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Intranasal insulin and cell-penetrating peptides in the treatment of Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is the most common cause of dementia and is responsible for up to 75% of the nearly 47 million dementia cases worldwide. AD is the clinical manifestation of toxic β-amyloid and neurofibrillary tangles accumulation caused by altered proteostasis. Due to not fully understood molecular mechanisms of AD only symptomatic treatment is available. Considering that cognitive decline is associated with insulin resistance, metabolic alterations may be the primary causes of the AD and insulin therapy is proposed as a novel approach in AD treatment. Intranasal administration of insulin appears to be a profitable solution for drug delivery to the brain due to blood-brain barrier bypassing, higher bioavailability and the lack of systemic side effects. When applying this method, insulin is sprayed into the nasal cavity, enters the mucosa and is transported along the axon bundles to the brain. Clinical trials of AD treatment with intranasal insulin revealed improvement in cognition, verbal memory and functional status. Notably, enhancement in memory and cognition were observed both in healthy adults and in AD patients and were ApoE ε4-related. The use of cell-penetrating peptides (CPPs) improve cellular uptake of insulin, enhance bioavailability and increase the direct insulin transport into the deeper regions of the brain such as the olfactory bulb and hippocampus. Animal studies revealed beneficial effects on AD symptoms of other molecules applied in connection with CPPs and most of them approve CPPs formulations for use in clinical trials. This review article summarize clinical trials results of intranasal insulin administration in AD treatment and propose the use of CPPs as an additive to conventional and experimental therapies for AD.

***Keywords*:** Alzheimer's disease, cognitive function, dementia, anti-diabetic drugs, intranasal insulin, GLP-1 agonists, cell-penetrating peptides

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1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder leading to cognitive impairment, neuropsychiatric symptoms and disability. AD is the most common cause of dementia and the most prevalent neurodegenerative disorder – currently accounts for up to 75% of the nearly 47 million people that suffer from dementia [1]. 1-3% of people aged 65-70 are diagnosed with AD, however above 80 years of age the prevalence increases to 30-50% [2]. According to the systematic review based on 119 studies reporting the original data, the prevalence of dementia due to AD among individuals older than 60 years of age was 40.2 per 1000 persons (CI95%: 29.1-55.6), which proves the substantial burden of AD on society [3].

1. 1. Pathogenesis of AD

The major mechanism of AD is protein dyshomeostasis caused by decline in the capacity of proteostasis process during aging. Enzymatic processing of neuroprotective amyloid precursor protein results in formation of toxic β-amyloids (Aβ) aggregates composed of improperly folded chains of proteins. The balance between production and disposal of proteins is impaired which exacerbate proteotoxic stress. Aβ usually coexist with brain tissue neuroinflammation [4].

Pathogenesis of AD is described as a "dual proteinopathy". In addition to extracellular Aβ fibrils, there are also hyperphosphorylated tau (P-tau) aggregates localized intracellularly termed neurofibrillary tangles (NFTs). The gradual spread of NFTs correlates better with progression of cognitive deficits than does Aβ deposition, which is often diffuse at the time of symptom presentation [5].

It should be noticed that the imbalance of the proteostasis network occurs years or decades before clinical manifestation of the disease. The precise molecular mechanisms of AD are still not fully understood [4].

1. 2. Major symptoms

One of the first alteration observed after long pre-clinical phase in which neuropathological changes develop is subjective cognitive decline (SCD), however there are no neuropsychological tests which could confirm this diagnosis. SCD leads to mild cognitive impairment (MCI) defined as occurring of subtle deficits in performing daily activities in combination with objective cognitive dysfunction confirmed by neuropsychological assessment. The AD dementia stage is a progressive cognitive deterioration leading to certain functional deficits. Therefore, AD is described as a continuum and includes pre-clinical phase, SCD, MCI, and dementia due to AD. The clinical diagnosis of AD is often based on excluding other systemic and brain disorders that may lead to cognitive deterioration and is characterized by 81 and 70% sensitivity and specificity respectively [6].

1. 3. Current treatment of AD

The conventional pharmacotherapy of AD includes two basic strategies: increasing levels of acetylcholine inside the nervous synapses and protecting the nervous tissue from glutamate mediated excitotoxicity. The lowered concentrations of acetylcholine result in a progressive loss of cognitive and behavioral function.

Most commonly used drugs of the first group are donepezil and rivastigmine which inhibit the enzyme called acetylcholinesterase and thereby prevent acetylcholine degradation. Memantine, a glutamatergic antagonist, belongs to the second group of medicines used in AD treatment [7].

However, both these classes of drugs provide only the symptomatic relief. Over the last decade, more than 50 drugs candidates treating the underlying causes of AD have successfully passed phase II clinical trials, but none has been successfully used in phase III [4].

2. INTRANASAL INSULIN IN AD TREATMENT

Recent therapeutic approaches have been strongly influenced by five neuropathological hallmarks of AD: acetylcholine deficiency, glutamate excitotoxicity, extracellular deposition of Aβ, formation of intraneuronal NTFs and neuroinflammation [8]. Many researchers have focused on the Aβ hypothesis, however have failed to treat or prevent AD even when the effective clearing of Aβ deposits in AD brain was demonstrated. Alternative therapies with antioxidants, anti-inflammatory agents were also ineffective [9].

Metabolites, such as insulin, adiponectin, and reduced antioxidant defense mechanisms, could be effective therapeutic targets for AD treatments, considering that metabolic alterations may be the primary causes of the disease [10]. The group of anti-diabetic drugs for AD treatment consists of intranasal insulin, glucagon-like peptide-1 receptor agonists, metformin, peroxisome proliferator-activated receptor-γ agonists, leptin and amylin analogues [9].

2. 1. Role of insulin in AD pathogenesis

Recent investigations proposed the term 'Type-3-Diabetes' for AD because of the shared molecular and cellular features among Type-1 and Type-2-Diabetes and insulin resistance associated with memory deficits and cognitive decline in elderly individuals. It was proven that insulin plays a crucial role in the formation of amyloid plaques. Insulin is also involved in the activation of glycogen synthase kinase 3β causing phosphorylation of tau protein and formation of NFTs [11].

Insulin participate in neuronal maintenance, energy metabolism, neurogenesis, neurotransmitter regulation and via other mechanisms like neuronal firing and long-term potentiation plays important role in memory processes and cognitive activity [12]. As expected, the AD patients have lower insulin levels in cerebrospinal fluid (CSF), higher plasma insulin levels and reduced CSF-to-plasma insulin ratio when compared to healthy adults. The differences are greater for patients with more advanced AD [13,14]. Post-mortem human brain samples present significantly reduced levels of insulin and IGF-I polypeptide genes and their corresponding receptors in advanced AD than aged control brains. The abovementioned study results were connected with neuronal loss and reduced expression of acetylcholinesterase [15].

The fundamental issue is whether hyperinsulinemia and alterations in insulin signaling are causes or consequences of AD. The possible hypothesis is if patients experience hyperinsulinemia before symptoms of MCI or AD, elevated insulin levels could contribute to both AD pathology and insulin resistance. Alternatively, initial Aβ accumulation can be the

cause of neuronal insulin resistance and secondary hyperinsulinemia, which further exacerbates AD progression [16].

2. 2. Intranasal insulin

Recent reports suggest that peptide drugs such as insulin have the treatment potential in AD and other neurodegenerative diseases. Insulin cannot be administered orally because of its enzymatic degradation in the gastrointestinal tract, also intravenous administration for AD treatment is not recommended due to strong peripheral side effects of relatively high doses such as hypoglycemia. Moreover, blood-brain barrier (BBB) limits penetration of drugs to a therapeutic target when administrated intravenous or subcutaneous. Intranasal insulin administration offers a relatively high bioavailability, avoidance of the first-pass effect and invasive administration with the lack of systemic side effects and appears to be an ideal solution for drug delivery to the brain, bypassing the BBB. [17,18]. When applying this method, insulin is sprayed into the nasal cavity, enters the mucosa and is transported extracellularly along the axon bundles of the olfactory receptor cells leading through the foramina of the lamina cribrosa to the olfactory bulb, hippocampus and other regions of the brain and upper spinal cord [19].

Notably, intranasal administration of insulin was found to improve memory in healthy adults by maintaining their serum insulin and glucose levels. This results indicate a direct action of prolonged intranasal administration of insulin on brain functions, memory (significantly improved delayed recall of words) and mood in the absence of systemic side effects. No differences between the placebo and insulin conditions in blood glucose and plasma insulin levels has been found [20].

Similarly, intranasal insulin administration has been shown to be beneficial in AD patients treatment enhancing cognitive functions. The duration of current available studies is from 3 weeks to 4 months and most of them use regular human insulin (RH-I). No treatment-related severe adverse events occurred and no changes in fasting plasma glucose and insulin levels were observed. The summary of these studies is presented in Table 1 [20-24]. One of the studies reported greater efficacy of rapid acting insulin (RA-I) compared with regular human insulin (RH-I). Intranasal insulin analog Aspart (ASP-I), which belongs to the RH-I group, has been found to have stronger beneficial effects on declarative memory than RH-I in humans, thus RA-I may have superior therapeutic effects compared with regular insulin types [24,25]. Insulin-dose response curve for memory had an inverse “U” shaped function with the apex of 20IU and null or negative effects when levels were too low or too high, therefore the intranasal insulin dose of 20IU per day is revealed to be the optimal dose [22,26,27].

Summarizing, intranasal administration in AD treatment is supported by the following research results:

- AD is associated with brain insulin resistance and insulin deficiency
- brain regions responsible for cognitive functions, such as olfactory bulb, hippocampus and hypothalamus, have the highest insulin receptors concentration
- intranasal application by-passes the BBB and directly enters the brain
- no side effects or only minor side effects of intranasal insulin administration were reported
- insulin therapy improved memory and cognition both in healthy adults and in patients with AD [9].

The Phase II/III trials are currently ongoing to examine whether intranasal insulin by nasal spray improves memory in patients with mild cognitive impairment or AD.

Table 1. Examples of clinical trials of intranasal insulin administration in the treatment of AD.

No of patients	Group characteristics	Intervention	Time	Result (in comparison with placebo)	Ref
24	AD and MCI	RH-I (Humulin R) 20 IU or placebo	21 days	improved verbal memory, attention and functional status ($p<0.05$)	[21]
36	healthy men	RH-I (Actrapid) and ASP-I 4 x 40 IU or placebo	8 weeks	ASP-I and RH-I: improved declarative memory ($p<0.01$ and $p<0.05$) ASP-I: even better than those of the RH-I-treated group ($p<0.05$)	[24]
38	healthy adults	RH-I (Actrapid) 4 x 40 IU or placebo	8 weeks	improved verbal memory ($p<0.05$), enhanced mood, reduced anger ($p<0.02$) enhanced self-confidence ($p<0.03$)	[20]
104	MCI and AD	RH-I (Novolin R) 20 or 40 IU or placebo	4 months	20IU and 40 IU: improved cognition functions ($p<0.05$), preserved caregiver-rated functional ability ($p<0.01$) 20 IU only: improved delayed memory ($p<0.05$) CSF: decreased tau protein to A42 ratios, increased A42 levels	[22]
Clinical trials including the impact of APOE-ε4 status					
9	AD and APOE-ε4 carriers	RA-I (Glulisine) 20 IU or placebo	administred only twice	no significant differences between the groups in neuropsychological assesment of learning, memory, executive function, language and visuospatial function	[25]
61	AD, MCI and normal adults	RH-I (Novolin R) 20 or 40 IU or placebo	administred only once	cognition tested 15 min post-treatment: both doses: verbal memory improvement ($p<0.01$) with stronger effect for non-APOE-ε4 carriers than APOE-ε4 carriers and normal adults	[30]
80	MCI and AD	LA-I detemir (Levemir)	21 days	40 IU: improved verbal memory ($p<0.03$), visuospatial memory ($p<0.04$) improvement for APOE-ε4 carriers	[23]

		20 or 40 IU twice daily or placebo		(p<0.02) and worsening for non-carriers (p<0.02)	
92	AD, MCI and normal controls	RH-I (Novolin R) 10, 20, 40, or 60 IU or placebo	5 days	cognition tested 15-min post-treatment: performance peaking at 20 IU non-APOE-ε4 carriers: verbal memory improvement (20 IU optimal, insulin-dose response curve had an inverse U shaped function), APOE-ε4 carriers: relative decline in verbal memory	[26]
104	MCI or AD	RH-I (Novolin R) 20 or 40 IU or placebo	4 months	men and women (20 IU): cognitive improvement only men (40 IU): cognitive improvement non-APOE-ε4 carriers (40 IU): men improved, women worsened	[27]

AD – Alzheimer's disease, MCI – mild cognitive impairment, RH-I – regular human insulin, ASP-I – insulin analog aspart, LA-I: long acting insulin, CSF – cerebrospinal fluid.

2. 3. The impact of ApoE ε4

Apolipoprotein E (ApoE) mediates hepatic and extrahepatic uptake of plasma lipoproteins and cholesterol efflux. There are 3 major varieties of ApoE:

- ApoE ε3 renders protection against cardiovascular diseases
- ApoE ε2 is associated with dysbetalipoproteinemia
- ApoE ε4 is a risk factor for AD

However, the direct mechanism of the above connections remains uncertain. One of the possible explanations for enhanced risk of AD in ApoE ε4 carriers is that ApoE ε4 has a lower affinity for amyloid β causing impaired clearance of Aβ aggregates [28].

ApoE ε4 carriers are presented in 40–65 % of the late-onset AD population, and these observations may influence the therapeutic application of intranasal insulin in AD patients [25]. One copy of the ε4 allele is associated with a 2 to 3 times increased risk for AD and two copies of the ε4 allele is associated with even 10 times increased risk [29]. The treatment response would differ between subjects with and without the ε4 allele. Not all but most of clinical trials of intranasal insulin administration prove better response for treatment in subjects without ApoE ε4 [25-27,30].

Evidence is still lacking to explain the mechanism by which the ApoE ε4 genotype attenuates the cognitive response to intranasal insulin [25]. Also, results indicate that men and women with memory impairment responded differently to intranasal insulin treatment and the differences are most apparent for ApoE ε4 negative individuals [27].These findings suggest that groups with different genetic risks for AD may show different response to insulin administration [26].

3. THE USE OF OTHER ANTI-DIABETIC DRUGS

So far only insulin-based clinical trials were performed and revealed beneficial effects in AD treatment [31]. Other currently investigated strategies based on anti-diabetic drugs are presented below.

3. 1. GLP-1RAs

GLP-1Ras (glucagon-like peptide-1 receptor agonists) are a class of incretin-based anti-diabetes drugs acting by glucose-dependent pancreatic islet cell hormone secretion. This group of medications is represented by natural exendin-4 or synthetic analogues: liraglutide, semaglutide and exenatide. The major benefits of GLP-1Ras use include improvement of fasting and postprandial blood glucose control and no significant risks for hypoglycemia. [32].

Several preclinical studies have demonstrated the protective effect of GLP-1 analogues on neurodegenerative diseases such as AD [33]. GLP-1 together with its receptor, GLP-1R, is expressed in the brain. In animal models, increase in GLP-1R expression in hippocampal area enhance learning, memory and associative functions. The confirmation of relations between above effects and GLP-1R expression was the use of GLP-1R antagonist linked by cognitive functions deterioration [34]. Exendin-4 has profound effects on synaptic plasticity, decreases levels of Aβ and protects synapses from Aβ toxicity [35]. Other beneficial effects on brain tissue include neuroprotective and anti-inflammatory effects, increase in synaptogenesis, neurogenesis and cell repair as well as the reduction of the chronic inflammatory response. Abovementioned results from animal studies become the basis of the theory that GLP-1 analogues could be an effective prophylactic treatment of AD [31].

The impact of subcutaneous injections of GLP-1RAs on condition in AD was evaluated in a 6-month clinical trial performed on 40 adults person. The dosage of 1.8 mg corresponds to the maximal recommended dose for DM-2. Unfortunately, primary outcome was no changes in the intracerebral Aβ deposition in the central nervous system measured by positron emission tomography and secondary outcome (neuro-psychological assessment) is not yet available [36]. Hovewer, the effectiveness of GLP-1Ras may depend on route of drug administration. It is worth mentioning that intranasal exendin was four times more effective in terms of delivery to the olfactory bulb compared to intravenous administration [35]. A clinical trial of subcutaneous exendin-4 in AD was performed to determine the effectiveness of twice daily administration of exendin-4 as a treatment for early-stage AD or MCI, but so far no study results have been posted [37]. Currently only one randomised double-blind placebo-controlled Phase IIb study is ongoing to evaluate the effects of subcutaneously administered liraglutide in AD patients [38]. Unfortulately, no clinical trials were performed to determine whether intranasal GLP-1Ras improved memory.

3. 2. Metformin, PPAR-γ and DPP-4I

Clinical studies concerning whether oral administration of metformin or PPAR-γ (Peroxisome proliferator-activated receptors γ) are inconsistent – positive effects in animal models of AD have not been proven in clinical data. DPP-4I (dipeptidyl peptidase 4 inhibitors) may provide neuroprotection by increasing blood GLP-1 levels and improving the learning and memory in connection with reduction in total Aβ levels or tau

hyperphosphorylation in animal models of AD [39]. One recent clinical study confirm the positive effect of 6-month sitagliptin (DPP-4I) therapy in 205 participants as it improved cognitive function in elderly diabetic patients with and without AD, but further trials are needed to support these results [40]. No clinical studies on intranasal administration were performed.

4. CELL-PENETRATING PEPTIDES

One of the most crucial problem concerning intranasal drugs administration is low penetration of peptide substances into neuronal and epithelial cells in the olfactory mucosa [17]. Cell-penetrating peptides (CPPs) have been designed to improve cellular uptake of therapeutic molecules in response to the fact, that some new potent therapeutic molecules do not reach the clinic due to poor delivery and low bioavailability [41].

CPPs are mainly short amphipathic and/or cationic peptides capable of transporting various hydrophilic molecules over the plasma membrane. There are four main events for CPPs uptake [42]:

- interactions with the cell surface
- translocation through the cell membrane mainly through different types of endocytosis or direct translocation through the cell membrane
- transport to different intracellular compartments
- endosomal release of the CPPs (following endocytic uptake).

The CPPs-based intracellular delivery was shown to be cell-type independent and able to penetrate across various barriers, such as retina, neurons, blood brain barrier, intestine wall and skin [43]. Animal studies clarified that CPPs can increase the direct transport of insulin into the deeper regions of the brain such as the olfactory bulb and hippocampus. One of the first research revealing the affect of CPPs on the nasal absorption of insulin was performed with the use of L- or D-forms of penetratin, or L- or D-forms of octaarginine. L-penetratin was the most effective promoter of insulin absorption with dose-dependent relationship compared with others CPPs and no detectable damage to the integrity of cells in the nasal respiratory mucosa was observed [44]. Other report evaluated the effect of penetratin on the brain transport and systemic absorption of insulin after intranasal administration. Both L- and D-penetratin elevated insulin levels in the olfactory bulb and other brain compartments with different time profiles after intranasal administration as compared to insulin alone. However, in this study D-penetratin was described as better peptide carrier for brain delivery of insulin due to less systemic insulin exposure [45]. The acceleration of insulin with CPPs direct transport from the nasal cavity to the brain parenchyma was also proven by the use of autoradiography and a gamma counter (the greatest radioactivity at 15 min after intranasal administration) [46].

In one of the recent studies evaluating the therapeutic potential of CPPs, insulin was administered intranasally with or without L- or D-penetratin every day for 8 weeks to senescence-accelerated mouse. Coadministration of insulin and CPPS increased the plasma insulin level and reduced the blood glucose concentration (similar effect observed in cases of subcutaneous insulin administration and intranasal coadministration of insulin with L-penetratin). Special tests confirmed that coadministration improve memory during the early

stage of dementia and probably prevent the progression of cognitive dysfunction. However, the deposition of Aβ was increased and there were no positive effect on severe cognitive dysfunction [47]. Intranasal administration of the physical mixture of insulin and exendin-4 was also found to accelerate absorption from the nasal cavity via electrostatic and/or hydrophobic intermolecular interaction. This effect was achieved without any acute or chronic mucosal and systemic toxicities [48].

In vitro studies on the cytotoxicity of CPPs show that cationic CPPs (used in cited research above) are less toxic and can be tolerated by the cells at much higher concentrations than amphipathic CPPs. Commonly used assays, such as cell viability, proliferation and leakage of lactate dehydrogenase (LDH) demonstrate virtually no toxic effects of penetratin [49]. The risk of adverse effects such as toxicity and undesired immunogenicity, during chronic CPPs administration, was evaluated in 1-month study of coadministration of insulin and CPPs. There was negligible biomarker leakage in nasal lavage fluid and the integrity of the nasal epithelium remained unaffected. No significant difference in the release of inflammatory and immunogenicity mediators in plasma was observed after nasal administration of CPPs with or without insulin. However, due to less leakage observed when the CPPs were administered alone than with insulin, the safety of CPPs should be evaluated in conjunction with its associated cargo [50].

Another strategy similar to CPPs tested for increased brain penetration through intranasal administration are nano- or microsize vesicles consisting of one or more lipid bilayers surrounding an aqueous compartment, called liposomes (Lp). There are some studies evaluating liposomal formulation of rivastigmine. Intranasal delivery of rivastigmine Lp was shown to prevent degradation of the drug in the nasal cavity and to carry it through the mucosal barriers and was described as a viable and effective route to improve drug bioavailability [51]. A significantly higher level of drug was found in the brain with intranasal liposomes of rivastigmine compared to the intranasal free drug and the oral route in rat models [52]. Several studies investigated the effectiveness of Lp with other drugs used in experimental therapy of AD. For example, intranasal administration of Lp with β-sheet breaker peptide results in 1.67-2.92 fold greater concentrations in tissues (hippocampus, olfactory bulb, cerebrum, cerebellum, plasma: $p<0.05$) than peptide solution without liposomes [53].

The therapeutic strategy with potential benefits of both CPPs and Lp are cell-penetrating peptide modified liposomes (CPP-Lp). CPP-Lp may improve brain delivery and enhance pharmacodynamics which respect to BBB penetration and nasal olfactory pathway into brain after intranasal administration. One of the studies compare the activity of acetylcholinesterase (enzyme inhibited by rivastygmine) after intranasal administration of 3 substances: Lp with rivastigmine, CPP-Lp with rivastigmine and rivastigmine solution. The lower acetylcholinesterase activity was observed after CPP-Lp administration. Other benefits were decreased hepatic first pass metabolism and rarer gastrointestinal adverse effects [54].

These results indicate that CPP-Lp are the most effective in enhancement of the pharmacodynamic efficacy of rivastigmine. There are small number of published animal studies, however most of them affirm the safety of CPPs molecules and approve them for use in clinical trials [49].

5. CONCLUSIONS

Intranasal insulin administration seems to be an effective intervention that delays loss of cognition in AD patients, however only short term clinical studies were performed. No treatment-related severe adverse events and no changes in fasting plasma glucose and insulin levels contribute to the safety of intranasal insulin therapy. The additive of CPPs results in acceleration of insulin transport from the nasal cavity to the brain parenchyma which could amplify the beneficial effects of intranasal insulin administered alone.

The use of nanotechnology, such as CPPs and Lp, in delivering drugs to the brain protects from poor delivery occurrence and low bioavailability, which are the essential factors influencing the failure of clinical stage of studies despite pre-clinical effectiveness. Further studies on large groups of patients are necessary to confirm effectiveness of intranasal insulin and the use of CPPs.

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