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Effect of Administration Method, Animal Weight and Age on the Intranasal Delivery of Drugs to the Brain

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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 (^3H -2-PAM) to the brain of rats.

Results—We found that using upright vs. supine posture did not significantly affect ^3H -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.

Keywords

blood brain barrier; posture; intranasal delivery device; glymphatic system; bioavailability

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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, Emory et al., 1995; Thorne, Pronk et al., 2004a). Drugs delivered intranasally reach the CNS parenchyma via perivascular convection along blood vessels associated with the olfactory and trigeminal nerves (Lochhead, Wolak 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, Arun et al., 2016). We have also shown rapid intranasal delivery of the bacterial enzyme chloramphenicol acetyltransferase in the active form to different brain regions as a model for enzyme therapy in the CNS (Appu, Arun 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, Pronk et al., 2004b). INDD circumvents first-pass hepatic metabolism (Miller, Ashford et al., 2008), can transport active enzymes (Appu, Arun et al., 2016), rapid onset to the CNS in therapeutic doses (Krishnan, Arun et al., 2016; Yamamoto, Iseki 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, Tambe et al., 2012; Yamamoto, Iseki 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, Boyden 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, Hickie 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, Pronk et al., 2004b) 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 labelled pralidoxime (^3H -2-PAM) in order to evaluate the feasibility of INDD of oximes for neuroprotection against organophosphate threat agents (Krishnan, Arun 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 ^3H -2-PAM in different brain regions. First, we tested the effect of age/weight by studying the bioavailability of ^3H -2-PAM in different age/weight groups of rats (100g, 200g, 400g and 600g) by intranasal delivery of the same dose of ^3H -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.

Materials and Methods

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 hour 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.

Reagents and sample preparation

High specific activity [^3H]-2-PAM (20 Ci/mmol) was custom synthesized (American Radiolabeled Chemicals, Inc., MO) using a published method (Balan, Barness 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 2mg of unlabeled 2-PAM in sterile saline.

a) Age and weight comparison—Adult male Sprague-Dawley rats 100 gm (~ 4 weeks), 200 gm (~ 6 weeks), 400 gm (~ 12 weeks) and 600 gm (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 ^3H -2-PAM per 100g of animal weight, with a specific activity of 1.65 $\mu\text{Ci}/\mu\text{mole}$. The

second group received 12.0 $\mu\text{moles/animal}$, regardless of weight, again with a specific activity of 1.65 $\mu\text{Ci}/\mu\text{mole}$. All the animals received ^3H -2-PAM in the same volume of sterile saline. Animals were anesthetized with 5% isoflurane (4min) 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 gm, ~ 6 weeks) and old ($2\text{Kg} \pm 250\text{g}$, ~ 18 months) were randomly assigned to groups with 3 animals in each group. Animals received 12 μmole of 3H-2-PAM, specific activity = 0.83 $\mu\text{Ci}/\mu\text{mole}$ in the same volume of sterile saline as above, using the POD. Animals were kept in the supine position until they recovered from anesthesia.

b) Technique comparison—In the next investigation we compared INDD between the more commonly used supine position (Apostolatos, Song et al., 2012; Baker and Spencer, 1986; Thorne, Pronk et al., 2004b) and an upright position (Xiao, Davis et al., 2013) during drug administration (Figure 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).

Tissue collection

Thirty minutes after intranasal [^3H]-2-PAM administration the rats were deeply anesthetized with a pentobarbital based preparation (Fatal Plus – 100 mg/kg, i.p) and perfused transcardially with PBS (200 ml), brains were dissected on ice into specific regions including olfactory bulb, frontal cortex (including frontal cortex and striatum), brainstem (including medulla and pons), cerebellum, midbrain (including mesencephalon and thalamus), hippocampus, trigeminal ganglia, spinal cord and cortex, (including parietal, occipital and external temporal cortex). Before perfusion with PBS, whole blood was collected in heparinized tubes. Samples were rapidly frozen on dry ice and stored at -80°C until analyzed. Tissue processing and sample counting were done as described previously (Krishnan, Arun et al., 2016).

Statistics

Values are expressed as means $\pm$ SD. One-way ANOVA (post-hoc-LSD) were used to compare the treatments among the 4 groups. For the data involving the older (18 month) animals, where we compared only two groups, we used independent student's t-tests. For both ANOVA and student's t-tests $p < 0.05$ was considered to be significant.

Results

The effects of age and weight on intranasal brain delivery are given in Figures 2 and 3. INDD was equally effective at all ages/weights tested. As previously reported (Krishnan, Arun et al., 2016), the highest concentration of ^3H -2-PAM was detected in the olfactory bulbs in all the age groups. Also, as expected, lower levels were detected in the younger age groups in the study in which ^3H -2-PAM was given on a per weight basis: younger groups have lower weights and hence received lower doses of ^3H -2-PAM. In contrast, in the experiment in which animals in each weight group all received the same dose of ^3H -2-PAM, the levels in different brain areas were similar indicating that intranasal delivery method is equally effective regardless of animal weight and the stage in development.

Results of the experiments comparing supine vs. upright posture of the younger rats with and without a delivery device are given in Figure 4. The two methods were found to be generally comparable, with the POD delivery device being more effective in the upright position in the olfactory bulbs, but not in other brain areas. Effectiveness of the device was less clear in the supine position. No differences in ^3H -2-PAM concentration were observed in the blood using the different delivery methods.

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 older rats (18 months). Based on the results of the IN technique comparison in 100 gm to 600 gm young adult rats we restricted the analysis of IN administration in older age rats to the supine position only (Figure 5 and Table 1). When given the same amount of ^3H -2-PAM (12.0 $\mu\text{moles/animal}$) irrespective of animal weight, we found that the concentrations of ^3H -2-PAM in different brain areas were somewhat lower in the older rats when compared to the young ones. However, when adjusted for the weight difference, the concentrations reached were somewhat higher in the older rats (Table 1B). This is consistent with our earlier results in which we found a dose dependent increase in the 2-PAM concentrations in different brain areas after intranasal brain delivery (Krishnan, Arun et al., 2016).

Discussion

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, Arun et al., 2016). This finding is highly relevant to civilian and military scenarios involving chemical accidents or terrorism (Jett, 2007). To the best of our knowledge, the present study is the first in which intranasal delivery of solutes into different areas of the brain has been compared in upright vs supine positions. In an earlier study, intranasal delivery of hydrocortisone (HC) into the cerebrospinal fluid (CSF) was compared in the upright vs. three supine positions: supine -90° angle, supine- 70° angle and supine- 45° angle (van den Berg, Romeijn et al., 2002). Placing the rat in the supine- 90° angle position increased absorption of HC into plasma and CSF about 2 – fold compared to the upright- 90° angle position. The supine- 70° angle did not change the HC CSF and plasma levels compared to the supine- 90° angle position. Thus, these studies showed that supine- 70° angle

and supine-90° angle positions are well suited for studying intranasal transport of chemicals and drugs into the CSF.

The present studies show that intranasal brain delivery of 2-PAM in both supine and upright postures provides similar results in rats (Figure 4). 2-PAM was detected in all regions examined but we observed substantial animal to animal variability in delivery. Animal weight significantly affected delivery to the blood, midbrain, cortex and spinal cord when animals were given an equal dose of drug, with lower concentrations observed in the larger rats (Figure 2). When the dose delivered was based on animal weight we only observed increased concentrations of 2-PAM in the olfactory bulbs and blood (Figure 3). We found that the POD delivery device was not superior to simple pipette application in most cases. The POD significantly increased 2-PAM delivery to the olfactory bulb in the upright position (Figure 4A). While the effect was modest, we did observe a significant improvement in drug delivery with the POD device to the spinal cord when used in the upright position as compared with the supine position (Fig. 4B). As expected, the effect of the delivery device was not detectable in the blood (Figure 4A). It should be emphasized that the present studies were based on radiolabeled 2PAM, a positively charged and small molecular weight molecule. While bulk flow mechanisms indicate that all molecules will behave more or less in a similar way, this question needs to be examined further and confirmed in future studies using molecules differing not just in size but also in charge. As mentioned above, we found that the delivery device is most effective for delivery to the olfactory bulb in the upright position. Whether this pattern holds true for all types of molecules and in all species remains unclear and needs further investigations.

Conclusion

INDD is a rapid and reliable method for brain delivery of therapeutic compounds. However, bioavailability is limited by the rate of bulk flow within the finite capacities of the olfactory and trigeminal pathways to the brain (Kumar, Gautam et al., 2016). Different positions and delivery techniques had minimal effects on the bioavailability of the drug in the brain areas measured. As expected, larger animals required higher doses to achieve the same drug concentration throughout the brain.

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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

Figure 1. Animal IN administration positions

Supine position, where the animal was anesthetized and kept flat on a table during and after drug delivery (A). Upright position, where the animal was held in hand perpendicular to the table during and after drug delivery (B).

Figure 2. The effect of animal weight on the concentration of 2-PAM in different brain regions after dosing with the same amount of 2-PAM per animal

2-PAM bioavailability was highest in the olfactory bulbs, trigeminal ganglia and blood (A) as compared with the brain regions examined (B). Error bars represent standard deviation ($n = 3$ per group; * = $P = 0.05$, ** = $P = 0.01$, *** = $P = 0.001$ relative to 100 gram group). Animals in both age groups were kept in a supine position for drug delivery using the aerosol POD device.

Figure 3. The effect of animal weight on the concentration of 2-PAM in areas examined and blood with dosing based on a μCi per gram animal weight basis

Values show 2-PAM concentrations in olfactory bulbs, trigeminal ganglia and blood (A) as well as in different brain regions (B). Animals in both age groups were kept in a supine position for drug delivery using the aerosol POD device. Error bars represent standard deviation ($n = 3-4$ per group, $** = P < 0.01$, P , $*** = P < 0.001$).

Figure 4. Delivery method comparison

The effect of different intranasal delivery techniques (micropipette vs. aerosol POD IN device) on the concentration of 2-PAM in olfactory bulbs, trigeminal ganglia and blood (A) and in different brain regions (B). Error bars indicate standard deviation (n=3 except for midbrain where n=2, * = P 0.05, ** = P 0.01).

Figure 5. Delivery method comparison in different age groups

The effect of different intranasal delivery techniques on the concentration of 2-PAM in the olfactory bulbs, trigeminal ganglia and blood (A and B) and in different brain areas (C and D). Values from young animals are shown in the graphs on the left, data for older rats are given in the graphs on the right. Animals were not given doses based on their body weight. We compared the effect of micropipette versus aerosol POD device application with animals in the supine position. Error bars represent standard deviation (n = 3 per group).

Table 1

A: Age comparison: We compared 2-PAM concentrations (micromolar) in the brains of young (~5 months) and old (18 months) rats. We found that the concentrations of 2-PAM in different brain areas were comparable despite the fact that the old age animals were not dosed based on their weight. Animals in both age groups were kept in a supine position for drug delivery using the POD device.

Animal weight	Olfactory bulb	Frontal cortex	Cortex	Midbrain	Hippocampus	Brainstem	Cerebellum	Spinal cord	Trigeminal ganglion	Blood
600 ±50g	38.4±10	1±0.1	1±0.4	0.4±0.2	0.7±0.1	0.9±0.4	1.1±0.2	0.6±0.5	13±1.3	11±2.1
2000 ±250g	15±6	0.8±0.5	0.5±0.1	0.9±0.1	0.7±0.2	0.7±0.2	0.7±0.5	0.4±0.1	3±1.6	6.2±1.4

B: 2-PAM uptake on a per gram basis. Adjusting for animal weight (from Table 1A), the 2-PAM concentrations in older rats were higher on a per gram basis than in the smaller, younger rats.

Animal weight	Olfactory bulb	Frontal cortex	Cortex	Midbrain	Hippocampus	Brainstem	Cerebellum	Spinal cord	Trigeminal ganglion	Blood
600 ±50g	38.4±10	1±0.1	1±0.4	0.4±0.2	0.7±0.1	0.9±0.4	1.1±0.2	0.6±0.5	13±1.3	11±2.1
2000 ±250g	49±19	3±1.6	2±0.5	3±0.3	2±0.7	2±0.7	2±0.1	1±0.5	9±5	20±5