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A strategy for bypassing the blood-brain barrier: Facial intradermal brain-targeted delivery via the trigeminal nerve

Xi-Chong Yu[a], Jing-Jing Yang[a], Bing-Hui Jin[a], He-Lin Xu[a], Hong-Yu Zhang[a], Jian Xiao[a], Cui-Tao Lu[b], Ying-Zheng Zhao[a,b,**], Wei Yang[a,*]

[a] School of Pharmaceutical Science, Wenzhou Medical University, Wenzhou, Zhejiang Province 325035, China
[b] The Second Affiliated Hospital of Wenzhou Medical University, Wenzhou City, Zhejiang Province 325000, China

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ABSTRACT

Although intranasal delivery bypasses the blood-brain barrier (BBB), the anatomical location of the olfactory mucosa and respiratory airflow interference lead to less brain-targeted drug delivery. In addition to intranasal delivery, evidence indicates that facial intradermal injection might be a novel strategy for bypassing the BBB via the trigeminal nerve (TN). The hypothesis was verified by pharmacokinetic evaluation, nasal injury, lymphatic vessels inhibition and immunohistochemistry. Intradermal injection into the rat mystacial pad (i.d.) elevated the brain sub-areas and trigeminal Evans Blue (EB) concentrations, C_{max} and $AUC_{(0-t)}$. I.d. also increased them in brain sub-areas beyond those of intranasal (i.n.) and intravenous injection (i.v.), especially the pons varolii and the medulla oblongata (sub-areas associated with TN). I.d. injection increased the brain drug targeting efficiency, brain direct transport percentage and brain bioavailability of EB while i.n. injection altered them slightly. Trigeminal transection and nasal injury reduced trigeminal EB with i.d. administration. Trigeminal perineurium, epineurium, perivascular spaces, neurons and Schwann cells were involved in the EB brain-targeted delivery. The lymphatic system mediated EB diffusion from the mystacial pad to the nasal mucosa and the brain. Thus, facial intradermal injection might be a promising strategy for brain-targeting delivery, bypassing the BBB via the trigeminal substructures.

1. Introduction

The blood-brain barrier (BBB) demonstrates a bottleneck effect for drug delivery in central nervous system (CNS) diseases via systemic administration, especially for hydrophilic drugs. Several strategies are available to permeate the BBB for systemic drug delivery to brain, but most brain-targeted delivery systems insufficiently improve CNS diseases, and higher dosages can cause the toxicity [1,2]. Bypassing the BBB is a strategy for brain-targeted delivery of drugs. Although invasive technologies such as intraventricular, intrathecal or perispinal injection directly deliver drugs to the CNS and improve CNS diseases [3–5], these methods can lead to brain infections, injury and glial scar. Intranasal delivery is a promising strategy for bypassing the BBB. However, the anatomical position of the olfactory mucosa and respiratory airflow interference lead to less brain-targeted drug delivery [2,6,7]. There are few intranasal delivery systems available for clinical application.

The olfactory nerve, trigeminal nerve (TN), nasal lymphatic vessels, etc. mediate intranasal drug delivery for CNS diseases [2,8,9]. The TN is the main pathway for intranasal drug delivery [10–12]. Moreover, the TN innervates the facial skin, facial muscles, meninges and respiratory mucosa (Fig. 1). Thus, we suppose that facial skin administration serves as an alternative strategy to bypass the BBB. Indeed, facial skin administration via TN innervation has demonstrated the ability to deliver horseradish peroxidase (HRP) or lectins to the TN [13–16], and the route has been used for treating trigeminal neuralgia [17,18]. Following injection into the ethmoidal nerve, a branch of the TN, HRP was found in the trigeminal ganglion and the trigeminal nucleus [19]. HRP was also retrograde-transported from the hypothalamus to the cortex, hippocampus and trigeminal sensory nucleus [20]. These works indicate that facial dermal delivery might be an option for bypassing the BBB to the CNS via the trigeminal neurons for hydrophilic drugs.

The trigeminal nerve is structured similarly to the olfactory nerve. Since the neurons, nasal lymphatic vessels, perivascular spaces, perineurium and epineurium of the olfactory nerve serve as pathways for intranasal delivery [10,21–23], whether these trigeminal sub-structures have the same function in facial dermal delivery remains to be

* Corresponding author.
** Corresponding author at: Wenzhou Medical University, Zhejiang Province 325035, China.
E-mail addresses: pharmatds@163.com (Y.-Z. Zhao), yw4565@163.com (W. Yang).

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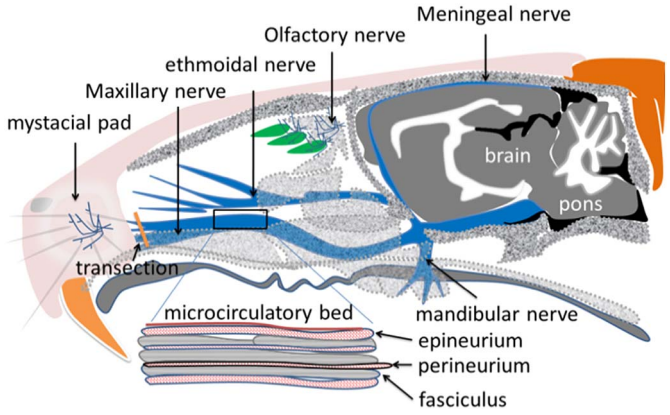

Fig. 1. The diagram of the trigeminal innervation in the rat head.Blue: the trigeminal nerve and its branches; yellow line: the transection level of maxillary nerve. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

discovered. Dermal lymphatic vessels serve as a route for drug absorption [24,25]. There are abundant microcirculatory beds and lymphatic vessels in the trigeminal perineurium [26,27]. The perineurium and the epineurium are the elongation of pia and dura mater, respectively. In the CNS, dural lymphatic vessels, perivascular spaces and paravascular microcirculation in the brain parenchyma function as exchange channels [28–30]. Therefore, trigeminal vasculature, perineurium and epineurium might connect facial skin and the brain and serve as pathways for facial intradermal brain-targeted delivery. In the fasciculus, Schwann cells wrap around trigeminal neurons and axons, but its role in the dermal drug delivery to brain is unknown. In this article, using intradermal injection in the mystacial pad of rats, we reported that the facial intradermal brain-targeted delivery of Evans blue via trigeminal substructures for bypassing the BBB.

2. Methods and materials

2.1. Animals and materials

Male Sprague Dawley Rats, 200–240 g, were acquired from the Animal Center of Wenzhou Medical University. All experimental protocols and procedures were approved by the Animal Care and Use Committee of Wenzhou Medical University. Rats were housed a specific-pathogen-free (SPF) breeding facility on a 12/12 h cycle (6:00–18:00 light), at 26 degrees and 50 ± 5 percentage air humidity. Evans blue (EB) was purchased from Sigma Aldrich and dissolved in double-distilled water (0.71%). NeuN, GFAP and S-100 antibody were purchased from Abcam, USA. LYVE1 polyclonal antibody was purchased from Santa Cruz Biotechnology, USA.

2.2. Intranasal delivery and intradermal injection in mystacial pad

Intranasal injection (i.n.) was performed based on the following protocol. The rat was anesthetized with isoflurane (2.5% isoflurane, with 80% oxygen and 20% room air). Intranasal delivery is influenced by airflow and the volume of solution [31]. After isoflurane anesthesia, the rats received a tracheal cannula to prevent airflow disturbance and EB entry into the airway (after cannulation, isoflurane was achieved through the cannula). Previous studies have reported that the upright position during intranasal delivery retained the substance in the nasal cavity [32]. In consideration of the tracheal cannula, the rat head was set to the upright 45 degrees-supine to retain the solution in the nasal cavity. Evan Blue (10 μL/100 g, 0.71%) was injected into the nasal cavity at 1 μL/min with micro-syringe via a PE10 tube. The rats received the EB intradermal injection in the left mystacial pad (i.d.) and intravenous injection (i.v.) via the tail vein, and rats maintained the same position during intranasal, intravenous and intradermal injection.

2.3. Pharmacokinetic evaluations

After EB i.n., i.d. or i.v. injection, rats were sacrificed with isoflurane at 0.0833, 0.25, 0.5,1, 2, 4, 6, 24, 48, 72, 120 and 168 h post-injection, additional point at 0.0416 h was supplemented for blood samples collection. A 1 mL blood sample was collected via cardiac puncture and infused into a heparinized plastic tube (50 μL 4% heparin in the tube dried at 37 degrees overnight). After exsanguination, the left and right trigeminal nerve, olfactory bulb, hippocampus, cortex and striatum, whole thalamus, pons varolii, medulla oblongata and were removed promptly and carefully. The brain sub-area was weighed and cut into pieces. The samples were precipitated with 2.0 mL alcohol for 0.5 h, and then the samples were centrifuged at 15,000 r/min and dried at 70 degrees for 24 h. Dried brain sub-areas were immersed in formamide (2–5 folds of wet tissue weight) and incubated at 70 degrees for 72 h. All brain samples were centrifuged at 3,000 r/min for 15 min. The supernatant was transferred to other tube. Blood samples were centrifuged at 10,000 r/min for 10 min. Then, 0.1 mL diluted plasma (1–3 folds of double-distilled water) was mixed with 2.0 mL alcohol and centrifuged at 10,000 r/min again. The alcohol was evaporated at 70 degrees for 24 h. Then, 0.4 mL formamide was added to the residue for 72 h and centrifuged again. The supernatant was used for EB determination. All samples were appropriately diluted with formamide for EB determination. EB was determined by high-throughout method as reported by Wang, H. L. and T. W. Lai [33]. EB in brain sub-areas and plasma were determined by Spectra Max (M2 molecular device) at an excitation wave length of 620 nm and an emission wave length of 680 nm. The method was validated before testing (Fig. S1A–C and Table S1), EB concentrations were calculated according to the corresponding standard curve (Fig. S1D and E).

2.4. Efficiency of intradermal and intranasal brain-targeted delivery

The brain and plasma bioavailability of EB and the ratio of brain to plasma were calculated using Eqs. (1) and (2). The drug targeting efficiency percentage (DTE) and the direct transport percentage (DTP) of brain were calculated using Eqs. (3) and (4), respectively [34].

$$F = \frac{AUC_{exe} \times D_{iv}}{AUC_{iv} \times D_{exe}} \times 100\% \quad (1)$$

where, AUC_{exe} is the area under the curve of plasma or brain EB from i.n. or i.d. administration. D_{exe} is the dosage of drug for i.n. or i.d. administration; and D_{iv} and AUC_{iv} are the dosage and area under curve of i.v. injection (excluding trigeminal $AUC_{(0-t)}$), respectively.

$$R_{Brain-Plasma} = \lg\left(\frac{AUC_{brain}}{AUC_{plasma}}\right) \quad (2)$$

where, AUC_{brain} and AUC_{plasma} are the sum of the brain sub-areas $AUC_{(0-t)}$ and plasma $AUC_{(0-t)}$ of EB from the same administration.

$$DTE = \frac{AUC_{exe(brain)}/AUC_{exe(plasma)}}{AUC_{iv(brain)}/AUC_{iv(plasma)}} \quad (3)$$

$$DTP = \frac{AUC_{exe(brain)} - (AUC_{iv(brain)}/AUC_{iv(plasma)}) \times AUC_{exe(plasma)}}{AUC_{exe(brain)}} \quad (4)$$

where, $AUC_{exe(brain)}$ is the sum of all EB $AUC_{(0-t)}$ of the brain sub-areas (excluding trigeminal $AUC_{(0-t)}$) after i.n. or i.d. injection. $AUC_{iv(brain)}$ is the sum of all EB $AUC_{(0-t)}$ of the brain sub-areas after i.v. injection.

2.5. Nasal injuries

Using $ZnSO_4$, we injured nasal mucosa with modification [35].