Review

Intranasal applications in Alzheimer's treatment

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative brain disease which does not currently have a fully known treatment. Research over the past 30 years has provided numerous treatment options to correct the underlying neurodegenerative pathology. The drugs investigated in these studies focus on Amyloid beta (Aβ) muscle acceleration, which prevents the accumulation of amyloid aggregates. Despite many critical discoveries, failures in late-phase clinical trials indicated that targeting Aβ pathology alone is not effective in altering disease progression. These discouraged researchers were prompted to search for new approaches, one of which was intranasal release methods. In this review, intranasal drug administration for the treatment of AD and the effects of insulin, erythropoietin, exosome, mesenchymal stem cells, and rifampicin administered by this route on AD will be discussed. It is foreseen that definitive treatment possibilities can be developed by further investigating this method.

Keywords: Alzheimer's disease, erythropoietin, exosome, insulin, intranasal administration, mesenchymal stem cells, rifampicin.

Alzheimer's disease (AD) generally affects individuals over age 65. With the growing elderly population, prevalence of the disease has increased.[1] While this has led to increased efforts to develop treatment, there is no definitive treatment of the disease.[2,3] More than 200 therapeutic agents have been evaluated as a result of studies, and no new drug has been approved by the FDA for the treatment of the disease since 2003.[4,5] Many speculations have been made regarding the failure of the applied treatments. The most important of these are the wrong choice of main treatment goals and misinterpretation of AD pathophysiology.[6]

Research suggests that the characteristic amyloid plaques and tau nodes in the brain are not a cause but an effect. Alzheimer's disease is a result of neuroinflammation and brain wound healing gone awry.[7] This neurologic circumstance results in cognitive and behavioral disorders. Conventional treatment strategies, such as acetylcholinesterase inhibitor drugs, are often ineffective due to their poor solubility, low bioavailability, and inability to permeate the blood-brain barrier.[8] These limitations associated with current therapy draw attention to the intranasal strategy. This strategy seems to be a promising route for the delivery of drugs to the brain.[9] Recent studies examine direct intranasal delivery of drug groups to the central nervous system (CNS) rather than oral or parenteral routes.[9]

INTRANASAL DRUG ADMINISTRATION IN THE TREATMENT OF ALZHEIMER'S DISEASE

Intranasal drug administration has emerged as an alternative to oral and parenteral routes. Since the olfactory nerve cells and trigeminal nerves are in direct contact with both the peripheral and the CNS, intranasally administration is a non-invasive method for allowing drugs to pass the blood brain barrier (BBB).[9] Accordingly, drugs can directly pass from the olfactory region and respiratory epithelium to the brain.[10,11] Thus, the treatment of neurological disorders is targeted.[12] Advantages compared to other drug administration routes include rapid onset of action, avoidance of the presystemic metabolism of the intestines and liver, reduced systemic exposure, direct administration to the brain and cerebrospinal fluid (CSF), ease of application, and better patient compliance.[9] Limitations of this route include weak nasal permeability and mucociliary clearance.[13] Studies show that drugs administered into the nasal cavity must have a more prolonged residence time to overcome nasal mucociliary clearance.[14]

Transport of drugs across the nasal barriers occurs intracellularly or extracellularly. The first step in intranasal transport is endocytosis to olfactory sensory neurons and then trigeminal ganglion cells respectively.[15] This pathway is referred to as intraneuronal transport. Since it is very slow, it may take 24 hours after nasal administration for agents to reach the CNS.[16] Then, intracellular transport to the olfactory bulb and the brain stem occurs. Transcytosis occurs by passive diffusion or by receptor-mediated endocytosis throughout the intracellular space and basolateral membrane. Diffusion into the olfactory bulb throughout the extracellular pathway associated with olfaction takes about 0.73-2.3 hours. Diffusion into the brain stem takes approximately 17-56 hours.[15] Substances acquired through this pathway are distributed through the nasal membranes to the blood, then to the olfactory mucosa and eventually to the CNS. This pathway is less effective than the transcellular route and is dependent on the molecular weight and size of the drug.[17] However, this mechanism is faster and allows drugs with low molecular weight to be delivered to the CNS in a few minutes.[18]

Nasal drugs can be cleared in the nasal cavity thanks to mucociliary clearance. The drug that enters blood circulation can be cleared with normal clearance mechanisms or may pass the BBB into the brain. The challenging aspect of application is ensuring adequate therapeutic levels of drug delivery to target areas of the brain. The drug's localization in the brain must target necessary receptors for managing CNS diseases such as Parkinson's disease, schizophrenia, AD, brain tumors, meningitis, and migraine.[9]

This method, proposed by Frey in 1989 for the treatment of AD and other CNS disorders,[19] is a promising approach for the delivery of existing drugs and alternative therapy molecules in AD.[9]

INTRANASALLY ADMINISTERED AGENTS IN ALZHEIMER'S TREATMENT

Insulin

The use of intranasal insulin in AD has been investigated. Studies have proven that intranasally administered insulin is rapidly distributed throughout the brain at the cribriform plate level and reverses learning and memory loss in the AD mouse model. Intranasal insulin poorly enters the bloodstream and has no peripheral metabolic effects. Protein kinase-C (PKC) inhibition occurs as a result of uptake from the cribriform plate into the brain. Thus, cellular pathway inhibitors play a role in the transport of insulin in the blood-brain barrier. These results suggest that the intranasal route is an effective means of delivering insulin to the brain.[20]

Patients with AD have reduced insulin receptor sensitivity in the brain.[21,22] Insulin has important functions in the CNS. Insulin receptors in the brain are found in the olfactory bulb, hypothalamus, hippocampus, cerebral cortex, and cerebellum.[23] Insulin signaling contributes to synaptogenesis and synaptic remodeling.[24] Acute uptake of insulin into the hippocampus improves spatial memory in a dependent manner on phosphatidylinositol 3-kinase (PI3K) by modulating glucose utilization.[25]

Intranasal administration or perfusion of insulin to healthy individuals improves cognitive

function.[26,27] Intranasal insulin administration before anesthesia prevented AD-like tau hyperphosphorylation in a widely used transgenic mouse model of AD.[28] Tau hyperphosphorylation, a pathological feature of AD, increases with anesthetic exposure.[29] This causes significant learning and memory impairments in elderly rodents.[30,31]

Enhanced brain insulin signal enhances memory in cognitively healthy people, indicating its neuroprotective features. These results indicate that increasing brain insulin concentrations in AD patients could prevent or slow down the development of this debilitating disease.[20]

In a clinical study on patients with AD,[32] patients given placebo or 20 or 40 IU insulin intranasally for four months and a positron emission tomography was performed before and after patients received intranasal insulin. Alzheimer's Disease Rating Scale was used to evaluate results, which showed that there was an improvement in delayed memory and cognitive function in the insulin group.

A single dose of intranasal insulin acutely improved memory in older adults with AD.[12] Insulin was effective in improving verbal memory test performance in the AD group. However, among these groups, only women with the Apolipoprotein-ε4 (APO-ε4) allele were unaffected by administration of high doses of insulin.[32] In another study, APO-ε4 positive AD patients were less responsive to insulin. Given that APO-ε4 carriers represent 40-65% of the late-onset AD population, it is suggested that intranasal insulin may have limited therapeutic use.[33] Although the studies conducted so far are promising, more clinical studies are needed. Little is known about the mechanism by which intranasally administered insulin reaches the brain. Some hypotheses regarding intranasal delivery of insulin to the brain include transport along axon bundles of olfactory receptor cells on the roof of the nasal cavity, transport along the trigeminal nerve pathway, and via the rostral migration flow.[34] Recent studies have shown that the rapid distribution of intranasally administered molecules via the olfactory tract or trigeminal pathway involves the bulk flow within the perivascular space of cerebral blood vessels.[35] All of these studies support that intranasally administered insulin is carried along the nasal epithelium. Ongoing studies are examining the regional distribution and course of action of administered insulin to investigate the effects on cellular mechanisms of brain intake and cognition in rodent models of AD.[20]

Erythropoietin

Erythropoietin (EPO) is a hematopoietic growth factor[36] and a glycoprotein cytokine involved in the regulation of erythropoiesis. It also has biological functions such as non-hematopoietic neuroprotection and neurogenesis.[37] For these reasons, EPO has begun to be used as a therapeutic agent for neurodegeneration.[36]

The use of EPO in clinical applications has not yielded positive results. This is due to the erythropoietic effect of EPO.[38] The use of recombinant human EPO (rh-EPO) in neurological diseases increases hematocrit and blood viscosity. This condition causes cardiovascular diseases such as infarction or stroke. To solve this problem, EPO derivatives lacking erythropoietic activity but preserve neuroprotective activity against neuronal injury have begun to be used.[39] Neuro-EPO was developed as a non-erythropoietic derivative of EPO with chemical modifications.[37]

Neuro-EPO is a recombinant human glycoprotein produced in Chinese hamster ovary cells and contains low sialic acid. Its most important feature is that it lacks erythropoietic activity while exhibiting high neuroprotective properties.[40] Neuro-EPO is rapidly degraded in the liver and is therefore more suitable for intranasal administration.[41] Thanks to the intranasal administration, it quickly reaches the brain and does not stimulate erythropoiesis after acute treatments.[40] The neuroprotective effects of Neuro-EPO, such as improving of cognitive function after stroke, have been studied in animal models of stroke.[40] In one study, Neuro-EPO was intranasally administered in doses of 62, 125 and 250 μg/kg in non-transgenic Alzheimer rodent models. Results of the study indicated that intranasal administration of Neuro-EPO prevented Aβ25-35 toxicity in the rodent hippocampus and showed a strong potential protection against amyloid toxicity. Apart from a few differences depending on dosage, Neuro-EPO showed a positive effect in the treatment of

memory loss. Doses such as 125 and 250 µg/kg were effective in most of the tests. However, the dose of 125 µg/kg showed a prolonged effect lasting up to 22 days.[42] Erythropoietin directly affects synaptic plasticity, especially in the hippocampus.[43,44] It has been determined that EPO increases primary hippocampal neuronal networks. That is, EPO improves hippocampus-derived memory by enhancing plasticity, synaptic connections, and neuronal networks.[44] Erythropoietin has a protective effect against inflammatory pathologies. TNFα and IL-1β released by A25-35 toxicity are blocked by Neuro-EPO. This neuroprotective ability of EPO is due to its ability to provide extrinsic cell homeostasis, by modulating microglial activation and controlling cytokine release.[45] Different forms of EPO show different affinities in activating EPO receptors. Higher concentrations are needed for the low sialic acid form of EPO to activate EPO receptors.[45] Another important feature of Neuro-EPO is that it can produced to be biologically similar to endogenous EPO without chemically modification, therefore causing less side effects in long-term use.[42]

Erythropoietin selectively induces the synthesis of the neuroglobin protein in damaged regions, providing angiogenesis and protection of the vascular endothelium. Lost functions in damaged regions of the brain are preserved by ensuring homeostasis.[42]

Intranasally EPO is currently being tested for the treatment of stroke.[40,46] Studies on intranasal EPO applications in neurodegenerative pathologies are ongoing.[42]

Exosomes

Microglial cells are the brain's macrophages. Microglial cells contribute to inflammation by producing interleukin in inflammatory diseases such as Alzheimer's. Therefore, it makes sense to target microglial cells for anti-inflammatory treatments.[47] No specific strategy for targeting brain microglial cells is in practice.[48] Drugs that target inflammatory cells without damaging normal tissues have been developed, but they do not cross the BBB.[49-51] Therefore, exosomes have been used to deliver anti-inflammatory drugs to the brain via a non-invasive intranasal route.[48] One of these anti-inflammatory drugs is curcumin, a natural polyphenol found in Curcuma

longa (turmeric) rhizomes. Curcumin is anti-inflammatory, antineoplastic, antioxidant, and chemopreventive.[52-54]

Since curcumin has poor solubility and poor bioavailability, it poses problems in clinical application.[55] To overcome this, exosomes have been used as nanoparticle drug carriers. Exosomes are 30-100 nm sized nanoparticles that can be secreted into the extracellular environment. Curcumin was loaded into exosomes at 22°C and then subjected to sucrose gradient centrifugation. Thus, curcumin was encapsulated to exosomes, increasing its *in vitro* solubility, stability, and bioavailability.[56] In order to demonstrate the therapeutic use of curcumin with exosomes (Exo-cur), mice in a lipopolysaccharide (LPS)-induced inflammatory state were used. Curcumin was loaded into exosomes and administered intranasally. The presence of curcumin in brain lysates was identified using high performance liquid chromatography one hour after Exo-cur application. The presence of curcumin in brain lysates was observed for up to 12 hours. This proves that microglial cells are selectively targeted by exosomes. Subsequently, a decrease in the number of activated inflammatory microglial cells was observed in the brains of LPS-treated mice. Since exosomes can be delivered to the brain in a rapid and selective manner, they are regarded as a vehicle of drug delivery.[47]

Studies have shown that brain microglial cells are targeted by exosomes, but do not address the mechanism of this selectivity. It is unknown whether exosomes enter recipient cells through the endosomal route,[57] or whether exosomes derived from T cells use the endosomal route for entry into microglial cells. The degree of exosomal entry into various regions of the brain and spinal cord, and whether or not glial cell populations like astrocytes are targeted by exosomes are also unclear. It is also unknown whether or not exosome uptake by microglial cells in the brain depends on inflammation, or whether it increases parallel to inflammation. When these questions are answered, it is thought that the applicability of intranasal exosome technology to neurological diseases will become clear.[47]

Mesenchymal stem cells

Alzheimer's disease is a disease of complex mechanisms that does not originate from a

single cause. Therefore, approaches to target the complex pathology of the disease are needed. It is thought that stem cells applications will provide multi-targeted therapies required for the solution of the disease.[58] The most important issue before starting stem cell therapy is selecting the correct stem cell. Mesenchymal stem cells (MSC) can be obtained from many types of cells and have ease of application. For these reasons, it is the most frequently studied type of stem cell.[59]

Mesenchymal stem cells are neuroprotective stem cells with anti-amyloidogenic activities.[60] Mesenchymal stem cells has a therapeutic effect on AD. Mesenchymal stem cells administration demonstrated anti-inflammatory and anti-amyloid properties in animal models of AD, resulting in improved memory.[59,60] Therefore, it is also promising for the treatment of various neurological diseases.[61]

Mesenchymal stem cells therapeutically restore degenerated neurons, provide neuroprotection through secretory factors, exert immunomodulatory effects on cells responsible for disease development, and proliferate endogenous cells.[62] However, implantation risks limit its clinical usage. These limitations include: the process of invasive cell isolation, loss of potency, limited lifetime, large-scale expansion cost[63] and a low possibility of uncontrolled cell proliferation.[63,64]

It has been stated that MSC act through paracrine mechanisms by releasing bioactive components when exposed to an injured environment, rather than through direct engraftment. Although studies have demonstrated the neuroprotective and anti-inflammatory effects of MSC in various disease models,[63,64] this was not observed in AD mouse models.[58] Studies have demonstrated that secretomes derived from MSC *in vitro* in an AD environment (MSC-CS) fully replicate multiple neuro-reparative activities in implanted mice.[58]

In transgenic mice with AD aged 22 to 25 months, repeated intranasal administration of MSC-CS resulted in a decrease in cortical and hippocampal plaque burden, the number of surrounding activated glial cells, and expression of the phagocytic marker CD68. However, the ability of MSC-CS to reduce the level of AβO suggests that MSCs have strong therapeutic

potential.[65,66] Mesenchymal stem cells-CS also reduces hippocampal atrophy and neuronal damage in the brains of transgenic mice.

Long-term MSC-CS treatment has yielded positive results.[58] This is attributed to the self-renewing, neuroprotective, and regenerative abilities of MSCs.[67] Mesenchymal stem cells treatment eliminates brain atrophy by plaque reduction and by decreasing in amyloid beta oligomer (AβO) concentrations. Thus, a preclinical, non-invasive, and continuous treatment model has been to reverse functional and structural damage in elderly AD mice.[58]

Rifampicin

Most studies up to now have investigate methods to eliminate the pathogenesis of neurodegenerative dementia such as cerebral accumulation of amyloid oligomers and tauopathy to treat AD.[68,69] Clinical studies have found that these treatments do not provide particularly good results in the cognitive levels of patients.[70,71] Such negative consequences have led to the introduction of rifampicin, an antibiotic that can be taken orally, has few side effects, and can reduce neurotoxic oligomers to a large extent, as a new method of preventing the disease.[72]

Rifampicin has been shown to have preventive effects against AD under certain conditions.[73] Therefore, preventive treatment should be initiated before the start of memory problems.[74]

Among patients without memory decline, having AD-type hypometabolism is sufficient reason to begin preventive therapy. Rifampicin has been used as a preventive treatment. Its effective dosage was considered to be 450 mg/day or higher (e.g. 600 mg/day). Rifampicin treatment must be continued for at least 12 months to achieve positive results in the elderly. It is thought that the memory impairment can be prevented if rifampicin is used continuously for several years.[73]

Orally administered rifampicin reduces amyloid beta (Aβ) and tau pathologies in mice. On the other hand, long-term use is not preferred because it has adverse effects such as liver damage. This situation has prompted scientists to seek a safer route of rifampicin administration. Therefore, the therapeutic efficacy and safety of rifampicin was evaluated by administering rifampicin

treatment for one month by oral, intranasal, and subcutaneous routes to transgenic AD mouse models. The results of the experiment indicated that intranasal or subcutaneous administration was better than oral administration at improving memory. In addition, a decrease was observed in neuropathologies such as Aβ oligomer accumulation, abnormal phosphorylation of tau, and loss of synapses. Pharmacokinetic studies show that the highest rifampicin level in the brain is achieved with the intranasal rifampicin administration strategy. Due to its ease of administration and non-invasive nature, intranasal administration is a reasonable option for long-term rifampicin dosages. The inexpensive availability of rifampicin also increases the drug's potential for long-term use.[75]

Many attempted methods to treat AD have failed. This has steered researchers towards different approaches. Intranasal administration provides direct delivery to the CNS unlike oral and parenteral routes. Since the olfactory nerve cells and trigeminal nerves are in direct contact with both the environment and the CNS, it is a promising non-invasive method to cross the blood brain barrier. In this manner, direct drug passage from the olfactory region and respiratory epithelium to the brain can be achieved, therefore allowing the treatment of the neurological disorder, AD. Advantages compared to other drug administration routes include rapid onset of action, avoidance of the presystemic metabolism of the intestines and liver, reduced systemic exposure, direct administration to the brain and CSF, ease of application, and better patient compliance. The limitations of this route are mucociliary clearance and poor nasal permeability of intranasally administered drugs. Many agents are applied or attempted to be applied with this route. Studies on mouse models have demonstrated that the intranasal administration of substances such as insulin, erythropoietin, exosomes, MSC, and rifampicin is applicable for the treatment of AD. Clarifying the uncertainties in treatment doses and mechanism of action will help to evaluate the applicability of these agents in AD treatment.

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