on a 30 m × 0.25 mm i.d. × 0.25 mm film DB-WAX capillary column. The polar column was maintained at 140 °C for 9 min, Split injection was conducted with split radio of 5:1; flow-rate, 1.00 $mlmin^{-1}$; nitrogen was used as carrier gas; injector temperature, 250 °C; detector temperature, 280 °C. Some individual components could be identified by co-injection of pure compounds and comparison of their retention times. There was a good linearity between A and C (Calibration curves were calculated by plotting the peak area ratios of analyte to internal standard versus analyte concentration. C = 0.1271A-0.0007, r = 0.9998). The validation parameters of precision (CV less than 2%) were acceptable and the lower limit of quantitation was 23.5 $\mu g \cdot mL^{-1}$.

### 4.4. Sample preparation

Tween-80 (0.5% (v/v)) was dissolved in physiological saline and used as the solvent for drugs. Borneol was dissolved according to the ratio of desired concentration for *in situ* nasal single pass perfusion experiments.

### 4.5. In situ nasal perfusion experiments

The absorption studies were carried out using a novel *in situ* nasal single pass perfusion technique (Fig. 2). Rats were anesthetized by intraperitoneal injections of urethane (1.2 $g \cdot kg^{-1}$ body weight). An incision was made in the rat neck. The trachea was cannulated with a polyethylene tube to allow breathing while another tube was inserted through the esophagus into the posterior part of the nasal cavity. The nasopalatine duct was closed with cyanoacrylate glue to prevent the drainage of solution from the nasal cavity to the mouth. The tube inserted into the esophagus was connected to a storage bottle containing 100 mL drug solution. The whole passage was sealed in order to avoid the volatilization of borneol.

The drug solution was infused into the nasal cavity by the pressure generated from a peristaltic pump at a certain speed. After running through the nasal cavity, the perfusate was collected into a receiving bottle in a certain interval minutes (5, 10, 20, 30, 45, 60, 90 and 120 min as scheduled). The bottle with perfusate was weighed immediately when it was substituted. Then, a certain amount of perfusate was sampled, and octadecane (103.1 $\mu g \cdot mL^{-1}$ in ethyl acetate, internal standard, the Alfa Aesar China (Tianjin, China) Co., Ltd. China, join) 0.4 mL was added to 0.4 mL of sample which was then vortexed for 1 min and centrifuged at 10000 rpm for 10 min. The 200 μL of supernatant organic layer were removed. An aliquot of 1 μL was injected into GC to determine the content of borneol. The first-order rate constant of the absorption of borneol, Ka, was estimated by Eq. (1).

$$Ka = \left(1 - \frac{\rho_{out}}{\rho_{in}} \bullet \frac{V_{out}}{V_{in}}\right) \bullet \frac{v}{V_{nose}} \quad (1)$$

where $V_{in}$ corresponds to the volume of drug solution perfused in every min; $V_{out}$ is the volume of outflow perfusate received in every min ($V_{out} \approx W_{out}$ for density of perfusate approximately equal to 1 $g\,mL^{-1}$, $W_{out}$ is the weight of outflow perfusate received in every min); $v$ is the speed of perfusion; $\rho_{in}$ and $\rho_{out}$ are concentrations of borneol ($mg \cdot mL^{-1}$) of buffers in the entrance and exit, respectively; $V_{nose}$ is the volume of the rat nasal cavity (calculated by the volume of drug solution contained in it). The in situ Ka of the nasal absorption groups were compared by one-way ANOVA at the 0.05 significance level, and the tests for statistical differences were paired t-tests. (Statistics Analysis System 8.0).

### 4.6. In vivo experiments

Ten male SD rats, weighing 250∼300 g, were randomly assigned to two groups. All animals were fasted for 12 h prior to the experiments and anesthetized with an intraperitoneal injection of urethane (1.2 $g \cdot kg^{-1}$ body weight). About 1 mL of injection sample (at a single dose of 12 $mg \cdot kg^{-1}$ borneol) was either injected via the tail vein or administered via the nostril by a modified micro-injector as a 20 μL i.n. solution. 0.35 mL of blood was collected from the left carotid artery at 1, 3, 5, 10, 15, 30, 60, 90, 120, 180, 240 min after drug administration. Blood samples were placed into heparinized tubes. After centrifugation, the plasma obtained was stored at -24 °C until determination. An aliquot of 150 μL of plasma sample was placed into a centrifuge tube and 20 μL of octadecane was added and vortexed for 30 s. Ethyl acetate (100 μL) was added to the mixture followed by shaking. After 1 min in a vortex, the mixture was centrifuged at 10,000 rpm for 10 min. Supernatant (1 μL) was injected into the GC system. The GC conditions were listed above. It could be seen that there was a good linearity between C and A (C = 9.6154A-0.0621, r = 0.9998). The validation parameters of precision (CV less than 3%) and accuracy (recovery of ± 20%) were acceptable and the lower limit of quantitation was 0.046 $\mu g \cdot mL^{-1}$.

### 4.7. Data analysis

Serum concentrations of i.n. and i.v. vs. time data were analyzed by a noncompartmental method using Kinetica 4.4 software. The *in vivo* pharmacokinetic parameters of the i.n. and i.v. groups were compared by one-way ANOVA at the 0.05 significance level, and the statistical difference was analyzed by paired t-tests (Statistics Analysis System 8.0).

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