Increased circulating insulin-like growth factor-1 in late-onset Alzheimer's disease

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Abstract

Background: Insulin-like growth factor (IGF)-1 has been implicated in the pathogenesis of Alzheimer's disease (AD).

Methods: We compared the level of circulating total and bioavailable IGF-1, by simultaneous measurements of IGF-1 and IGF binding protein (IGFBP)-3, between 87 patients diagnosed with AD and 126 age and sex matched control subjects without cognitive impairment. Blood samples were collected and IGF-1 and IGFBP-3 measured by ELISA. Subjects were also genotyped for apolipoprotein E.

Results: Total circulating IGF-1 levels were significantly raised in the AD group as compared to the control group ($p=0.022$). There was no significant difference in the circulating level of IGFBP-3 between the two groups. When the IGF-1 levels were ratioed against IGFBP-3 levels as an indicator of unbound, bioavailable circulating IGF-1, there was a significant increase in the molar IGF-1:IGFBP-3 ratio in the AD subjects (0.181 ± 0.006) as compared to the controls (0.156 ± 0.004) ($p<0.001$). Logistic regression analysis revealed that an increase in the IGF-1:IGFBP-3 molar ratio increased the risk of AD significantly.

Conclusion: The results of increased total and free circulating IGF-1 support the hypothesis that in its early stages late-onset AD reflects a state of resistance to IGF-1.

Key words: Alzheimer's, insulin-like growth factor-1, insulin-like growth factor binding protein-3, apolipoprotein E, insulin resistance.

Introduction

Alzheimer's disease (AD) is the most prevalent form of dementia and understanding the factors which influence the pathophysiology of the disease is essential for impacting on incidence and defining new therapeutic strategies. Insulin-like growth factor (IGF)-1 modulates growth and metabolic function in the brain, enhances neuronal survival and exerts neurotrophic activities in the hippocampus, which is involved in learning and memory [10, 11]. At a cellular level, disruption of IGF-1 input lowers neuronal resistance to amyloid β (Aβ) peptide toxicity, increases cellular susceptibility to cell death signals, enhances phosphorylation of tau and leads ultimately to accumulation of Aβ in the brain [5]. Aβ, which is proteolytically cleaved from the larger amyloid β-protein precursor (AβPP) [30], is the key constituent of the amyloid plaque and hyperphosphorylation of tau is integral to the formation of neurofibrillary tangles, both of which are diagnostic of AD pathologically.

Although IGF-1, its receptor and binding proteins are present and locally produced in the brain [27], IGF-1 is actively transported across the blood-brain barrier, and therefore changes in circulating IGF-1 can lead to changes in IGF-1 input to the brain [4]. The bioavailability and bioactivity of IGF-1 is regulated by six IGF binding proteins (IGFBPs) and several IGFBP-proteases [7, 8]. Quantitatively the most important binding protein in the circulation is IGFBP-3 which binds more than 80% of the circulating IGF-1 [15]. Thus measurement of circulating IGFBP-3 levels, in addition to IGF-1 levels, allows the amount of bioavailable IGF-1 to be determined [16, 23]. Although other studies have measured circulating IGF-1 levels in AD subjects as compared to control subjects, the results have been conflicting with one showing an increase [29] and two a decrease [22, 32] in IGF-1 levels in AD. However, none of these studies measured the circulating levels of IGFBP-3 which would reflect the amount of bioavailable IGF-1. This lack of measurement of bioavailable IGF-1 was identified as a potential limitation with these studies [32]. Therefore, the present study was designed to

compare the level of circulating total and bioavailable IGF-1, by simultaneous measurements of IGF-1 and IGFBP-3, between patients with late-onset, sporadic AD and age-matched controls without dementia.

Materials and Methods

Subjects

Two hundred and thirteen elderly subjects were enrolled for the purposes of this study. These subjects consisted of 87 patients diagnosed with AD and 126 age and sex matched control subjects without cognitive impairment. AD patients were recruited through memory clinics in Leeds and Dewsbury (West Yorkshire, England). Control subjects were recruited through the Leeds Family Health Services Authority, day hospitals and elderly medicine outpatient clinics within the Leeds area. All participants were of European Caucasian extraction and gave written informed consent (assent from relatives of the AD patients was given where appropriate) according to a protocol approved by the Leeds (East) Research Ethics Committee. Diagnosis of probable AD was made in accordance with international diagnostic criteria (National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association Work Group; NINCDS-ADRDA) [18]. All patients and control subjects underwent a standardized clinical evaluation which included medical history, anthropometric measurements and cognitive function assessment (Mini-Mental State Examination; MMSE and Modified Mini-Mental State examination; 3MS) [12, 28]. Demographic and clinical profiles of the subjects participating in this study are summarized in table 1.

Assays

Blood samples were collected between 8 am and noon after an overnight fast in lithium heparin on ice, centrifuged at 4°C at 2997*g* for 30 min and the plasma samples stored at -40°C until analysis. Enzyme-linked immunosorbent assay (ELISA) kits were used to measure insulin (Biosource, Nivelles, Belgium), total IGF-1 and IGFBP-3 (R&D Systems, Oxford, UK). According to the manufacturer's data sheets, the minimum detection limit for the IGF-1 assay was 0.026 ng/ml (3.42 pM) and for the IGFBP-3 assay was 0.05 ng/ml (1.72 pM), and the IGF-1 assay does not detect IGF-2, insulin, IGFBP-2, -3 or -4, and the IGFBP-3 assay does not detect IGF-1 or -2, IGFBP-1, -2, -4, -5 or -6. The apolipoprotein E (ApoE) genotype was determined by polymerase chain reaction as described previously [6].

Statistical Analysis

All statistical analyses were performed using SPSS for windows version 12.0. Distribution of variables was tested for skewness using the Kolmogrov-Smirnov test. Normal data are presented as mean and standard error of the mean; non-parametric data are presented as median and range. The unpaired Students t-test was used to compare mean values of normally distributed data; the Mann-Whitney U-test was used to compare non-parametric data. Differences in categorical data (expressed as percentages) groups were assessed using the chi-squared test. Logistic regression analysis was used to investigate factors contributing to the risk of AD.

Results

There were no significant differences between the AD group and the control group in terms of age, gender, alcohol intake (past or present), education (years of schooling or university attendance), prevalence of diabetes mellitus, prevalence of cerebrovascular or

cardiovascular disease, fasting blood sugar, fasting plasma insulin or insulin resistance as defined by the homeostasis model assessment (HOMA) [31] (Table 1). HOMA is a validated standard method of assessing insulin resistance using glucose and insulin concentrations, calculated by dividing the product of fasting plasma insulin concentration and fasting plasma glucose concentration by 22.5 [31]. The low prevalence of diabetes mellitus in the AD subjects and control group (Table 1) is very similar to general population figures quoting prevalence to be 3.45% in England [19]. AD subjects were significantly shorter in stature than the control group ($p=0.038$), had significantly smaller head circumference ($p=0.039$) and were significantly lighter in weight ($p<0.001$) than control subjects (Table 1). Body mass index (BMI) was significantly lower in the AD subjects as compared to the control group ($p=0.001$) (Table 1). There was a significantly greater proportion of subjects with at least one copy of the ApoE4 allele in the AD group ($p<0.001$) (Table 1).

Comparison of total plasma IGF-1 between AD subjects and control subjects revealed it to be significantly raised in the AD group as compared to the control group ($p=0.022$) (Table 1). There was no significant difference in the plasma level of IGFBP-3 between the two groups. When the IGF-1 was ratioed against IGFBP-3 as an indicator of unbound, bioavailable circulating IGF-1 [16], there was a significant increase in the IGF-1:IGFBP-3 molar ratio in the AD subjects (0.181 ± 0.006) as compared to the controls (0.156 ± 0.004) indicating a greater amount of circulating bioavailable IGF-1 in the AD subjects ($p<0.001$) (Fig 1).

Logistic regression analysis was performed to look for independent associations between particular parameters and the risk of AD (Table 2). Of the factors in the model, the presence of an ApoE4 allele and also an increase in the IGF-1:IGFBP-3 molar ratio each increased the odds of AD significantly. In particular, for an increase of one standard deviation of IGF-1:IGFBP-3 molar ratio, the estimated odds ratio of being a patient with AD was 1.90 ($p=0.001$). No interaction between ApoE4 status and IGF-1:IGFBP-3 molar ratio was

detected and there was no significant difference in IGF-1:IGFBP-3 molar ratio when the subjects were grouped based on ApoE4 status. Although weight was also significantly associated, ischaemic heart disease, cerebrovascular disease, diabetes, smoking status and plasma insulin were not associated with the risk of AD (Table 2). There was no correlation between MMSE and IGF-1, IGFBP-3 or IGF-1:IGFBP-3 molar ratio in the AD group.

Discussion

In this study, the primary findings were that circulating total IGF-1 was raised in late-onset, sporadic AD subjects as compared to controls. Furthermore, the IGF-1:IGFBP-3 ratio, indicative of bioavailable IGF-1, was raised significantly in the AD subjects as compared to control subjects. Secondary findings included significant associations between stature, head circumference, weight, BMI, ApoE4 allele and AD consistent with previous studies [1, 2, 21, 26]. There was no significant difference between the groups in terms of plasma insulin measurements and insulin resistance as measured by HOMA [31]. Although other studies have shown plasma insulin to be raised in AD [3, 9, 20], these studies included smaller numbers of subjects (12-25 AD patients; 14-66 controls) and also excluded diabetic subjects. A larger study found cognitive impairment with subcortical features to be associated with biochemical and clinical features of the insulin resistance syndrome [13], however, this work was not limited to AD.

Our data are consistent with a previous small study with 10 Caucasian sporadic AD subjects (age range 63-74 years with probable AD according to the NINCDS-ADRDA criteria and mean MMSE ± S.D. 15 ± 9) and 10 controls (age range 65-83 years) which found circulating total IGF-1 to be raised in the AD group [29]. Increased circulating IGF-1, both total and bioavailable, is consistent with the hypothesis that late-onset AD may reflect a state of resistance to IGF-1 signalling, with the primary pathogenic event being the loss of

sensitivity to IGF-1 action [5], possibly due to reduced receptor expression or function in the AD brain [25]. In an attempt to overcome this resistance to IGF-1 signalling, circulating IGF-1 increases. Although it has been suggested that the IGF-1 resistance in late-onset, sporadic AD may either originate from prior insulin resistance or lead to the development of insulin resistance [5, 14], there was no difference in measures of peripheral insulin resistance between the AD subjects and controls in our study.

In contrast to the increase in IGF-1 observed in our study and that of Tham et al. [29], reduced circulating total IGF-1 was observed in a study involving 106 Japanese sporadic AD subjects (mean age 79 years with probable AD according to NINCDS-ADRDA criteria and mean MMSE ± S.D. 11 ± 7) compared with 227 control subjects (mean age 76 years) [32]. One possible explanation for the observed difference between our study and that of Watanabe et al. [32] is that in the earlier stages of AD IGF-1 is raised, as seen in our study where the mean MMSE score was 21, whereas in the later stages of the disease IGF-1 decreases, as seen in the study by Watanabe et al. [32] where the mean MMSE score was 11. Although it should be noted that we did not observe any correlation between either total or free circulating IGF-1 and MMSE score.

Circulating total IGF-1 was reported to be reduced in 5 familial AD subjects (mean age 60.8 years with probable AD according to NINCDS-ADRDA criteria) compared with 10 controls (mean age 55.8 years) [22]. However, all the patients in this study carried the Swedish AβPP 670/671 mutation that causes early onset AD. It has been suggested that alterations in IGF-1 in this case may be due to a greater demand for IGF-1 neuroprotection due to the primary pathogenic event (the mutation in AβPP) in this case causing an increase in the production of the neurotoxic Aβ peptide [5].

In conclusion, the increased circulating IGF-1 observed in the present study provides evidence that in the early stages late-onset, sporadic AD may reflect a state of resistance to

IGF-1 signalling. At what stage in the dementia process resistance to IGF-1 develops and whether this state predates the onset of cognitive impairment have yet to be determined. However, it should be emphasised that the results presented here refer to plasma IGF-1 levels and, as cerebrospinal fluid samples were not taken, we cannot be certain that these levels of IGF-1 would be replicated in the brain and thus whether blood vessels, brain parenchymal cells or both are IGF-1 resistant. Also as higher total IGF-1 has also been associated with better cognitive performance and lower risk of cognitive decline in older individuals [17, 23, 24], the measurement of circulating IGF-1 is unlikely to be a useful biomarker to screen for the onset of AD. If in the early stages late-onset, sporadic AD is indeed a state of resistance to IGF-1 signalling, this may explain why MK-0677, a compound that induces secretion of IGF-1 and increased circulating IGF-1 by 60%, failed to alter cognitive function in a double-blind trial in 563 AD patients with baseline MMSE scores between 14 and 26 (http://www.alzforum.org/new/detail.asp?id=1458). Rather, it may be that therapeutics that increase the sensitivity of cells to IGF-1 would hold greater potential in the pharmacological treatment of patients in the early stages of AD.

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Table 1. Clinical and biochemical characteristics of study subjects

Characteristic	AD (n=87)	Control (n=126)	p value
Male %	47	47	NS
Age (yrs)	78.7 ± 0.7	78.8 ± 0.6	NS
MMSE score	21 (7-27)	29 (27-30)	<0.001
3MS score	63 (12-88)	97 (82-100)	<0.001
Current smoker %	2.3	7.9	NS
Alcohol current (units/week)	0 (0-28)	2 (0-60)	NS
Alcohol past (max. units/week)	2 (0-80)	4.5 (0-140)	NS
Diabetes mellitus %	4.6	4.1	NS
Ischaemic heart disease %	17.2	26.2	NS
Cerebrovascular disease %	8.0	11.9	NS
BMI (kg/m^2)	24.9 ± 0.4	26.9 ± 0.4	0.001
Weight (kg)	66.4 ± 1.5	74.1 ± 1.3	<0.001
Height (cm)	163.1 ± 1.1	166.0 ± 0.9	0.038
Head circumference (cm)	56.0 (51.0-61.0)	56.5 (52.0-62.0)	0.039
Schooling (years)	10 (7-14)	10 (7-14)	NS
University attendance (%)	6.9	5.6	NS
Apolipoprotein E4 allele positive (%)	64.4	19.8	<0.001
Fasting plasma glucose (mmol/l)	5.0 (3.8-12.8)	5.2 (4.2-8.9)	NS
Fasting plasma insulin (μIU/ml)*	7.9 (3.3-44.7)	8.0 (3.0-56.0)	NS
HOMA*	1.8 (0.9-21.0)	1.8 (0.6-16.7)	NS
IGF-1 (nM)	11.3 ± 0.3	10.3 ± 0.3	0.022
IGFBP-3 (nM)	65.8 ± 2.1	68.7 ± 1.6	NS

Data are shown as mean ± SEM or median (range). *Those taking synthetic insulin were excluded from the analysis.

Table 2. Association of IGF-1:IGFBP-3 ratio and other parameters with AD

Parameter	Estimated Odds ratio	95% CI	p value
IGF-1:IGFBP-3 molar ratio	1.90*	1.30-2.79	0.001
Age	1.03	0.97-1.09	0.316
Gender	2.26	0.87-5.92	0.096
Height	0.96	0.92-1.01	0.139
Weight	0.96	0.92-0.99	0.008
ApoE4 allele	8.10	3.89-16.87	<0.001
Ischaemic heart disease	0.50	0.20-1.24	0.135
Cerebrovascular disease	0.78	0.23-2.67	0.697
Diabetes mellitus	1.00	0.21-4.70	0.997
Smoking status	0.17	0.02-1.19	0.074
Plasma insulin	1.05	0.99-1.12	0.096

* Estimated Odds ratio for an increase of one standard deviation.

Figure 1. Plasma IGF-1:IGFBP-3 molar ratio in controls and AD subjects.

Bars represent mean ± SEM. * = p<0.001.