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First clinical data of the neuroprotective effects of nasal insulin application in patients with Alzheimer's disease

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Abstract Previous reviews have outlined the important role of insulin in the brain, and the observation that insulin signaling is desensitized in patients with Alzheimer's disease (AD). Because insulin is used to treat diabetes and insulin desensitization in the periphery, this motivated the design and execution of clinical pilot trials in patients with AD and mild cognitive impairment. Because insulin has powerful effects on blood sugar levels, a new technique was used by which insulin is applied as a spray. This method avoids high levels of insulin in the periphery and makes use of the transport system, via the nasal epithelium, into the brain. First trials in healthy subjects showed improvement in attention and memory tasks, and confirmed the concept that insulin signaling plays an important role in neuronal function and cognition. In a series of small clinical trials in patients with mild cognitive impairment/AD, nasal application of insulin or long-lasting insulin analogs showed improvements in memory tasks, cerebrospinal fluid biomarkers, and in a fluorodeoxyglucose positron emission tomographic study. In a more recent trial, two patient subgroups were identified, in which the insulin-resistant group improved after drug treatment whereas a subgroup that did not show insulin desensitization deteriorated. This highlights the need to conduct additional studies and demonstrates clearly that the hypothesis that insulin signaling plays in important role in cognition and AD has merit, and that this is a worthwhile target that shows great promise for future drug developments that improve insulin signaling. Insulin itself may not be the best choice, and other drugs that have been developed to treat diabetes that do not enhance insulin desensitization may be a better choice.

Keywords: Neurodegeneration; Cxidative stress; Growth factors; Clinical trial

1. Introduction

Previous reviews have documented the importance of insulin signaling in the brain, and the fact that insulin signaling is compromised in the brains of patients with Alzheimer's disease (AD). These findings motivated the testing of drugs that were developed initially to treat diabetes if they are able to reverse the desensitization and to normalize neuronal functions. The obvious first choice of drug is insulin itself, which is used successfully to treat diabetes. Because insulin lowers blood sugar levels, it cannot be given to people who do not have diabetes. To circumvent this problem, a nasal application technique has been developed that funnels the insulin predominately to the brain by absorption via the nasal epithelium, with only minimal peripheral exposure to insulin. This brief summary gives an overview of the results of the clinical trials that have been conducted so far.

A number of studies have shown that insulin has direct affects on brain activity and cognitive processes. In animal models, a decrease in the insulin receptor signaling system produces cognitive impairments and a reduction in hippocampal synaptic neurotransmission [1,2]. Conversely, insulin injected into the brain intracerebro-ventricular (ICV) can improve performance in memory tasks in animals and also performance of attention tasks in humans when applied via the nasal passages, where it can enter the brain more directly [3]. This effect might also be linked to the fact that long-term potentiation of neuronal synaptic transmission is impaired if insulin signaling is affected, as shown in animal models of diabetes. Treatments of diabetic animals with insulin or incretins

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rescued the impairment in neurotransmission and memory impairments [4–7]. People with type 2 diabetes mellitus also have cognitive impairments, and treatment with diabetes medication improves these impairments [4,8].

Reduced insulin sensitivity and efficacy is also observed in the majority of elderly people [8,9]. As described in previous reviews, several studies demonstrate that insulin signaling is impaired in patients with AD [10–12]. This unexpected connection between type 2 diabetes mellitus and AD opened up novel research avenues to investigate what the underlying mechanisms for this may be. Insulin is a hormone that has a range of functions in the body. Its general physiological profile is that of a growth factor. Insulin is crucial for cell growth and survival. Neurons also carry insulin receptors, and activating them induces dendritic sprouting, neuronal stem cell activation, and general cell growth, repair, and neuroprotection [3,8,13–16]. Furthermore, insulin has potent neuroprotective factors and also regulates GS-kinase 3β, the main kinase that phosphorylates tau, which is the major component of neurofibrillary tangles found in the AD brain [14,17]. Insulin also improves brain activity, such as attention, memory formation, and cognition in humans [18–21].

To avoid hypoglycemia, insulin is applied by nasal spray application. With this technique, insulin is sprayed into the nose of the subject with the help of a nasal spray nebulizer. The insulin then enters the nasal mucosa and is transported extracellularly along the olfactory receptor cells in the roof of the nasal cavity, leading through the lamina cribrosa into the olfactory bulb [22]. This method of application transports insulin into the brain via the blood–brain barrier [23], and can be detected at physiologically active concentrations in cerebrospinal fluid (CSF) 30 to 40 minutes after intranasal application [24].

2. Insulin treatment improves memory in normal subjects

Using the nasal spray technique in psychological tests of healthy subjects, insulin showed improvements in several tests of attention, cognition, and memory formation. One study investigated the effects of 8 weeks of intranasal administration of insulin on memory, testing immediate and delayed recall of word lists, and on attention (Stroop test) in 38 healthy subjects in a double-blind, between-subject comparison trial. More important, blood glucose and plasma insulin levels did not differ between the placebo and insulin conditions. After treatment, the delayed recall of words improved significantly. These results demonstrate a cognitive effect of chronic treatment, improving memory in the absence of systemic side effects [25]. This facilitating effect on memory was even greater using insulin aspart, a fast-acting insulin analog [26]. A single, acute dose of insulin also showed memory enhancement in both spatial and working memory tasks [27]. Furthermore, verbal working memory was also improved after a single dose of insulin [28].

3. Clinical trials of nasally applied insulin in patients with mild cognitive impairment/AD

Based on the encouraging findings of these first trials in healthy human subjects that demonstrated that the method of application was safe and effective even in non-demented subjects, first clinical studies have been conducted in patients with mild cognitive impairment (MCI), which develops further to AD in the majority of cases.

A first study testing nasal application of insulin in patients with MCI/AD included 26 memory-impaired subjects (13 diagnosed with early AD and 13 with MCI) and 35 control subjects allocated randomly to groups with a double-blind design. They received three intranasal treatments, consisting of saline (placebo) or insulin (20 IU or 40 IU). Cognition was tested 15 minutes after treatment, and blood was acquired at baseline and 45 minutes after treatment. The insulin treatment had no effect on plasma insulin or glucose levels. Insulin facilitated recall on two measures of verbal memory in memory-impaired apolipoprotein E (*APOE*) ε4 carriers. These effects were stronger for memory-impaired non-*APOE* ε4 carriers than for memory-impaired *APOE* ε4 carriers and control subjects. Interestingly, memory-impaired *APOE* ε4 carriers showed poorer recall after insulin administration on one test of memory. These findings are a proof of concept and demonstrate that insulin can, indeed, improve memory impairments in patients with MCI/AD even after acute treatment. The results also suggest that *APOE* ε4 has an effect on insulin signaling [29].

The same group conduced a pilot study with 24 patients with MCI/AD assigned randomly to insulin or placebo groups, again in a double-blind design. The insulin-treated group retained more verbal information after a delay compared with the placebo group. Insulin also improved attention and functional status (e.g., orientation, judgment, social interactions, home activities, personal care, speech/language) as assessed by caregivers. Insulin also increased the amyloid-β (Aβ) 40/42 ratio. These encouraging results also confirm the hypothesis that insulin has positive effects on patients with MCI/AD [30].

In a follow-up study, 33 patients with MCI/AD and 59 control subjects received five intranasal treatments of insulin or placebo. Cognition was tested 15 minutes after treatment, and blood was acquired at baseline and 45 minutes after treatment. Insulin improved recall on two measures of verbal memory in memory-impaired non-*APOE* ε4 carriers. In contrast, memory-impaired *APOE* ε4 carriers displayed a decline in verbal memory. Interestingly, insulin also affected plasma Aβ levels in memory-impaired subjects and control subjects, effects that again differed by *APOE* genotype. These results confirmed the previous pilot study and further demonstrate that *APOE* affects the insulin response [20].

These small studies that tested insulin for only a short time already document that insulin can affect cognition and amyloid levels in a positive way, which supports the insulin signaling hypothesis and can be seen as a proof of

concept. This is an important aspect because a number of very promising drug trials that showed good effects in preclinical trials unfortunately showed no improvement whatsoever. The fact that genuine cognitive improvements are found in patients with MCI/AD greatly supports the notion that insulin signaling impairment in the brain may be linked causally to the development of AD, and that improving insulin signaling may be the key to a genuine improvement of memory and attention deficits in these patients. However, the number of patients was low, and the actual treatment was very short, making it unlikely that the treatment improved any structural losses and made permanent improvements in the brain of these patients.

To address these issues, a randomized, double-blind, placebo-controlled clinical pilot trial was conducted. A total of 104 patients with either MCI (n = 64) or mild to moderate AD (n = 40) were used. Patients received either placebo (n = 30), 20 IU insulin (n = 36), or 40 IU insulin (n = 38) for 4 months. Primary measures consisted of delayed story recall score and the Dementia Severity Rating Scale score, and secondary measures included the Alzheimer Disease's Assessment Scale Cognitive Subscale score and the Alzheimer's Disease Cooperative Study Activities of Daily Living scale. CSF samples were taken in 23 patients, and ^{18}fluorodeoxyglucose positron emission tomography was conducted in 40 patients before and after treatment. Treatment with 20 IU insulin improved memory, and both doses of insulin (20 IU and 40 IU) preserved caregiver-rated functional ability. Both insulin doses also preserved general cognition as assessed by the Alzheimer Disease's Assessment Scale Cognitive Subscale score for younger participants, and functional abilities as assessed by the Alzheimer's Disease Cooperative Study Activities of Daily Living scale for adults with AD. AD biomarkers in CSF samples did not change for insulin-treated participants as a group, but changes in memory and function were correlated with changes in the Aβ42 level and in the tau protein-to-Aβ42 ratio. Placebo-assigned participants showed decreased ^{18}fluorodeoxygluce positron emission tomographic uptake in the parietotemporal, frontal, precuneus, and cuneus regions, and insulin-minimized progression. More important, the improvements in episodic memory were still present 2 months after cessation of treatment [31]. This chronic study testing a larger group of patients and also assessing AD biomarkers and glucose use in the brain supports the concept that impairment of insulin signaling is at least one crucial parameter in the disease progression of AD, and that improving insulin signaling indeed has beneficial effects on the chronic developments, AD biomarkers, and energy use of neurons. This suggest that the insulin effect is not just an acute improvement of neuronal and cognitive function that is not permanent, but that, over time, cell metabolism and perhaps neuronal function and cognition are changed on a structural level, too.

In a recent clinical trial called the Study of Nasal Insulin to Fight Forgetfulness–Long-Acting Detemir (or SNIFF-Long), patients with MCI/AD who received 40 IU/day of the insulin analog detemir (Levemir) showed improvements in memory relative to the placebo group, but only if they had high levels of insulin resistance at baseline. The PK (the affinity constant value of receptor/ligand interaction) of insulin detemir (Levemir) is superior to insulin, and detemir remains active in the body for up to 24 hours after a single dose, peaking about 5 hours after dosing, thus mimicking the natural basal secretion of insulin in the pancreas. The study allocated randomly 60 patients with MCI/AD either to 20 IU/day or 40 IU/day of intranasal insulin detemir or placebo, treated for 3 weeks. Patients were tested at baseline and at the end of treatment with two verbal memory tests, with results combined into a composite score, as well as Benton Visual Retention Test recognition task and a dot-counting test of working memory. At baseline, the patients' mean age was 72 years, with Mini-Mental State Examination scores averaging 26 points. Surprisingly, neither dose of insulin was more effective than placebo on the primary outcome, which was change from baseline in memory composite scores. However, when patients in each treatment arm were stratified into those with low vs. high homeostasis model of assessment–insulin resistance values for insulin resistance, the two subgroups in the 40-IU/day arm both differed significantly from the stratified placebo groups—in opposite directions. Patients with high insulin resistance that were given 40 IU/day showed an improvement of about 0.75 point in the memory composite, compared with an increase of less than 0.1 point in patients with high insulin resistance administered a placebo. The 40-IU/day group with low insulin resistance, meanwhile, had a decrease of 1 point in memory score, compared with a decrease of about 0.2 point in the low-resistance placebo subgroup ($P = .015$ for both placebo vs. 40-IU comparisons). A similar pattern was seen when the treatment arms were stratified by *APOE* genotype, with carriers of the *APOE* ε4 allele benefiting significantly from 40 IU insulin detemir relative to placebo, whereas noncarriers showed worsened memory compared with placebo [32].

This study showed some surprising results that shed some light on the mechanisms that underlie the insulin treatment. Again, the result confirms that insulin signaling plays a crucial part in cognition and in the impairment of brain functions in AD. The two groups that showed opposite results demonstrate that patients with high insulin resistance benefited from the insulin treatment. However, patients that did not show insulin resistance fared worse. This suggests that the insulin given to these patients made matters worse rather than better, triggering insulin resistance (which may have been developing already) and making neuronal communication and energy use worse, rather than improving it. This suggests that the underlying mechanism that triggered AD in this subgroup is independent from insulin signaling. Because we do not know what triggers AD initially, and because there is a list of potential mechanisms that initiate the process and lead to the same end result of AD, it may be that, in a subgroup of patients with AD,

insulin desensitization is not the primary cause for disease development, and that insulin treatment will facilitate insulin desensitization.

This highlights an important issue that needs to be discussed in this context. The use of insulin to treat insulin desensitization has been copied from the treatments of diabetes. Insulin and its long-acting analogs are commonly used. However, the long-term prognosis in diabetes is not good for treatment with insulin because it facilitates insulin desensitization further, to the point where insulin is no longer active [33]. Therefore, research of novel drug treatments have moved away from insulin analogs to new signaling pathways—in particular, to the incretin hormones (glucagon-like polypeptide 1 and 2 [GLP-1, GLP-2] and glucose dependent peptide [GIP]) [34,35]. These drugs do not enhance insulin desensitization because they do not activate insulin receptors. They are separate signaling pathways and have considerable advantages over insulin in the treatment of diabetes (e.g., they improve pancreatic functions long term) [35,36]. In addition, GLP-1 analogs do not affect blood sugar levels in normoglycemic people [37], and therefore can be given safely to nondiabetic patients with AD or Parkinson's disease. If the unexpected observation of the SNIFF trial—that a subset of patients deteriorated after insulin treatment—is actually a result of the fact that insulin signaling was desensitized by the insulin treatment, then treatments with other drugs such as the incretins, which do not accelerate insulin desensitization, may show more positive effects.

Several other clinical trials testing the nasal application of insulin are currently running or in the planning stages [38-40]. These trials with a longer treatment duration (6 months) and greater patient numbers will shed more light on the actual processes that govern insulin signaling and cognitive function in patients with AD. Because there is large variation within the patient group, greater numbers will most likely produce more reliable results.

In conclusion, the clinical evidence presented in these studies of nasal application of insulin give evidence that improving insulin signaling in the brain of patients with MCI/AD shows positive effects, with clear improvements in at least a subset of patients. These clinical data are an important proof of principle that encourage further research, and demonstrate that the improvement of insulin signaling is a worthwhile target that is very likely to lead to an improvement in cognition and perhaps neuroprotection in these patient groups.

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