The In Situ and In Vivo Study on Enhancing Effect of Borneol in Nasal Absorption of Geniposide in Rats

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The objective of this research was to study the in situ and in vivo nasal absorption of Geniposide (Ge) co-administered with borneol. A rat in situ nasal perfusion technique with a novel volume-adjusted calculation was used to examine the absorption rate and extent of Ge. The influence of different experimental conditions such as purity of extract, drug concentration, co-administration with synthetic borneol or natural borneol were also investigated. Results indicated nasal absorption of Ge was primarily by passive diffusion that resembled first order kinetics. Following co-administration with bornenol, the drug absorption was increased by 1.4 and 1.7 folds for natural borneol and synthetic borneol, respectively. However, the effect of other factors on drug absorption was not significant. In addition, it was also observed that there is a positive correlation between the absorption of water and Ge by the nasal route. In vivo studies carried out in rats where Ge was co-administered with NB and the pharmacokinetic profile obtained following intranasal administration were compared with those after intravenous administration. The bioavailability of Ge by intranasal was 101.5% and \(T_{\text{max}}\) was 2.04 ± 0.64 min. MRT was 218.7 ± 74.1 min and 44.4 ± 8.9 min for intranasal and intravenous, respectively. Combined with the borneol, Ge can be promptly and thoroughly absorbed intranasally in rats.

Key words: Geniposide, Nasal absorption, Bornel, Enhancement, Pharmacokinetics

INTRODUCTION

Geniposide (Ge) is the biologically active ingredient of Gardenia fruit (Gardenia jasminoides Ellis, Rubiaceae). Ge is widely used in China as an important active ingredient of multi-component injection in the treatment of cerebrovascular and cardiovascular diseases for its antithrombotic and anti-inflammatory effects (Suzuki et al., 2001; Koo et al., 2006). However, as more and more adverse reactions are reported following multi-component Chinese herbal medicine (MCHM) injection, it is important to identify alternative routes for drug administration, that are safer and more convenient. Among the many routes of drug administration, intranasal (i.n.) route could serve as a promising substitution for MCHM injection. This route is beneficial in that the drugs are absorbed sufficiently and rapidly into systemic circulation (Duchine and Ponchel, 1993) and could be transported in sufficient concentrations to the brain for action on central nervous system (Ilium, 2000). The i.n. route is safer than intravenous (i.v.) route due to the barricade effect of nasal mucosa. A preliminary study (Zhang et al., 2009) conducted by us indicated that the apparent partition coefficient (\(P\)) of Ge between octanol and water is about 0.108, suggesting that the mucosal absorption of Ge may not be sufficient to achieve the desired concentrations.

Borneol is a monoterpenoid component of the medicinal plant. It is widely used in traditional Chinese medicine (TCM), and often in combination with Gardenia for the treatment of stroke. Some studies have shown that borneol could improve nasal and oral bioavailability by accelerating the opening up the blood-brain-barriers (BBB) and enhancing the distribution of drugs in brain (Chen et al., 2004, 2006; Dai et al., 2009). Both, synthetic borneol (SB) as well as natural...
borneol (NB) are widely used in Chinese medicine. SB consists of d-borneol and isoborneol while NB only contains d-borneol.

In this study, a rat in situ nasal perfusion technique was adopted to determine the rate and extent of Ge absorption, alone and in combined with borneol. The in vivo studies of Ge with borneol were carried out in rats to map the pharmacokinetic parameters of i.n., which were then compared with that of i.v. route.

**MATERIALS AND METHODS**

**Materials and animals**

Ge was obtained from National Institute for the Control of Pharmaceutical and Biological Products (NICPBP), NB and SB were obtained from Tong Rentang Inc. Male Sprague Dawley (SD) rats (250–280 g) were obtained from WeiTong Biotechnology Inc. Acetonitrile, methanol and water were of HPLC grade (Qualigens) and all other reagents were of analytical grade.

**In situ nasal perfusion experiments**

**Preparation of nasal solutions**

Tween-80 solution of 0.5% (v/v), dissolved in physiological saline was used as the solvent for drugs. Ge and borneol were dissolved in the ratios of desired concentrations for in situ nasal perfusion experiments.

The groups of in situ experiments

Twenty Four Male SD rats, randomly assigned into four groups of six rats, were conducted in situ nasal perfusion experiments with Ge solutions of 40, 100, 400 µg/mL and gardenia extract solution of 67 µg/mL (60% purity for Ge, w/w).

Twelve Male SD rats, randomly assigned into three groups of four rats were conducted in situ nasal perfusion experiments as normal saline group (100 µg/mL of Ge), NB addition group and SB addition group (100 µg/mL of Ge and 200 µg/mL of borneol).

**Method of in situ nasal perfusion**

The absorption studies were carried out according to the in situ nasal perfusion technique as described by Hirai et al. (1981) and Huang et al. (1985). Rats were anesthetized by intraperitoneal injection of urethane (1.2 g/kg body weight). An incision was made in the neck of rat and trachea was cannulated with polyethylene tube to keep continuity in breathing. Another tube was inserted through the esophagus into the posterior part of the nasal cavity. The nasopalatine duct was closed with a cyanoacrylate glue to prevent the drainage of solution from nasal cavity into the mouth. The tube inserted into the esophagus was connected with a volumetric cylinder contained 5 mL drug perfusate in a water-bath at 37°C. The perfusate was circulated by peristaltic pump from the volumetric cylinder through the nasal cavity then out of the nostrils and back into the volumetric cylinder. An aliquot (0.25 mL) was sampled at predetermined time point while the same volume of stock solution was refilled. The volume of remaining perfusate was recorded before every sampling to measure the amount of water absorbed by rat.

The amount of Ge were expressed as the percentage of the initial amount (not concentration for volume changed) according to Eq. (1). The first-order rate constants ($K_{obs}$) of Ge absorption were estimated by linear regression analysis of ln $Q_n$ versus time data.

$$Q_n = \frac{(V_n + V_D)C_n + \left(\sum_{i=1}^{n-1} C_i (n-1)C_{ini}\right) \times 100}{Q_{ini}} \times 100\%$$ (1)

where $Q_n$ corresponds to amount of Ge at the sampling point of $n$, $Q_{ini}$ and $C_{ini}$ as the initial amount and concentration of Ge in initial perfusate, $V_n$ and $C_n$ as recorded volume of perfusate and concentration of Ge at the sampling point of $n$, $V_D$ as the dead volume.

**Analytical procedure of in situ experiments**

The concentration of Ge was quantitated by RP-HPLC (Agilent1100, HP Inc. USA, Diamonsil® C18 column, 250×4.6 mm, 5 µm, Dikma Technology company, China). The mobile phase used were acetonitrile and water (15:85, v/v), the signal was monitored at 238 nm. The flow rate was maintained at 1.0 mL/min (Ye et al., 2006). All the samples were centrifugated (12000 r/min) for 15 min before determination.

**In vivo Experiments**

Twelve male SD rats, weighing 270 ± 12 g, were randomly assigned into two groups of six rats. All the animals were fasting for 12 h prior to initiation of the experiment and were anesthetized by intraperitoneal injection of urethane (1.2 g/kg body weight). About 0.5 mL of injection sample was injected via tail vein and 50 µL of nasal solution was administrated via nostril by a modified microinjector, both at the single concentration of 4 mg/kg (for Ge). Total 0.25 mL of blood was collected from the left carotid artery at 0.5, 1, 3, 5, 10, 20, 30, 60, 90, 120, 180, 240 min after drug administration. Blood samples were placed into heparinized tubes. After centrifugation, the obtained plasma was stored at -20°C until determination. An aliquot of 100 µL plasma sample was placed into a centrifuge tube and 200 µL acetonitrile was added. After vortexed for