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Effect of administration method, animal weight and age on the intranasal delivery of drugs to the brain

Jishnu K.S. Krishnan, Peethambaran Arun, Bhadra Chembukave, Abhilash P. Appu, Nivetha Vijayakumar, John R. Moffett, Narayanan Puthillathu, Aryan M.A. Namboodiri*

Department of Anatomy, Physiology and Genetics, and Neurosciences Program, Uniformed Services University School of Medicine, USUHS, Bethesda, MD, United States

HIGHLIGHTS

- Intranasal delivery of drugs to the CNS bypasses the blood brain barrier.
- Variation in the techniques used to administer drugs intranasally may affect uptake.
- We examined effects of animal weight, age and delivery device on pralidoxime uptake.
- The use of an aerosol delivery device improved uptake only in the olfactory bulbs.
- Older animals had increased uptake on a per weight basis.

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ABSTRACT

Background: The intranasal route of administration has proven to be an effective method for bypassing the blood brain barrier and avoiding first pass hepatic metabolism when targeting drugs to the brain. Most small molecules gain rapid access to CNS parenchyma when administered intranasally. However, bioavailability is affected by various factors ranging from the molecular weight of the drug to the mode of intranasal delivery.

Comparison with existing methods: We examined the effects of animal posture, intranasal application method and animal weight and age on the delivery of radiolabeled pralidoxime (^{3}H-2-PAM) to the brain of rats.

Results: We found that using upright vs. supine posture did not significantly affect ^{3}H-2-PAM concentrations in different brain regions. Older animals with higher weights required increased doses to achieve the same drug concentration throughout the brain when compared to young animals with lower body weights. The use of an intranasal aerosol propelled delivery device mainly increased bioavailability in the olfactory bulbs, but did not reliably increase delivery of the drug to various other brain regions, and in some regions of the brain delivered less of the drug than simple pipette administration.

Conclusion: In view of the emerging interest in the use of intranasal delivery of drugs to combat cognitive decline in old age, we tested effectiveness in very old rats and found the method to be as effective in the older rats.

1. Introduction

Intranasal drug delivery (INDD) is an effective method for bypassing the blood brain barrier (BBB) and targeting therapeutic agents to the central nervous system (CNS) (Casettari and Illum, 2014; Thorne et al., 1995, 2004). Drugs delivered intranasally reach the CNS parenchyma via perivascular convection along blood vessels associated with the olfactory and trigeminal nerves (Lochhead et al., 2015). This method of drug administration has been shown to be effective with a wide array of drugs, as well as with polypeptides such as insulin (Frey, 2013). We have recently shown that intranasally administered oximes bypass the BBB and provide an effective method for protecting the brain from organophosphate mediated CNS damage (Krishnan et al., 2016). We have also shown rapid intranasal delivery of the bacterial enzyme chloramphenicol

* Corresponding author at: Department of Anatomy, Physiology and Genetics, and Neurosciences Program, Uniformed Services University School of Medicine, USUHS, Bethesda, MD 20814, United States.
E-mail address: anamboodiri@usuhs.edu (A.M.A. Namboodiri).

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acetyltransferase in the active form to different brain regions as a model for enzyme therapy in the CNS (Appu et al., 2016). Different INDD application methods have been reported in the literature but few studies have examined the relative advantages of the different methods. Some studies have used intranasal catheters with aerosol propellant to deliver therapeutics to the olfactory epithelium. Other studies employed application by pipette through the nares to the entire sinus cavity. Additionally, studies have differed with respect to the positioning of the animals in either an upright posture or supine position. Another important question is if intranasal brain delivery is effective in the different age groups in view of the therapeutic importance of this delivery method for brain disorders including Alzheimer disease.

INDD has gained significant therapeutic application in recent years. This delivery technique enables rapid distribution to the CNS non-invasively. INDD of therapeutics provides direct nose to brain delivery via the olfactory and trigeminal pathways (Thorne et al., 2004). INDD circumvents first-pass hepatic metabolism (Miller et al., 2008), can transport active enzymes (Appu et al., 2016), rapid onset to the CNS in therapeutic doses (Krishnan et al., 2016; Yamamoto et al., 2001). This approach is particularly advantageous because charged molecules and high molecular weight compounds, which cannot penetrate the BBB can be rapidly delivered to the CNS (Baker and Spencer, 1986). In comparison with other mucosal membranes in the body the nasal mucosa provides more rapid absorption (Anderson et al., 2012; Yamamoto et al., 2001). Most intranasal administration can be self-administered and does not require any technical skill.

INDD studies involve challenges including accuracy and consistency of administration which is made difficult by the small volumes involved (5–30 μl), the positioning of the animal during dosing, the proper insertion and positioning of the INDD catheter and the drainage of fluid from the nasal cavity to the esophagus. It has been reported that the dose reaching brain may be less than 1% of the total administered (Landis et al., 2012). Conventional intranasal administration of therapeutics delivers drug only into the nasal cavity without reaching the upper and posterior regions of the nasal cavity where more cranial nerves are exposed, which can facilitate transport drugs to the brain (Guastella et al., 2013).

Despite the inherent difficulties when dosing animals, INDD is considered the most safe and efficient procedure to bypass the BBB and deliver therapeutics to the brain (Lochhead and Thorne, 2012). The olfactory and trigeminal cranial nerves distribute various nerve endings to the nasal cavity (Thorne et al., 2004) thereby facilitating transportation of solutes to the CNS. The current investigation focuses on comparing the effect of INDD using the Precision Olfactory Delivery (POD, Impel NeuroPharma) device as compared with pipette application to the nares as well as the effects of age and body weight on intranasal drug transport to the brain.

Previously we investigated the bioavailability of intranasally delivered tritium labeled pralidoxime (^{3}H-2-PAM) in order to evaluate the feasibility of INDD of oximes for neuroprotection against organophosphate threat agents (Krishnan et al., 2016). In the current study we used this method to address the effects of animal weight and the positioning of the head during INDD on the bioavailability of ^{3}H-2-PAM in different brain regions. First, we tested the effect of age/weight by studying the bioavailability of ^{3}H-2-PAM in different age/weight groups of rats (100 g, 200 g, 400 g and 600 g) by intranasal delivery of the same dose of ^{3}H-2-PAM or the dose adjusted on a body weight basis, all using the same delivery volume. Second, we compared different methods from the literature, namely supine vs. upright posture of the rat with and without the intranasal POD device. Finally, we tested effectiveness in very old rats in view of the emerging interest in the use of intranasal delivery of drugs to combat cognitive decline in old age.

2. Materials and methods

2.1. Animals

All animal experiments were conducted following the NIH Guidelines for the Care and Use of Laboratory Animals, and the animal protocol was approved by the animal care and use committee (IACUC) of the Uniformed Services University of the Health Sciences, Bethesda, MD. Male Sprague-Dawley rats were used for all studies (Taconic Biosciences, NY). Animals were housed individually in an environmentally controlled room (20–23 °C, ~44% humidity, 12 h light/dark cycle, 350–400 lux, lights on at 6:00 am), with food (Teklad Global 18% protein #2018 rodent diet; Harlan Laboratories, IN) and water available continuously.

2.2. Reagents and sample preparation

High specific activity [^{3}H]-2-PAM (20 Ci/mmole) was custom synthesized (American Radiolabeled Chemicals, Inc., MO) using a published method (Balan et al., 1993). Other reagents were from Sigma–Aldrich. Preparations for intranasal administration were made by mixing 10–20 μCi of [3H]-2-PAM with 2 mg of unlabeled 2-PAM in sterile saline.

2.2.1. Age and weight comparison

Adult male Sprague-Dawley rats 100 g (~4 weeks), 200 g (~6 weeks), 400 g (~12 weeks) and 600 g (over 5 months) were randomly assigned to two groups with 3–4 animals in each group. The POD device was used for all drug administrations in this set of experiments. The first group of rats received 2.0 μmoles of ^{3}H-2-PAM per 100 g of animal weight, with a specific activity of 1.65 μCi/μmole. The second group received 12.0 μmoles/animal, regardless of weight, again with a specific activity of 1.65 μCi/μmole. All the animals received ^{3}H-2-PAM in the same volume of sterile saline. Animals were anesthetized with 5% isoflurane (4 min) prior to the intranasal application procedure. For these experiments rats were kept in supine position, and 20 μl of solution were administered bilaterally using the POD device; 10 μl/nostril with an interval of 1 min between the two administrations. Rats were kept in a supine position after the drug delivery until they were conscious (5–10 min).

To test the effect of age on INDD, male Sprague-Dawley rats, young (200 ± 50 g, ~6 weeks) and old (2 kg ± 250 g, ~18 months) were randomly assigned to groups with 3 animals in each group. Animals received 12 μmole of ^{3}H-2-PAM, specific activity = 0.83 μCi/μmole in the same volume of sterile saline as above, using the POD. Animals were kept in the supine position until they recovered from anesthesia.

2.2.2. Technique comparison

In the next investigation we compared INDD between the more commonly used supine position (Apostolatos et al., 2012; Baker and Spencer, 1986; Thorne et al., 2004) and an upright position (Xiao et al., 2013) during drug administration (Fig. 1). We also compared administering the drug by micropipette application through the nares to administration using the POD device with aerosol propellant (Brown and Liu, 2014). The micropipette technique involved similar procedures as above except that the same volume of the drug (10 μl per nostril) was given drop-wise using a micropipette. Animals in the upright position group were held in upright after drug delivery until they became conscious (approx. 5 min).

2.3. Tissue collection

Thirty minutes after intranasal [^{3}H]-2-PAM administration the rats were deeply anesthetized with a pentobarbital based prepara-