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Wheat germ agglutinin enhanced cerebral uptake of anti-Aβ antibody after intranasal administration in 5XFAD mice

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ABSTRACT

Alzheimer's disease (AD) is the 6th leading cause of death in United States afflicting >5 million Americans. This number is estimated to triple by the middle of the century if effective treatments are not discovered. Current therapy for AD is mainly symptomatic. Effective disease-modifying treatments are needed that would eliminate the cause rather than the symptoms of the disease. Polymerization of monomeric beta-amyloid peptide (Aβ) into dimers, soluble oligomers and insoluble fibrils is considered the prime causative factor in triggering AD pathogenesis. Based on these facts, removal/reduction of Aβ has gained importance as a primary therapeutic target in treating the cause of the disease. In that regard, passive immunotherapy with direct delivery of anti-Aβ antibodies to the brain has shown great promise, but awaits the challenge of overcoming greater influx of anti-Aβ antibody into the brain. This investigation was undertaken to maximize direct delivery of immunotherapeutics to the brain by using wheat germ agglutinin (WGA) as a novel axonal transporter-carrier to be conjugated with anti-Aβ antibody (6E10) raised against EFRHDS 3–8 amino acid (aa) epitopes of Aβ known to react with 1–16 aa residues of mono-/di-/oligomeric Aβ. This is the first report showing the use of WGA as an efficient axonal transporter carrier that not only enhanced the influx of anti-Aβ antibody directly into the brain but also resulted in greater reduction of cerebral Aβ compared to the unconjugated anti-Aβ antibody delivered intranasally in Alzheimer's 5XFAD model.

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1. Introduction

Alzheimer's disease (AD) is an age-dependent progressive neurodegenerative disorder functionally characterized by mild cognitive impairment (MCI) at its onset leading to subsequent cognitive decline; and pathologically characterized by the deposition of β-amyloid (Aβ) neuritic plaques (NP) derived from β-amyloid precursor protein (APP), and deposition of neurofibrillary tangles (NFTs) resulting from abnormal phosphorylation of tau proteins within the brain parenchyma [1,2]. Since formation of Aβ is considered the key causative seeding event in Alzheimer's pathogenesis that produces neurotoxicity, synaptic degeneration, neuroinflammation, and tau phosphorylation, with concomitant cognitive deficits [3–7], removal/reduction of Aβ has been explored as the prime therapeutic target in Alzheimer's pre-clinical research. In that regard, immunotherapeutic strategies have shown great progress and promise over the past few decades. Antibodies to Aβ derived from active or passive immunization showed reduction of cerebral Aβ and improvement in cognitive deficits [8–14]. Although partially successful, all immunization strategies explored this far are posed with various limitations. By and large, passive immunization using anti-Aβ antibodies delivered directly to the brain has shown greater benefits. More specifically, selection of antibody and facilitation of greater influx of antibody into the brain are critical in advancing immunotherapy for Alzheimer's disease.

Intranasal route is largely considered as a non-invasive, simple and practical route for the delivery of therapeutics to the central nervous system (CNS) that can bypasses the blood–brain barrier (BBB) and systemic adversities. The unique anatomic and physiologic characteristics of nasal mucosa such as the large surface area available for drug absorption and close proximity to CNS and CSF [15–18] facilitate drug uptake despite minor limitations posed by nasal milieu itself, i.e. exo-/endo-peptidase(s)-mediated degradation of drugs or mucociliary clearance [16,18]. The olfactory epithelium is located just below the cribriform plate separating the nasal cavity from the cranial cavity (Fig. 1). Besides olfactory supporting cells and basal cells, the olfactory epithelium contains olfactory sensory bipolar neurons (OSNs) (Fig. 1, blue double-lined

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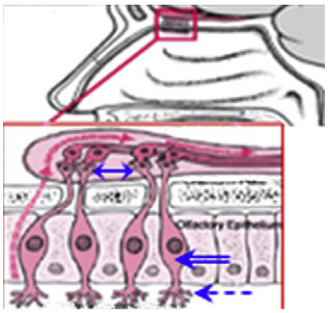

Fig. 1. Schema showing the intranasal route of transfer of materials to the brain. Pink outlined inlet showing olfactory epithelium located just below the cribriform plate separating the nasal cavity from the cranial cavity. The olfactory epithelium contains olfactory supporting cells, basal cells, and olfactory sensory bipolar neurons (OSNs) (blue double-lined arrow) with a single dendritic process bearing non-motile cilia (blue dotted arrow), and 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 cribriform plate (blue two-sided arrow). A pink dotted arrow indicates receptor-mediated endocytosis into the OSNs followed by intracellular transport to olfactory bulb. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

arrow) with a single dendritic process bearing non-motile cilia (Fig. 1, blue dotted arrow), and 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 cribriform plate (Fig. 1, blue two-sided arrow) [16] which merge with the afferent axons connected to the olfactory tracts of the olfactory bulb. Thus, OSNs congregate directly with the CNS.

Intranasal administration conventionally utilizes 3 potential pathways to reach CNS [19]: (i) adsorptive or receptor-mediated endocytosis into the OSNs followed by intracellular transport to the olfactory bulb (Fig. 1, pink dotted arrow); (ii) non-specific fluid phase endocytosis into the OSNs followed by intracellular transport to olfactory bulb (Fig. 1, pink dotted arrow); (iii) extracellular diffusion along the open inter-olfactory clefts directly to the olfactory bulb/subarachnoid space/CSF. The intranasal route has been used to deliver neurotrophic factors [19,20], cytokines [21], neuropeptides [22], and antibodies [23,24] to CNS. Enhancing intranasal delivery of therapeutics includes conjugation of candidate drugs with microspheres, liposomes, chitosan, cytodextrins, bile salts/surfactants and lectins [18]. Among all, the lectin wheat germ agglutinin (WGA) is unique in that it contains N-acetyl glucosamine and sialic acid, both of which are abundant in nasal mucosa, reducing the chances for self-rejection/removal by nasal mucosa [22,25]. Most importantly, WGA has a preferred selectivity for adsorptive endocytic uptake by OSN anterograde axonal transport to the olfactory bulb [19,26]. Thus, conjugation of a candidate drug with WGA is expected to enhance intranasal delivery of therapeutics to the brain both by enhancing active endocytic uptake and by passive diffusion.

Despite rigorous pre-clinical immunization approaches explored in the models of AD, there are additional challenges to be met in order to improve/advance immunotherapy. Considering the potential toxicity produced by mono/di/oligomeric Aβ, the immunotherapy that would target monomers, dimers, and oligomers of Aβ while simultaneously enhancing influx of immunotherapeutics into the brain, is expected to overcome most limitations of passive immunotherapy for AD. With this consideration, we chose anti-Aβ antibody 6E10, that is raised against EFRHDS 3–8 amino acid (aa) epitopes of Aβ, and is know to react with 1–16 aa residues of mono-/di-/oligomeric Aβ. Furthermore, in order to increase the influx of anti-Aβ antibody, we tested if conjugation of anti-Aβ antibody (6E10) with the unique axonal transporter carrier plant lectin-WGA [19,26] will enhance influx of intranasally delivered anti-Aβ antibody (6E10) into the brain.

This study investigated if conjugation of anti-Aβ antibody (6E10) with WGA will enhance cerebral uptake of anti-Aβ antibody and will efficiently reduce cerebral Aβ levels after intranasal delivery in 5XFAD mice, as evaluated by binding ability of WGA labeled anti-Aβ antibody to native Aβ plaques and by measuring of histological and biochemical profiles of cerebral Aβ.

2. Materials and methods

The 5XFAD mice harboring mutations APP K670N/M671L + I716 V + V717I and PS1 M146L + L286 V, were bred by crossing 5XFAD heterozygous male(s) (original founder breeder males obtained from Dr. Vassar, Northwestern University, Chicago, IL), with B6/SJL F1 females (Jackson labs, Bar harbor, ME) [27]. Presence of transgene was identified by PCR genotyping of tail genomic DNA with specific forward and reverse primers (Eurofins Operon, Fermentas Life Sciences). Since this study involved examination of binding of WGA-labeled anti-Aβ antibody to the cerebral Aβ plaques, only transgenic mice (Tgs) were included in the study.

Transgenic mice (Tgs) were divided into 4 groups ($N = 5$/group), each group intranasally administered with horse radish peroxidase (HRP)-labeled: (1) non-immune immunoglobulin (IgG) only (Covance) (Group 1: HRP + IgG); (2) WGA only (Group 2: HRP + WGA); (3) anti-Aβ antibody only (6E10, Covance) (Group 3: HRP + anti-Aβ antibody); (4) and WGA labeled anti-Aβ antibody (6E10, Covance) (Group 4: HRP + WGA + anti-Aβ antibody). Tagging IgG or anti-Aβ antibody either with WGA or with HRP, separately or together, was performed commercially (NOVUS Biologicals, LLC). Mice were administered with a total dose of 40 μg/mouse/week [(10 μg/5 μl/naris) = (20 μg/mouse), 2 × week = 40 μg/mouse, on Day 1 and Day 3/week]. All mice were euthanized at the end of the week on Day 7. Brains were harvested after decapitation under mild sedation and processed for frozen sectioning. The sections were subjected to standard diaminobenzidine chromogen development procedure to reveal HRP label [28]. No nuclear counter stain was used. Sections were subjectively observed by 3 independent investigators. Images were captured with the use of ImagePro and the densitometric or area measurements were performed with the use of NIH/ImageJ software. Total number and diameter of cortical Aβ plaques within 10 high power fields (hpfs) (200 μm^2/each hpf) bilaterally per animal with a total of 50 hpfs per group ($N = 5$/group) were analyzed.

Another set of experiment was conducted to measure cerebral levels of Aβ40/42 with the use of ELISA. Transgenic mice (Tgs) were divided into 4 groups ($N = 5$/group), each group intranasally administered with: (1) non-immune immunoglobulin (IgG) (Covance) alone (Group 1: IgG); (2) WGA alone (Group 2: WGA); (3) anti-Aβ antibody (6E10, Covance) (Group 3: anti-Aβ antibody); (4) WGA labeled anti-Aβ antibody (6E10, Covance) (Group 4: WGA + anti-Aβ antibody). Mice were administered with a total dose of 40 μg/mouse/week [(10 μg/5 μl/naris) = (20 μg/mouse), 2 × week = 40 μg/mouse, on Day 1 and Day 3/week]. All mice were euthanized on Day 7 at the end of the week.

All animal procedures were performed in accordance with the Jesse Brown VA Medical Center institutional Animal Care and Use Committee approval, National Institute of Health Guide for the Care and Use of Laboratory Animals, and policy and guidance of the Society for Neuroscience.

Brains were harvested after decapitation under mild sedation, homogenized in the modified RIPA buffer (85 mM HEPES, 250 mM NaCl, 5 mM $MgCl_2$, 10 mM EDTA, 5 mM NaF, 5 mM Na pyrophosphate, 1 mM PMSF, 1%Triton X100, 10% glycerol) con-