Effects of liraglutide on neurodegeneration, blood flow and cognition in Alzheimer's disease – protocol for a controlled, randomized double-blinded trial

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ABSTRACT

INTRODUCTION: Type 2 diabetes (DM-2) increases the risk of developing Alzheimer's disease (AD), and patients with AD are more likely to develop DM-2. DM-2 and AD share some pathophysiological features. In AD, amyloid-β (Aβ) is accumulated as extracellular plaques in the gray matter of the brain, while in DM-2 islet amyloid polypeptide (IAPP) is accumulated in the pancreas. Premature cellular degeneration is seen in both diseases. Glucagon-like peptide-1 (GLP-1) reduces the amount of Aβ and improves cognition in animal studies. The present study tests the hypothesis that treatment with the long-acting GLP-1 receptor agonist liraglutide affects the accumulation of Aβ in patients with AD.

MATERIAL AND METHODS: This is a randomized, controlled, double-blinded intervention study with AD patients treated for six months with liraglutide (n = 20) or placebo (n = 20). The primary outcome is change in deposition of Aβ in the central nervous system (CNS) by Pittsburgh compound B positron emission tomography (PET). The secondary outcome is evaluation of cognition using a neuro-psychological test battery, and examination of changes in glucose uptake in the CNS by ^{18}F-fluoro-deoxy-glucose PET. Finally, a perfusion-weighted magnetic resonance imaging with contrast will be performed to evaluate blood flow.

CONCLUSION: No registered drug affects the deposition of Aβ in the brain of AD patients. Our goal is to find a new therapeutic agent that alters the pathophysiology in AD patients by decreasing the formation of Aβ plaques and thereby presumably improves the cognitive function.

FUNDING: The trial is investigator-initiated and investigator-driven and is supported by Novo Nordisk Scandinavia.

TRIAL REGISTRATION: ClinicalTrials.gov: NCT01469351.

Over the past few years, a vast increase in the incidence of both type 2 diabetes (DM-2) and Alzheimer's disease (AD) has been observed, leading to increased morbidity and mortality. Epidemiologically and pathophysiologically, DM-2 and AD share several features. DM-2 increases the risk of AD and vascular dementia two to five fold [1, 2]. It has been suggested that the risk correlates with plasma glucose [2] and hyperinsulinaemia in the early stages of DM-2 [1]. There is an increased incidence of DM-2 in patients with AD [3]. In both diseases, premature cell degeneration occurs. Among other things, DM-2 is characterized by a loss of β-cell function and mass in the pancreas, while a neuronal loss of function and cell death are seen in AD. Glucagon-like peptide-1 (GLP-1) is an incretin hormone with numerous effects on the glycaemic response. It is released in the intestinal mucosa in response to food intake and stimulates insulin and decreases glucagon. In animal and cell studies, GLP-1 stimulates β-cell neogenesis, growth, and differentiation; and in vitro, GLP-1 has demonstrated inhibition of β-cell apoptosis [4].

The GLP-1 receptor (GLP-1R) is expressed abundantly in the central nervous system (CNS), it is widely distributed from the olfactory bulb to the spinal cord, particularly in the hypothalamus and hippocampus [5]. Primary hippocampal neurons exhibit a robust increase in cyclic adenosine monophosphate (cAMP) levels when exposed to a single dose of GLP-1, which indicates the presence of functional GLP-1R. Moreover, GLP-1R stimulation in rats results in neurite growth and protects against nerve cell apoptosis [6]. Mice that overexpress GLP-1R in the hippocampus show increased neurite growth and improved learning [7]. A recent study has shown memory impairment in GLP-1R knock out (KO) mice, which indicates a lack of a neuro-protective effect [8]. GLP-1 also acts as an anorectic neurotransmitter, regulates energy homeostasis, including glucose homeostasis, lowers core body temperature and plays a role in the activation of central stress responses [5].

AD is characterized by an increased level of amyloid-β (Aβ) and an increased number of neuro-fibrillar tangles [9]. In amyloid precursor protein/presenilin 1 transgenic (APP/PS1) mice, liraglutide (eight weeks, i.p.) prevented memory impairments and synaptic loss and reduced the number of Aβ plaques [10]. Another common feature in AD and DM-2 is metabolic and vascular dysfunctions, including oxidative stress [11]. Oxidative stress is an impor-

PROTOCOL ARTICLE

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TABLE 1

Inclusion and exclusion criteria.

Key inclusion criteria
Adult competent persons
Diagnosed with Alzheimer's disease. With an MMSE score of 18-21. The diagnosis should be entirely based on clinical findings, while diagnosis by MMSE with a score > 22 should be diagnosed by spinal puncture
Age ≥ 50 years and ≤ 80 years
Caucasians
Key exclusion criteria
Diabetes mellitus
Clinically significant liver or renal impairment (serum alanine aminotransferase> 2 times upper reference or creatinine-clearance < 60 ml/min, assessed on Cockcroft-Gault normogram)
Clinically significant anaemia
Current or former presence of one of the following diseases with clinical relevance: another central nervous system illness other than diagnosed depression treated with SSRI or SSRI similar drugs; liver disease; kidney disease; endocrinological disease other than well-controlled hypothyroidism
Current or history of chronic or acute pancreatitis
Patients treated with tricyclic antidepressants or neuroleptics
Significant abnormities in the brain detected by magnetic resonance imaging

MMSE = Mini-Mental State Examination; SSRI = selective serotonin re-uptake inhibitors.

TABLE 2

Flow chart visit 1-5.

	Visit no.				
Key measurements	**1: screening**	**2: randomisation and startup on Victoza**	**3. clinical monitoring**	**4: clinical monitoring incl. blood samples**	**5: conclusion**
Time perspective	Time = 0	Start	After 1 month	After 3 months	After 6 months
Weight	X	X	X	X	X
Blood pressure and heart rate	X	X	X	X	X
Blood samples	X			X	X
10 ml urine for later analysis	X				X
FDG and PIB PET scanning		X			X
Magnetic resonance imaging	X				X
Cognitive tests		X			X

PIB PET = Pittsburgh compound B positron emission tomography; FDG = ^{18}F-fluoro-deoxy-glucose.

tant pathogenic event in AD which is in particular related to changes in oxidation of nucleic acids [11].

PURPOSE

- To investigate whether six months of treatment with the long-acting GLP-1R agonist liraglutide will affect the intra-cerebral Aβ deposition in the CNS of patients with AD assessed by Pittsburgh compound B (PIB) positron emission tomography (PET).
- To examine changes in glucose uptake in the CNS by 18F-fluoro-deoxy-glucose (FDG) PET before and after treatment with liraglutide.
- To validate the cognitive functions using a specific neuro-psychological test battery before and after treatment with liraglutide.
- To evaluate changes in blood flow using magnetic resonance imaging (MRI) and to measure global oxidative RNA and DNA stress by measuring oxidated guanine nucleoside before and after treatment with liraglutide.

MATERIAL AND METHODS

Design

This is a randomized, controlled double-blinded intervention study including 40 patients with AD who will be treated with liraglutide (n = 20) or placebo (n = 20) for six months. Treatment with liraglutide/placebo will be administered as an "add on" to the patients' usual medications, including their present treatment for AD.

Patient recruitment and randomization

Patients will be recruited from the dementia clinics in Western Denmark. Key inclusion and exclusion criteria are shown in **Table 1**.

Patients willing to participate will be screened after written informed consent has been obtained, and those fulfilling the inclusion criteria will be randomized to either treatment. During the study, participants will attend a total of five visits. See **Table 2** for detailed information.

Study drug

Liraglutide is a long-acting human GLP-1 analog registered and approved for the treatment of DM-2.

Dose: Initially, 0.6 mg subcutaneously daily for one week; hereafter 1.2 mg daily for one week before finally increasing to 1.8 mg daily after another week. The dosage of 1.8 mg corresponds to the maximal recommended dose for DM-2.

Outcome measures

Positron emission tomography

FDG PET will be undertaken in the same manner as previous FDG PET studies performed on the brain [12, 13]. Similarly, ^{11}C labelled PIB PET will be carried out in accordance with previous PIB-PET studies performed on the brain [12, 14].

The first scan will be performed with 400 MBq PIB. Hereafter, a metabolic scan with 200 MBq FDG will be performed [15].

Quantitative assessments of glucose uptake and Aβ deposits will be performed. Image processing will be performed by using 2D acquisition, which reduces the need for radioactive tracer administration. The brain's glucose uptake will be calculated on the basis of the PET images and tracer kinetic studies of the FDG uptake.

Magnetic resonance imaging

At the first visit, an anatomical magnetic resonance imaging (MRI) of the brain will be performed. Immediately

hereafter, a perfusion-weighted MRI with contrast will be conducted. The contrast medium consists of complexed gadolinium.

Cognitive tests

Validation of the patients' cognitive function will take place using the neuropsychological test battery "Brief Cognitive Examination" from the Wechsler Memory Scale (WMS-III). This test is accepted and validated and used to assess the severity of AD. The test illustrates all cognitive domains.

Biochemical measurements

Blood and urine will be sampled in the morning after an overnight fast. The following blood tests will be performed: plasma glucose, insulin, C-peptide, pro-insulin, cortisol, cholesterol, haemoglobin A_{1c}, C reactive protein, haemoglobin, leukocytes, platelets, sodium, potassium, albumin, urea, creatinine, alanine aminotransferase, alkaline phosphatase and lactate dehydrogenase.

A total of 10 ml of urine will be drawn for analysis of oxidative guanine nucleoside and 20 ml of whole blood and 12 ml of plasma will be drawn and kept in a freezer for any later analysis.

Sample size consideration and statistical methods

Based on previous studies and the following power calculation, we estimate that a study will be informative with two groups of 20 participants each treated with liraglutide up-titrated to a maximum recorded dose or liraglutide placebo. In order to see structural changes and find reduced progression of cognitive skills, the treatment period has been set to six months.

The power calculation is based on a risk of type 1 error of 0.05 and a risk of type 2 error of 0.20 with a clinically relevant difference in glucose uptake of 15% [13] and a change in Aβ deposition of 15% [14] with a standard deviation (SD) set at 15%. This gives a sample size of n = 2(1.96-0.84) –2 × 0.15∧2/0,15∧2 = 16. We expect a certain dropout.

The ANCOVA test will be used for comparison between the groups and the paired t-test for comparison within the two groups at different times. Intention to treat analyses will be performed. p-values below 0.05 will be assumed to be statistically significant.

Ethics

The study will be conducted in accordance with the principles of the Helsinki Declaration. The Central Denmark Region Committees on Biomedical Research Ethics, the Danish Data Protection Agency and the Danish Medicines Agency have approved the study protocol.

Trial registration: ClinicalTrials.gov: NCT01469351

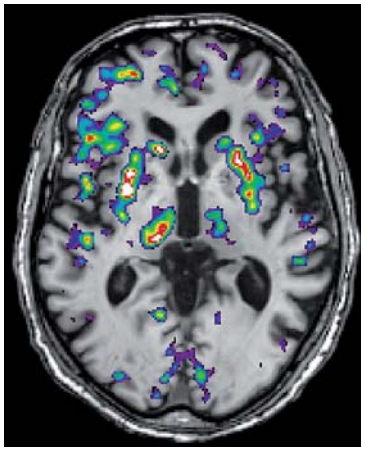
DISCUSSION

At present, AD is only treated symptomatically; no attempt at curative treatment is made. No registered drug affects the deposition of Aβ plaques in the brain of AD patients. Current therapeutic options are based on neurotransmitters and therefore do not affect neurodegeneration. The acetylcholinesterase inhibitors (AChEIs) prevent the degradation of progressively decreasing acetylcholine (ACh) levels. The other approved medication is memantine, which regulates the excitatory glutamatergic function and improves cognition. Current therapeutic options aim at transmitter targets secondary to AD pathology. They offer limited efficacy and do not slow disease progression. Liraglutide has potential protective effects in the brain. This clinical trial will provide new information about the effects of liraglutide in the CNS and, at a very early stage, explore the possibility of a new treatment strategy for patients with AD.

PET scans can monitor clinical evidence of CNS changes induced by AD [14, 16].

The PIB PET tracer has recently been developed and its use makes it possible to measure the amount of Aβ deposition in the living brain and thereby evaluate the treatment response for drugs affecting the Aβ deposition [12]. The FDG PET tracer can be used to monitor the progressive reduction in cerebral glucose metabolism in patients with pathologically proven AD before clinical symptoms can be detected. During disease progression, FDG PET studies have shown that glucose metabolism continues to decline, and that this deterioration is associated with decreasing patient scores in cognitive tests [16].

In AD, the hippocampus is the primary region of the brain to suffer from damage resulting in characteristic early symptoms such as amnesia and disorientation. FDG PET studies have shown reduced glucose metabolism in the parietemporal, frontal and posterior cingulate cortices of patients with AD compared to healthy



The image of a single subject with Alzheimer's disease shows the ^{11}C-Pittsburgh compound B (PIB) binding potential (BP_nd) within the 0.04-0.4 range overlayed on the subject's own T1-weighted magnetic resonance imaging. The ^{11}C-PIB binding potential was calculated using the HYPOTIME method [20].

individuals, and this decline in glucose metabolism is correlated with the severity of dementia [16].

Klunk et al compared PIB PET with FDG PET and found that PIB retention correlated inversely with cerebral glucose metabolism measured by FDG PET. Significant increases in PIB retention in patients with Aβ deposits in comparison to patients without Aβ deposits correlate well with amyloid deposits in brain tissue removed by autopsy from Alzheimer's patients [13]. PIB was one of the first PET tracers for visualization of Aβ and it is still the best studied of these radio-ligands. Changes in PIB retention have been detected at earlier stages of AD than changes in functional parameters, such as cerebral glucose metabolism and cognition. Overall, it appears that the PET tracer PIB is a reliable marker for AD in its early stages, but also that it is exceeded by FDG when it comes to monitoring of disease progression. The total radiation load during PET is 12 mSv. For persons over 50 years, this represents an effective dose of 12 mSv corresponding to an extra risk of getting a fatal cancer of 0.01% (overall risk of a fatal cancer is 25%). Visualization of Aβ using PET may help to select patients for anti-Aβ treatment in the future [16].

Perfusion-weighted MRI will be performed before and after treatment with liraglutide. These scans will be used to determine capillary transit time heterogeneity and fuel extraction [15]. In the present study, we will use gadobutrol which is a low-risk contrast medium for development of nephrogenic systemic fibrosis.

The renal function will be determined before the MRI by estimating the glomerular filtration rate (GFR), and only participants with an estimated GFR > 60 ml/min are included.

The oxidation of nucleic acids, in particular RNA, has recently been shown to be dramatically elevated in the brains of patients with AD, for a review see [11]. Also, it has been shown that high oxidation of RNA is associated with poor survival in patients with DM-2 [17].

Overall, liraglutide is known to have few side effects. The risk of hypoglycaemia would be expected to be very low for liraglutide as its effects on insulin secretion are glucose dependent. Treatment with liraglutide in obese, non-diabetic subjects with doses of liraglutide nearly twice the registered maximum dose did not lead to hypoglycaemia [18].

A moderate weight loss of between 1.85 and 3.39 kg has been reported in diabetes patients treated with liraglutide. This weight reduction increased with increasing body mass index at baseline [19]. Weight loss is predominantly due to the reduction in adipose tissue, especially visceral adipose tissue, rather than to a reduction in muscle mass [20]. Hence, we consider the risk of hypoglycaemia or significant weight loss negligible in non-diabetic patients who are being treated with liraglutide.

The most common side effect reported in patients treated with liraglutide is gastrointestinal upsets, mostly transient nausea of a mild to moderate character. Most cases of nausea occur during the first four weeks when the dose is up-titrated.

We here present a protocol aimed at investigating the effect of GLP-1 receptor agonism on a number of AD features.

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