Brain Uptake of Neurotherapeutics after Intranasal versus Intraperitoneal Delivery in Mice

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Abstract

There is a growing global prevalence of neurodegenerative diseases such as Alzheimer’s disease and dementia. Current treatment for neurodegenerative diseases is limited due to the blood brain barrier’s ability to restrict the entry of therapeutics to the brain. In that context, direct delivery of drugs from nose to brain has gained emerging interest as an important alternative to oral and parenteral routes of administration. Although there are considerable reports showing promising results after intranasal drug delivery in various disease-models and investigatory human clinical trials, there are very few studies showing a detailed pharmacokinetics with regard to the uptake and retention of intranasally delivered material(s) within specific brain regions, which are critical determining factors for dosing conditions and optimal treatment regimen. This investigation compared a time-dependent brain uptake and resident time of various radiolabeled candidate neurotherapeutics after a single bolus intranasal or intraperitoneal administration in mice. Results indicate that the brain uptake of intranasally delivered therapeutic(s) is > 5 times greater than that after intraperitoneal delivery. The peak uptake and resident time of all intranasally delivered test therapeutics for all brain regions is observed to be between 30min-12h, depending upon the distance of brain region from the site of administration, followed by gradual fading of radioactive counts by 24h post intranasal administration. Current study confirms the usefulness of intranasal administration as a non-invasive and efficient means of delivering therapeutics to the brain to treat neurodegenerative diseases including Alzheimer’s disease.

Keywords: Alzheimer’s disease; Nose to Brain delivery; GLP1; Anti–Aβ antibodies; Erythropoietin; Curcumin

Introduction

Growing world population with longer life expectancy has resulted in increased number of “aged” population with a greater prevalence of neurodegenerative diseases (ND) such as Alzheimer’s disease (AD). Currently, there is no cure for ND/AD [1]. The greatest challenge in curing ND/AD is the accessibility and bioavailability of therapeutics to the brain. In that context, intranasal delivery of therapeutics to the central nervous system (CNS) has emerged as a prospective alternative to parenteral routes of administration in treating ND/AD [1–9]. Intranasal delivery bypasses blood brain barrier (BBB) and circumvents systemic extraction of drugs, targeting therapeutics to the brain via olfactory, rostromigratory stream (RMS) and trigeminal pathways [4–13]. PubMed Literature search from late 1990s–to date, indicates that there have been > 200 studies reported thus far showing the utility of intranasal route as an effective means of delivering therapeutics to the CNS, some of which include intranasal delivery of benzodiazepine(s) [14,15], glucocorticoids/steroids/hormones [16–19], neurotrophic growth factors [20–31], vaccine antigens [32,33], Aβ immunogens [34–37], insulin [38–45], insulinomimetics/incretins [46–48], acetyl cholinesterase (AChE) inhibitors (AChEI) [49–58], and other candidate therapeutics [59–64]. Out of all reported intranasal studies, only few have demonstrated delivery of therapeutic antibodies utilizing intranasal route [65–69] including our recently published work [11,70]. Among few studies showing brain transit and pharmacokinetic of intranasally delivered materials [15,56,57,71,72], only one study showed brain-region-specific time-dependent uptake of intranasally delivered materials [46]. This investigation compares brain-region-specific time-dependent uptake of intranasally versus intraperitoneally delivered selected neurotherapeutics in the mouse brain including human recombinant erythropoietin (rhEpo), Curcumin, glucagon-like peptide 1 (GLP1) and anti– Aβ antibodies raised against specific amino acid (aa) epitopes of Aβ peptide.

Materials and Methods

Animals

Three month old mice (C57BL/6j), obtained from Jackson labs, Inc. (Bar Harbor, ME), are used in this study. This study compares the uptake of I-125 labeled test therapeutics in different brain regions of mice at different time points after intranasal (IN) or intraperitoneal (IP) administration. All experiments are approved and authorized by the local Institutional Animal Care and Use Committees at the Jesse Brown VA Medical Center and University of Illinois at Chicago. Animals are divided into nine major groups, analyzed at five different time points after IN and IP administration (N = 4/each time-point/group). Each group is studied and analyzed as an independent experiment and compared for IN vs IP delivery of all test materials at each time point for each group.

- Group 1: Mouse non-immune IgG (NG) (Abcam, Cat. # ab37355)
- Group 2: N-terminal anti–Aβ IgG2b MOAB-2 antibody raised against recombinant oligomeric Aβ 42 (not specific for Aβ42) (MOAB-2) (Abcam, ab126649)
- Group 3: N-terminal anti–Aβ IgG1 antibody (1-17aa Aβ epitopes) (N-anti–Aβ IgG1) (Abcam, ab11132)
- Group 4: N-terminal anti–Aβ IgG2a antibody (5-16aa Aβ epitopes) (N-anti–Aβ IgG2a) (Abcam, ab17250)
- Group 5: N-terminal anti–Aβ IgG2b antibody (19-22aa Aβ epitopes) (N-anti–Aβ IgG2b) (Covance Research products, SIG-39200)
- Group 6: C-terminal anti–Aβ IgG1 antibody (38-43aa of C-terminus Aβ1-43) (C-anti–Aβ IgG1) (Abcam, ab22258)
- Group 7: GLP1 (Alpha Diagnostics International, RP-1506)
- Group 8: Curcumin (90% Pure, Cayman Chemical, Item # 81025, CAS # 458-37-7)
- Group 9: Human recombinant Erythropoietin (rhEpo) (R&D Systems, 287-TC-500)
Treatment

Each group is administered with a single bolus IN (5μg/5μl/nostril = total dose of 10μg/10μl per mouse) or IP (100μg/100μl per mouse) of I-125 labeled test neurotherapeutics listed above. The radio-labeling is performed at the institutional core facility by technical experts using “Iodobead” kit (Pierce) as per manufacturer’s instructions, which ensures ~90% efficiency of iodination. The samples from different brain regions including olfactory lobes (OL), cerebral cortex (CTX), hippocampus (HP), and cerebellum (CBM) are collected at different time-points (30 min, 4h, 8h, 12h, 24h) following a single bolus IN or IP administration. The brain regions are homogenized in sterile saline (µg brain tissue/µl sterile saline) and 100µl of homogenate equating 100µg of brain tissue is recorded in the gamma scintillation counter. Data are statistically analyzed using GraphPad Prism Program to obtain respective group means with standard deviation (SD), and expressed as Mean ± SD (cpm/100µg) (Figures 1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B). Means are analyzed by 2-tailed t-test to compare the brain-regional uptake of IN vs IP administration. A value of p < 0.05 is considered statistically significant. Means are used to derive the ratio of cerebral uptake after IN administration vs IP administration, and are represented as “fold-increase”. (Table 1)

(Fold Increase = Brain-Region Uptake after IN Delivery / Brain-Region Uptake after IP Delivery)

Results

Results show that olfactory uptake of neurotherapeutics after IN delivery was ~7 ± 2 times greater than that after IP delivery. The olfactory uptake of all IN delivered neurotherapeutics is observed to peak at 30 min with a total resident time up to 12h which gradually is found to decrease by 24h post IN administration (Table 1, Figures 1A & 1B); while IP delivered neurotherapeutics, exhibit highest uptake in olfactory lobes at 4 h post-delivery with gradual decrease by 24 h. On the other hand, cortical uptake of IN delivered neurotherapeutics is observed to peak at 4h post-delivery with a total resident time up to 12h which is found to fade by 24h. Cortical uptake of IP delivered neurotherapeutics is observed to peak between 4-8 h post-delivery fading by 24 h. Cortical uptake of neurotherapeutics after IN delivery is ~6 ± 2 times greater than that after IP delivery (Table 1, Figures 2A & 2B). The hippocampal uptake of IN delivered neurotherapeutics is observed to peak at 8 h post-delivery with a total resident time up to 12 h declining by 24 h. IP delivery exhibits similar trend of hippocampal uptake. Hippocampal uptake of IN delivered neurotherapeutics is observed to peak at 12 h rapidly declining by 24 h. IP delivery does not exhibit peak cerebellar uptake at any particular time point, rather it is observed to be at the same level at all time-points. Cerebellar uptake of neurotherapeutics after IN delivery is ~5 ± 2 times greater than that after IP delivery (Table 1, Figures 3A & 3B). In summary, current results show significant low uptake of IP delivered materials in all brain regions analyzed. Although the peak uptake of all IN delivered neurotherapeutics in different brain regions is spaced out according to the distance of brain region from the site of administration, all test therapeutics are retained up to
12 h in all brain regions. The ratios of IN/IP delivery indicate peak uptake for olfactory lobes at 30 min, 4 h for cerebral cortex, 8 h for hippocampus and 12 h for cerebellum (Table 1). All values for IN delivery are significantly higher than those for IP delivery for all brain regions, all time points (p < 0.0001). Currently observed fading of IN delivery materials by 24 h is consistent with previously observed clearance of IN delivered horse radish peroxidase (HRP) labeled anti-Aβ antibodies [11,70].

Discussion

Historically, intranasal drug delivery has been utilized for local treatments such as allergies, etc. Recently, the use of intranasal route as a means of delivering therapeutics to the CNS has gained tremendous interest and momentum [73]. The blood cerebrospinal fluid (CSF) barrier (BCSFB) and BBB protect the CNS by limiting the entry of toxic substances into the CNS, limiting the entry of therapeutics into the CNS [74–76]. In that regard, intranasal route holds a great potential as a non-invasive practical approach of delivering drugs to the CNS that circumvents systemic extraction/alteration [73]. The major part of the nasal cavity both in human and rodents is covered by respiratory epithelium, across which drug absorption can be efficiently achieved. The unique anatomical and physiological characteristics of nasal mucosa such as the large surface area for drug absorption and close proximity to CNS and CSF [4,77–79] facilitate drug uptake despite minor limitations posed by nasal milieu itself, i.e. exo-/endo-peptidase(s)-mediated degradation of drugs or mucociliary clearance [77,79]. The olfactory epithelium is located just below the cribiform plate separating the nasal cavity from the cranial cavity. The olfactory epithelium (besides olfactory supporting cells and basal cells), contains olfactory sensory bipolar neurons (OSNs) with a single dendritic process bearing non-motile cilia, and with fine non-myelinated axons that connect with neighboring axons forming a bundle surrounded by glial cells penetrating into the cranial cavity through small holes in the cribiform plate which merge with the afferent axons connected to the olfactory tracts of the olfactory bulb [77]. Thus, OSNs congregate to connect with the CNS. Intranasal administration is known to utilize three potential pathways i.e. olfactory, trigeminal and RMS routes to reach CNS [11,80]. In addition, intranasally delivered materials also utilize extracellular diffusion along the open inter-olfactory clefts directly to the olfactory bulb/subarachnoid space/CSF [11,80]. IN route of administration has been exploited to deliver neurotrophic factors [80,81], cytokines [3], neuropeptides [82], and antibodies [11,83]. Despite considerable research in the field of intranasal administration targeted at nose to brain delivery of therapeutics, there are scant studies showing detailed brain-region-specific time-dependent uptake of intranasally delivered therapeutics, which is a critical determining factor for dosing conditions and optimal treatment regimen. In that regard, current study has significantly contributed detailing the entry, uptake and resident-time of intranasally delivered neurotherapeutics in mouse brain in comparison with the intraperitoneally delivered materials reaching the brain. Intranasally delivered therapeutics certainly has advantages not only with regard to efficient delivery but also with regard to their availability as an unaltered material since it bypasses systemic/hepatic extraction. Our observations that the
Table 1: Increased brain uptake of various neurotherapeutics after intranasal (IN) delivery.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Non-Immune Globulin (NG)</th>
<th>N-term MOAB Anti-Aβ antibody</th>
<th>N-term IgG1 Anti-Aβ antibody</th>
<th>N-term IgG2a Anti-Aβ antibody</th>
<th>N-term gG2b Anti-Aβ antibody</th>
<th>C-term Anti-Aβ antibody</th>
<th>Recombinant Human Erythropoietin (rhEpo)</th>
<th>Curcumin</th>
<th>Glucagon-Like Peptide 1 (GLP1)</th>
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<tr>
<td>30 min</td>
<td>6.99 (T)</td>
<td>5.13 (T)</td>
<td>5.77 (T)</td>
<td>4.59 (T)</td>
<td>4.66 (T)</td>
<td>5.67 (T)</td>
<td>5.08 (T)</td>
<td>6.79 (T)</td>
<td>6.55 (T)</td>
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<tr>
<td>4h</td>
<td>6.81 (T)</td>
<td>4.99 (T)</td>
<td>3.05 (T)</td>
<td>5.68 (T)</td>
<td>4.36 (T)</td>
<td>4.13 (T)</td>
<td>5.33 (T)</td>
<td>5.19 (T)</td>
<td>5.17 (T)</td>
</tr>
<tr>
<td>8h</td>
<td>6.67 (T)</td>
<td>4.87 (T)</td>
<td>2.59 (T)</td>
<td>4.02 (T)</td>
<td>3.44 (T)</td>
<td>3.75 (T)</td>
<td>3.48 (T)</td>
<td>4.68 (T)</td>
<td>4.87 (T)</td>
</tr>
<tr>
<td>12h</td>
<td>5.12 (T)</td>
<td>3.14 (T)</td>
<td>2.42 (T)</td>
<td>3.98 (T)</td>
<td>2.88 (T)</td>
<td>3.11 (T)</td>
<td>2.34 (T)</td>
<td>3.15 (T)</td>
<td>3.72 (T)</td>
</tr>
<tr>
<td>24h</td>
<td>3.66 (T)</td>
<td>2.48 (T)</td>
<td>1.47 (T)</td>
<td>2.11 (T)</td>
<td>2.48 (T)</td>
<td>2.19 (T)</td>
<td>1.32 (T)</td>
<td>2.11 (T)</td>
<td>2.13 (T)</td>
</tr>
</tbody>
</table>

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References


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