Reduced TNF-α and increased IGF-I levels in the serum of Alzheimer’s disease patients treated with the neurotrophic agent Cerebrolysin

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Abstract

According to current scientific knowledge, excess tumour necrosis factor-α (TNF-α) and low insulin-like growth factor-I (IGF-I) are pathogenic-risk factors that constitute therapeutic targets for Alzheimer’s disease (AD). Changes in serum TNF-α, total and dissociable IGF-I levels were determined by ELISA in 207 AD patients completing a 24-wk, double-blind, placebo-controlled trial to evaluate the effects of the neurotrophic compound Cerebrolysin (Cere: 10, 30 or 60 ml for 12 wk). At week 24, Cere reduced TNF-α and enhanced dissociable IGF-I with respect to placebo in a dose-related manner. TNF-α decreased in parallel with behavioural disturbances. Increases in total IGF-I were induced by 60 ml Cere and correlated significantly with improvements in global function, disabilities and behaviour in late-onset AD patients. These results showing for the first time the opposite influence of one anti-dementia treatment on serum TNF-α and IGF-I suggest the contribution of both factors to the clinical effects of Cere, and probably other drugs.

Received 11 March 2009; Reviewed 14 April 2009; Revised 15 April 2009; Accepted 11 May 2009; First published online 17 June 2009

Key words: Alzheimer’s disease, Cerebrolysin, IGF-I, neuropsychiatric symptoms, TNF-α.

Introduction

Elevated tumour necrosis factor-α (TNF-α) and reduced insulin-like growth factor I (IGF-I) levels are considered as pathogenic-risk factors and therapeutic targets for Alzheimer’s disease (AD). TNF-α over-expression in AD brains, cerebrospinal fluid and serum, as well as decreased serum levels and brain gene expression of IGF-I have been found in AD patients (Alvarez et al. 2007; Del Villar & Miller, 2004; Rivera et al. 2005). An increased production of peripheral TNF-α was also reported to be associated with an increased risk of developing AD (Tan et al. 2007) and with a reduced brain volume in subjects without dementia (Jefferson et al. 2007). Furthermore, the recent finding of cognitive improvement after the administration of a TNF-α antagonist supports the therapeutic use of anti-TNF strategies in AD (Tobinick & Gross, 2008). IGF-I was also proposed as a target for AD treatment (Torres-Aleman, 2007), but a recent trial failed to demonstrate the clinical efficacy of enhancing peripheral IGF-I with a growth hormone secretagogue (Sevigny et al. 2008). In a recent study we found a negative correlation between increased TNF-α and reduced IGF-I levels in the serum of AD patients (Alvarez et al. 2007). This finding together with experimental data showing the opposite effects of both factors on neuronal apoptosis and survival and on amyloid-β production, suggests that the signalling pathways of TNF-α and IGF-I are functionally interrelated and might be involved in the clinical response to anti-AD treatments (Carro et al. 2002; Kenchappa et al. 2004; Venters et al. 1999).
Cerebrolysin (Cere) is a peptide compound with a neurotrophic-like mode of action that reduces synaptic loss and amyloid-β production and enhances neurogenesis in several experimental conditions (Chen et al. 2007; Masliah et al. 1999; Rockenstein et al. 2006). According to recent clinical studies, Cere is an effective treatment for AD (Alvarez et al. 2006). The ability of Cere to reduce neuroinflammation has been demonstrated in \textit{in-vitro} and \textit{in-vivo} experiments showing that Cere attenuates microglial activation and the release of interleukin-1β induced by lipopolysaccharides (Alvarez et al. 2000).

Changes in peripheral TNF-α and/or IGF-I levels in response to anti-AD treatments have not been evaluated in previous clinical trials. Based on experimental data indicating that Cere reduces microglia activation and the production of interleukin-1β, we hypothesized a reduction of serum TNF-α in AD patients treated with Cere. According to scientific evidence of a negative interaction between TNF-α and IGF-I, it was also postulated that IGF-I might increase in Cere-treated patients undergoing TNF-α decreases. Therefore, the effects of Cere on the serum levels of TNF-α, total IGF-I and dissociable IGF-I (dIGF-I) were investigated in 207 AD patients completing, according to the protocol, a 24-wk, randomized, double-blind, placebo-controlled, dose-finding trial conducted to evaluate the safety and efficacy of three doses of Cere (10 ml, 30 ml and 60 ml) in comparison with placebo (Alvarez et al. 2006).

\section*{Method}

Our study involved 207 patients [146 female/61 male, average age (± S.D.) 74.06 ± 8.61 yr, range 52–91 yr] with mild to moderate probable AD according to DSM-IV (APA, 1994) and NINCDS-ADRDA (McKhann et al. 1984) criteria that completed a 24-wk, double-blind, placebo-controlled clinical trial to evaluate the safety and efficacy of three dosages of Cere. Exclusion criteria were any other significant neurological or psychiatric disease, allergies, unstable medical conditions or significant laboratory abnormalities. Patients were not taking systemic corticosteroids, anti-parkinsonian agents, narcotics or cholinesterase inhibitors for at least 4 wk prior to baseline. Inclusion and exclusion criteria as specified in a previous publication (Alvarez et al. 2006) were as usual for this kind of clinical trial. This clinical study was performed in compliance with Good Clinical Practice (GCP) and the principles of the Declaration of Helsinki (1964) and its subsequent revisions. Ethical approval for the study was granted by Comité Etico de Galicia (Santiago de Compostela, Spain) and the patients’ informed consent was received. Patients were recruited through selection from the site’s patient database and from new patients attending the site. During the trial patients received intravenous infusions of Cere (10, 30 or 60 ml) or placebo on 5 d/wk for 4 wk and on 2 d/wk for the following 8 wk, and were without treatment for a further 12 wk.

Blood samples were obtained at baseline, after active treatment (week 12) and at the end of the study (week 24). Clinical evaluations were done at the same time-points by using the Clinical Interview Based Impression of Change with Caregiver Input (CIBIC +, ADCS version; Knopman et al. 1994), the Alzheimer’s Disease Assessment Scale – cognitive subpart (ADAS-cog +, ADCS version; Mohs et al. 1997), the Disability Assessment in Dementia (DAD; Gelinas et al. 1998) and the Neuropsychiatric Inventory (NPI; Cummings et al. 1994).

A butterfly-21 G (Vacutainer brand, Becton Dickinson Vacutainer Systems, USA) was inserted into the antecubital vein and fasting blood samples were taken using evacuated blood collecting tubes (Venojet, Terumo Europe N.V., Belgium). Serum samples were then extracted and stored at −40 °C until required for assay. TNF-α, total IGF-I and dIGF-I were measured in serum by using specific enzyme-linked immunosorbent assay (ELISA) kits as previously described (Alvarez et al. 2007). TNF-α concentrations were measured by ELISA using a high sensitivity immunoassay kit with a 0.12 pg/ml detection limit (Quantikine HS, R&D Systems Inc., Minneapolis, USA). Total IGF-I levels were quantified by using an ‘one-step’ sandwich type ELISA kit with extraction having a detection limit of 0.03 ng/ml (Diagnostic Systems Laboratories Inc., USA). dIGF-I levels were determined by using a ‘two-step’ sandwich type non-extraction ELISA kit with a detection limit of 0.01 ng/ml (Diagnostic Systems Laboratories Inc.).

Changes from baseline in TNF-α, total IGF-I and dIGF-I concentrations were analysed by ANCOVA using baseline levels as covariates. The least squares means (LS mean, ± standard error) for each treatment arm were estimated and the difference between each dose of Cere and placebo were calculated together with the corresponding 95% confidence interval (CI). Further, subgroup analyses were performed for total IGF-I levels in the population of patients with late-onset AD and in the subgroup of late-onset AD females. Correlations between biological and clinical parameters were analysed using Pearson’s lineal correlation test. Probability values < 0.05 were considered statistically significant.
Results

As shown in Table 1, baseline levels of TNF-α, total IGF-I and dissociable IGF-I (dIGF-I) were similar in all treatment groups. Age, gender distribution, disease duration and severity, and baseline scores for cognitive performance, disability and neuropsychiatric symptoms (data not shown) were also similar in groups.

In comparison with placebo, Cere reduced TNF-α levels significantly at week 12 with the 60-ml dose, and at week 24 with the 30-ml and 60-ml doses (Table 1, Fig. 1).

Cere treatment induced a significant increase in dIGF-I values with respect to placebo at week 24 with dosages of 10 ml and 60 ml. Similar, but not significant changes were observed for dIGF-I at week 12 (Table 1).

No significant changes in total IGF-I concentrations were observed after treatment with Cere in the whole sample of patients (Table 1). However, compared to placebo Cere enhanced total IGF-I levels with the 60-ml dose in the population of late-onset AD patients at week 12 (treatment difference: 14.41, 95% CI 0.68–28.15, \( p = 0.040 \)), and in the subgroup of females with late-onset AD at both week 12 (treatment difference: 23.55, 95% CI 8.54–38.55, \( p = 0.002 \)) and week 24 (treatment difference: 24.18, 95% CI 4.97–43.38, \( p = 0.014 \)).

Changes in TNF-α and IGF-I levels did not show any significant correlation with scores of change in the clinical parameters when evaluated for the complete dataset of patients. However, in the subgroup of patients with late-onset AD, increases in total IGF-I

Table 1. Effects of Cerebrolysin (Cere) on serum TNF-α, total IGF-I and dissociable IGF-I (dIGF-I) at week 12 (end of treatment) and at week 24 (end of the study) in Alzheimer’s disease patients

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 12</th>
<th>Week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Score</td>
<td>LS mean difference ± S.E. (95% CI)</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>43</td>
<td>3.89</td>
<td>0.49 ± 0.21</td>
</tr>
<tr>
<td>10 ml Cere</td>
<td>49</td>
<td>3.86</td>
<td>−0.05 ± 0.18</td>
</tr>
<tr>
<td>30 ml Cere</td>
<td>56</td>
<td>3.95</td>
<td>0.04 ± 0.17</td>
</tr>
<tr>
<td>60 ml Cere</td>
<td>59</td>
<td>4.32</td>
<td>−0.46 ± 0.17</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>43</td>
<td>135.33</td>
<td>2.74 ± 5.50</td>
</tr>
<tr>
<td>10 ml Cere</td>
<td>49</td>
<td>138.77</td>
<td>3.41 ± 5.20</td>
</tr>
<tr>
<td>30 ml Cere</td>
<td>56</td>
<td>123.02</td>
<td>−4.01 ± 4.87</td>
</tr>
<tr>
<td>60 ml Cere</td>
<td>59</td>
<td>142.34</td>
<td>4.37 ± 4.77</td>
</tr>
<tr>
<td>dIGF-I (ng/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>43</td>
<td>123.84</td>
<td>−4.40 ± 4.37</td>
</tr>
<tr>
<td>10 ml Cere</td>
<td>49</td>
<td>112.18</td>
<td>2.41 ± 4.04</td>
</tr>
<tr>
<td>30 ml Cere</td>
<td>56</td>
<td>103.50</td>
<td>0.28 ± 3.91</td>
</tr>
<tr>
<td>60 ml Cere</td>
<td>59</td>
<td>115.61</td>
<td>6.20 ± 3.81</td>
</tr>
</tbody>
</table>

CI, Confidence interval; LS, least squares; TNF-α, tumour necrosis factor-α; IGF-I, insulin-like growth factor-I.

Treatment difference: Difference of LS means between Cere dose groups and the placebo group.

p values in bold face indicate a significant treatment difference to placebo.
Treatment differences with respect to placebo [least squares (LS) mean] at the end of the study (week 24) are represented. Exact $p$ values for comparisons to placebo are given.

Values at week 24 correlated significantly with improvements in activities of daily living (ADL) (positive correlation with DAD scores of change; $r = 0.191$, $p = 0.034$) and neuropsychiatric symptoms (negative correlation with NPI scores of change; $r = -0.194$, $p = 0.031$), and almost significantly with global clinical outcome (negative correlation with CIBIC+ scores; $r = -0.158$, $p = 0.079$). These correlations were even more evident in female late-onset AD patients for scores of change in total IGF-I vs. DAD ($r = 0.223$, $p = 0.032$), NPI ($r = -0.239$, $p = 0.022$) and CIBIC+ ($r = -0.205$, $p = 0.050$).

Discussion

The present results show that Cere reduces TNF-α and increases IGF-I levels with respect to placebo in the serum of patients with mild to moderate AD in a dose-related manner. The highest dose of Cere (60 ml) inducing the more pronounced effects. As previously published (Alvarez et al. 2006), significant improvements in global clinical function ($p < 0.001$) and favourable treatment differences in cognition, disability and behaviour were observed with all Cere dosages at the end of the study (week 24). Doses of 10 ml and 30 ml Cere induced the biggest effects on cognition and ADL, and the 60-ml dose was the most effective in improving neuropsychiatric symptoms.

Serum TNF-α increased in the placebo group and decreased in Cere-treated patients. Treatment differences accounted for a TNF-α reduction of 25% at week 12 and 20% at week 24 in patients treated with 60 ml Cere. This finding indicates that the effects of Cere on TNF-α levels are mainly maintained 3 months after stopping its administration at week 12. The mechanism by which Cere reduces circulating TNF-α is unknown, but our clinical results are in agreement with experimental data demonstrating that Cere inhibits microglial activation and the production of interleukin-1β in vitro and in vivo (Alvarez et al. 2000). Although there are no previous studies evaluating the effects of anti-dementia drugs on serum TNF-α, the decrease of peripheral TNF-α might contribute to the clinical benefits of Cere. This possibility is consistent with reports of increased TNF-α levels in AD and its association with reduced brain volume measures in subjects without dementia (Alvarez et al. 2007; Jefferson et al. 2007), and is also supported by the cognitive improvement recently found in AD patients treated with a TNF antagonist (Tobinick & Gross, 2008). In addition, since Cere induces similar dose-dependent reductions in TNF-α and in neuropsychiatric symptoms (Alvarez et al. 2006) the decrease of TNF-α might be relevant for the positive effects of Cere on behaviour. Otherwise, changes in TNF-α could reflect an antidepressant-like activity of Cere as suggested by findings of reduced TNF-α after successful treatment with antidepressants (O’Brien et al. 2007).

DIGF-I values, unlike TNF-α, decreased in the placebo group and increased in patients treated with 10 ml and 60 ml Cere. However, total IGF-I levels showed no apparent change in the complete sample of patients. Since low IGF-I is considered as a risk factor for late-onset AD (Torres-Aleman, 2007), we decided to analyse IGF-I data in the subgroup of 149 patients (111 female, 38 male) with late-onset AD. Interestingly, total IGF-I also decreased progressively in placebo-treated patients and increased in those receiving 60 ml Cere, these changes were more evident in females. Taken together, these results indicate that Cere counteracts the progressive decrease of serum IGF-I observed in AD patients on placebo treatment. Furthermore, the activation of IGF-I might contribute, in some part, to the clinical effects of Cere as suggested by the fact that increases in IGF-I correlate with improvements in ADL, behaviour and global function. The impact of serum IGF-I on brain functions is supported by experimental data demonstrating that peripheral IGF-I reduces brain amyloidosis and cognitive impairment in AD animal models (Carro et al. 2002). There are no previous studies on the effects of current AD treatments on serum IGF-I, but a recent paper has reported a worsening response to donepezil in AD.

Fig. 1. Dose-related effects of Cere (10 ml, 30 ml, 60 ml) on the serum levels of dissociable insulin-like growth factor-I (dIGF-I; – – –) and tumour necrosis factor-α (TNF-α; – – –) in patients with mild to moderate Alzheimer’s disease.
patients with low IGF-I levels (Tei et al. 2008). This finding is consistent with a potential influence of circulating IGF-I on the therapeutic response to some AD drugs. However, the increase of serum IGF-I seems to be insufficient to improve the clinical manifestations of AD as evidenced by the failure of a recent clinical trial performed with a growth hormone secretagogue (Sevigny et al. 2008).

Cere–placebo treatment differences obtained for TNF-α and IGF-I determinations were almost similar at the end of the active treatment period (week 12) and after 3 months without Cere administration (week 24). These long-lasting effects of Cere on TNF-α and IGF-I are consistent with the precluded neurotrophic mode of action of the compound and with the maintenance of its cognitive effects during the same period (Alvarez et al. 2006). Changes induced by 60 ml Cere on TNF-α and IGF-I levels tended to disappear slowly over time, being more pronounced at week 12 than at week 24 (Table 1), which seems to support a direct influence of Cere on these biological parameters. On the other hand, the increase of TNF-α and the decrease of IGF-I values observed in placebo-treated patients are in line with the expected changes for AD patients (Alvarez et al. 2007) and also contribute to the net effects of Cere reported here.

In summary, results of our study demonstrate for the first time the opposite effects of one anti-dementia drug on serum TNF-α and IGF-I levels. Whether changes in both factors are interrelated or correspond to the influence of Cere on different mechanisms remains to be elucidated. There is experimental evidence of a negative interaction between TNF-α and IGF-I systems, and the serum levels of these two parameters correlate negatively in AD patients (Alvarez et al. 2007). Thus, it is likely that Cere might induce changes in TNF and IGF through a common mechanism. According to results of two recent studies demonstrating that Cere reduces glycogen synthase kinase-3β (GSK-3β) activity in the brain of APP transgenic mice (Rockenstein et al. 2006) and that insulin inactivates GSK-3β in the hippocampus and reduces plasma levels of TNF-α in a rat model of ischaemia/reperfusion (Collino et al. 2009), exploration of the involvement of GSK-3β inhibition in the effects of Cere on TNF-α and IGF-I levels in AD patients is warranted. Further studies might also investigate the influence of Cere on other components of the peripheral TNF-α and IGF-I systems, e.g. soluble TNF receptors and IGF binding proteins. Since dIGF-I represents an indirect measurement, free IGF-I levels have to be determined in order to confirm the increase of bioavailable IGF-I after Cere treatment.

Acknowledgements

The study has been funded by a grant from EBEWE Neuro Pharma, Unterach, Austria.

Statement of Interest

Herbert Moessler is an employee of EBEWE Neuro Pharma, Research Department. He has no ownership in the company and his remuneration is not linked to the outcome of research projects.

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