Increase of BDNF Serum Concentration in Lithium Treated Patients with Early Alzheimer’s Disease

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Abstract. Preclinical and clinical studies gave evidence that lithium could be useful in the treatment of Alzheimer’s disease (AD). In experimental investigations, lithium induces brain-derived neurotrophic factor (BDNF). Recent studies have found a decrease of BDNF in the serum and brains of AD patients with potentially consecutive lack of neurotrophic support. We assessed the influence of a lithium treatment on BDNF serum concentration in a subset of a greater sample recruited for a randomized, single-blinded, placebo-controlled, parallel-group multicenter 10-week study, investigating the efficacy of lithium treatment in AD patients. In AD patients treated with lithium, a significant increase of BDNF serum levels, and additionally a significant decrease of ADAS-Cog sum scores in comparison to placebo-treated patients, were found. Diminution of cognitive impairment was inversely correlated with lithium serum concentration. Upregulation of BDNF might be part of a neuroprotective effect of lithium in AD patients. The results of the present investigation encourage performing studies with longer treatment phases to observe potential positive long-term effects of lithium in AD patients.

Keywords: Alzheimer’s disease, brain-derived neurotrophic factor, lithium, neuroprotection, neurotrophic effect, placebo-controlled trial

INTRODUCTION

Alzheimer’s disease (AD) is the most common cause of dementia in older people characterized by the accumulation of amyloid plaques and neurofibrillary tangles in the brain [1]. Currently, three cholinesterase inhibitors (ChEI) and one N-methyl-D-aspartate (NMDA) receptor antagonist are approved for the treatment of AD. These drugs have demonstrated benefits in cognition and global function over 6–12 months in a proportion of patients, but do not substantially change the course of the disease [2,3]. Thus, there is a need for the development of new therapeutic strategies.

Recently, preclinical and clinical studies gave evidence that lithium, a drug with proven efficacy in the therapy of affective disorders, has neuroprotective and neurotrophic effects and could be useful in the treatment of AD [4]. It has been shown that lithium inhibits the activity of glycogen synthase kinase-3 (GSK-3) [5, 6], which is involved in the aberrant hyperphosphorylation of the microtubule-associated protein tau, a major constituent of neurofibrillary tangles [7], and also in amyloid deposition [8]. Moreover, lithium induces...
BDNF belongs to the family of nerve growth factors and plays an important role in neuronal survival, differentiation, and synaptic plasticity in the central nervous system (CNS). In the last few years, there has been growing evidence for its involvement in the pathogenesis of AD [11], and the possible role of BDNF as a therapeutic target for AD is discussed [12].

Several postmortem analyses have shown decreased cerebral BDNF levels in pre-clinical stages of AD [13] and decreased levels of BDNF mRNA or protein in the hippocampus and cortex in later stages of the disease [14–17]. In previous studies, we could demonstrate significantly diminished BDNF serum concentrations in patients with beginning AD compared to normal controls [18,19]. In addition, Yasutake and colleagues [20] showed this for AD patients in late stages compared to vascular dementia and healthy controls. It can be concluded that BDNF levels are diminished both in the brain and serum of AD patients. This may reflect a lack of trophic support and thus contribute to progressive neurodegeneration in AD.

Bearden et al. [21] showed increased cortical gray matter density in lithium-treated patients with bipolar disorder compared to healthy controls as well as bipolar patients not taking lithium and concluded that these neuroanatomic differences are possibly due to neurotrophic effects of lithium. In a longitudinal volumetric magnetic resonance imaging study, bilateral increases in the volume of hippocampus of patients with bipolar disorders receiving lithium over a period of two to four years were detected [22].

Two case control studies delivered contradictory results concerning the question whether lithium treatment protects against the onset of dementia. While an investigation in the United Kingdom found a trend towards an increasing risk of a diagnosis of dementia in patients receiving lithium [23], a Brazilian study detected that lithium treatment reduced the prevalence of AD in patients with bipolar disorders [24]. A correlative study in non-demented elderly patients found a significantly better Mini-Mental State Examination (MMSE) score [25] in the group with current or previous lithium intake compared to matched controls without lithium prescription [26]. A single-case study reported that the administration of lithium alleviated symptoms of aggression and agitation in a patient with AD. Decreased cognitive function remained after 1.5 years of treatment [27]. However, up until now, no controlled prospective studies of the effect of lithium in AD patients have been performed.

In the present investigation, the influence of a lithium treatment on BDNF serum concentration was assessed in a subset of a greater sample recruited for a randomized, single-blinded, placebo-controlled, parallel-group multicenter 10-week study, investigating the efficacy of lithium treatment in AD patients (Hampel et al., unpublished). It was an additive examination undertaken at one of the six sites participating in the multicenter study.

SUBJECTS AND METHODS

Subjects and clinical assessment

27 patients with mild AD (defined as MMSE ≥ 21 and ≤ 26) were enrolled at the University Hospital of Tübingen (Department of Psychiatry and Psychotherapy, Geriatric Center, Department of Neurodegenerative Diseases, Hertie Institute of Clinical Brain Research) from November 2004 until June 2005. Patients were diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable AD [28] and fulfilled the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) [29] criteria for primary degenerative dementia of the Alzheimer type. Routine assessment involved physical, neurological, and psychiatric examination, neuropsychological assessment using the MMSE [25] and the Alzheimer’s Disease Assessment Scale, Cognitive subsection (ADAS-Cog) [30], electrocardiogram (ECG), and laboratory tests (alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, sodium, creatinine, osteocalcin, potassium, thyroid stimulation hormone, thyroxine, and haematology).

For inclusion in the study, patients had to fulfill each of the following criteria: 1) Provision of informed consent; 2) Female, without childbearing potential (post-menopausal for at least one year or surgically sterile), or male, aged 50–85 years; 3) Clinical diagnosis of AD of mild severity (MMSE total score ≥ 21 and ≤ 26); 4) DSM-IV criteria for primary degenerative dementia of the Alzheimer type; and 5) Willingness and ability to complete all study-related procedures and to understand patient information.

Exclusion criteria encompassed presence of abnormal values on the laboratory tests that may indicate contraindication for lithium treatment, untreated hypothyroidism, ECG changes indicative of cardiovascular dis-
ease, concomitant use of particular drugs (valproic acid, memantine, neuroleptics, coumarin anticoagulants, or non-steroidal anti-inflammatory drugs), salt-restricted diet, clinically significant liver disease or elevation in ALP, ALT, AST or total bilirubin 1.5 times the upper limit of the reference range, renal disease or creatinine elevated by 1.5 times the upper limit, drug or alcohol abuse, and participation in another drug trial within four weeks prior to enrollment.

Ethical approval was given by the Institutional Review Board (IRB) and the local Ethics Committee. Informed consent was obtained from each subject after oral and written information about the nature, purpose, possible risks, and benefits of the study. The study was performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with the International Conference on Harmonisation (ICH)/Good Clinical Practice (GCP).

In this study, patients were either treatment naïve or had received treatment with ChEI in a stable dose for at least six months. At Tübingen, all 27 patients were successfully randomized either to the lithium (13 patients) or placebo condition (14 patients). Three patients receiving placebo and four patients receiving lithium were previous on ChEI treatment.

One patient of the lithium group discontinued the study eight weeks after randomization not willing to continue the treatment. He had been on the lithium target concentration for four weeks. One serious adverse event occurred in the lithium group consisting of severe aggression with non-serious hallucinations in a 75-year old male patient seven weeks after start of the study. The co-medication consisted of ramipril, bisoprolol fumarate for arterial hypertension, ginkgo biloba extract for dementia, and potassium for hypopotassemia. The patient was hospitalized and discharged after complete recovery 17 days later. The study medication continued unchanged in this case. The adverse event was not attributed to the study medication.

15 control subjects without any organic brain disorders and with a MMSE score \( \geq 27 \) (mean of 28.5 ± 1.5 SD) underwent a physical, neurological, and psychiatric examination. From these subjects, a blood sample was taken to assess BDNF serum concentration. The local Ethics Committee approved to assess BDNF serum concentration in the control subjects and the AD patients and informed consent was obtained from each subject.

Study design

Following an enrollment visit and baseline assessments, eligible patients were randomized to receive lithium sulphate (Lithionit®) or placebo, and entered into a titration phase of six weeks. During the titration phase, there were weekly visits to adjust the lithium dose to the target serum lithium concentration of 0.5–0.8 mmol/L. The starting dose of lithium sulphate 42 mg (6 mmol Li\(^+\)) was 1 + 1 tablets daily (one tablet in the morning and one tablet in the evening approximately in a 12 hours interval). Dosages were escalated at weekly intervals until the target serum lithium concentration of 0.5–0.8 mmol/L (measured 12 hours from last dose). If dose-limiting toxicity was observed, the dose could be reduced, according to the clinical judgment, to a maximum tolerated dose. Also titration had to be stopped if the patient had completed the maximum dose of 4 + 4 tablets daily (i.e., a maximum total daily dose of 336 mg lithium sulphate). Drug concentration levels were assessed weekly for both lithium and placebo patients to maintain the blinding of all patients. The investigator was aware of the patients receiving lithium or placebo. Thereafter, a maintenance phase followed for four weeks (where drug concentration levels were assessed bi-weekly) and end-of-treatment assessment occurred after a total of ten weeks of treatment. A follow-up visit or telephone contact occurred approximately two weeks after the end-of-treatment visit. The serum lithium concentration range of 0.5–0.8 mmol/L was in accordance with the recommendations for use in bipolar disorder and well below those levels associated with lithium toxicity. Patients randomized to receive placebo remained at the starting dose of 1 + 1 tablets daily throughout the titration phase. At Tübingen, all 13 patients receiving lithium reached the targeted serum lithium concentration while the placebo group remained stable at a level below the lower limit of the target serum lithium concentration.

Measurement of lithium concentration in serum

Assessment of serum lithium concentration was performed with a colorimetric test (VITROS system, Orthoclinical Diagnostics GmbH Neckargemünd, Germany) according to the manufacturer’s instructions in the morning 12 hours from the last dose.

Measurement of BDNF concentrations in serum

Peripheral venous blood of the fasted study subjects was sampled into serum tubes between 8:00 and 9:00 am in order to take in account a possible circadian rhythm. Tubes were immediately immersed in melting ice. To minimize the source of platelets, serum
was centrifuged within 30 minutes after gaining and stored at \(-18^\circ\text{C}\) until further analysis. Serum levels of BDNF were measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems GmbH Wiesbaden-Norderstadt, Germany) according to the manufacturer’s instructions. All samples and standards were measured in duplicates, and the means of the duplicates were used for statistical analyses. The detection limit for BDNF was 31.25 pg/ml. The intra- and interassay coefficients of variation were <10%.

In the AD patients, BDNF was measured at baseline before treatment with lithium or placebo and at the end of the treatment phase.

Statistical analysis

All statistical analyses were carried out using the statistical analysis software package SPSS 14.0® (Munich, Germany). The data are presented as the mean ± standard deviation (SD). The two-tailed t-test for unpaired samples was used to compare the BDNF serum levels between the groups and the ADAS-Cog sum scores between the AD groups. The statistical analysis of differences between the two AD groups was performed using a multivariate analysis of variance (MANOVA) with the within-subject factor time and the inter-subject factor treatment (placebo vs. lithium) and the interaction of time and treatment. The exact Fisher’s test was applied for comparison of gender distribution. After controlling for Gaussian distribution by the Levene test, correlations between variables were determined using Pearson tests. Significance for the results was set at \(p<0.05\).

RESULTS

The demographic data and the mean MMSE scores of the AD patients receiving lithium as well as the ones receiving placebo and of the healthy controls are displayed in Table 1. All three groups were comparable regarding age and gender. Both AD groups did not differ in mean MMSE scores. However, the mean MMSE scores of the healthy controls were significantly higher than that of both AD groups.

In Fig. 1 BDNF serum concentrations of the healthy controls before treatment with lithium (AD-T0-Li) or placebo (AD-T0-Pl), and after treatment with lithium (AD-T1-Li) or placebo (AD-T1-Pl). *AD patients showed a significant increase of BDNF serum levels with time (df = 1, \(F = 10.193; p = 0.004\)) and a significant interaction of time and treatment (df = 1, \(F = 4.977; p = 0.035\)) of lithium treatment compared to the placebo group.

At the end of the treatment, the BDNF serum concentrations were significantly increased in the AD patients receiving lithium showing no more difference to the healthy controls (24.3 ± 8.5 ng/ml, \(p = 0.724\)). In the placebo group, BDNF serum concentrations remained significantly diminished after ten weeks of treatment in comparison to healthy controls (19.8 ± 6.3 ng/ml, \(p = 0.015\)). According to MANOVA, AD patients showed a significant increase of BDNF serum levels with time (df = 1, \(F = 10.193; p = 0.004\)) and a significant interaction of time and treatment (df = 1, \(F = 4.977; p = 0.035\)) of lithium treatment compared to the placebo group.

At baseline, both AD groups did not differ in mean ADAS-Cog sum scores (lithium group 19.2 ± 5.7, placebo group 16.5 ± 5.1, \(p = 0.195\)). At the end of the treatment, the patients receiving lithium showed a slightly better performance compared to baseline (17.7 ± 5.8), while the placebo group scored poorer (18.0 ± 5.1). According to MANOVA, AD patients showed no significant decrease of ADAS-Cog sum scores with time (\(F = 0.001; p = 0.974\)) but a significant interaction of time and treatment (df = 1, \(F = 6.751; p = 0.015\)) of lithium treatment compared to the placebo group.
Demographic and clinical parameters of Alzheimer’s disease patients (AD, before treatment with lithium or placebo) and the healthy control group.

<table>
<thead>
<tr>
<th>Variables</th>
<th>AD patients Lithium (1)</th>
<th>AD patients Placebo (2)</th>
<th>Healthy controls (3)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>1-2/1-3/2-3</td>
</tr>
<tr>
<td>Male/Female, n</td>
<td>6/7</td>
<td>5/9</td>
<td>8/7</td>
<td>0.704/1.00/0.46²*</td>
</tr>
<tr>
<td>Age (years), mean ± SD</td>
<td>69.4 ± 8.5</td>
<td>71.0 ± 9.0</td>
<td>69.2 ± 8.6</td>
<td>0.636/0.955/0.586²</td>
</tr>
<tr>
<td>MMSE, mean ± SD</td>
<td>23.6 ± 1.4</td>
<td>23.5 ± 1.7</td>
<td>28.5 ± 1.5</td>
<td>0.854/0.0001/0.0001²</td>
</tr>
</tbody>
</table>

MMSE = Mini-Mental State Examination, n = number of subjects, SD = standard deviation. *exact Fisher’s test; ‡two-tailed t-test.

We found a significant inverse correlation between lithium serum concentrations after ten weeks of treatment and changes of ADAS-Cog sum scores between baseline (T0) and end of treatment (T1; \( r = -0.641; p = 0.025 \)) in the AD patients treated with lithium (Fig. 2). However, we did not find a significant correlation between lithium serum levels after ten weeks of treatment and BDNF changes (\( r = 0.175; p = 0.568 \)) (Fig. 3). In addition, we could not show a significant correlation between ADAS-Cog sum score changes and BDNF changes (\( r = -0.187; p = 0.349 \)).

**DISCUSSION**

To our knowledge, this is the first prospective randomized study describing the impact of a treatment with lithium on BDNF serum concentration in patients with AD. As main results, AD patients treated ten weeks with lithium showed a significant increase of BDNF serum levels and a significant decrease of cognitive impairment measured by a decrement of ADAS-Cog scores compared with placebo-treated AD patients. In addition, our finding of an inverse correlation between lithium serum concentrations after ten weeks of treatment and changes of ADAS-Cog sum scores between baseline and end of treatment indicates that the level of lithium serum concentration may have influenced the degree of clinical improvement of cognitive functions in AD patients.

The results of our study are in accordance with pre-clinical investigations evaluating an induction of BDNF by lithium in rodents and cultured neurons. Fukushima et al. [9] showed that chronic lithium treatment...
significantly increases the expression of BDNF in the hippocampus as well as the temporal and frontal cortex of rat brains. Hashimoto et al. [10] found that lithium induces BDNF in primary cultures of rat cerebral cortical neurons and protects those against glutamate excitotoxicity.

The main source of serum BDNF are human platelets [31–33], which are responsible for the 100-fold higher average serum BDNF levels compared to plasma [34]. The protein can cross the blood-brain barrier [35,36], and an animal study found a positive correlation between serum and cortical BDNF concentrations [37]. Thus, it is reasonable to assume that serum BDNF changes are paralleled by changes of BDNF levels in the brain.

There are several data indicating that BDNF protects neurons in CNS against different forms of brain injury caused, for example, by cerebral ischemia, hypoglycemic coma [38], HIV [39], and bacterial meningitis [40]. Reactive glial cells (microglia and astrocytes) produce neurotrophins such as BDNF [41], which selectively regulates microglial proliferation and function [42,43], suggesting a possible involvement of these cells in the mechanism of neuronal survival.

Lithium may provide neuroprotective effects through several mechanisms. Based on this study and previous experimental evidence, the induction of BDNF could be one relevant mechanism in AD patients. BDNF has been demonstrated to contribute to increased amyloid-β degradation by promoting the expression of somatostatin [44,45]. Somatostatin increases neprilysin activity in primary cortical neurons, which is the key in vivo enzyme degrading amyloid-β [46]. In addition, as lithium itself, [5,6] BDNF is capable of inactivating GSK-3β [47], which is involved in hyperphosphorylation of tau protein [48] and also in amyloid deposition [8]. Moreover, lithium inhibits NMDA-receptor-mediated calcium influx, upregulates anti-apoptotic Bcl-2, down-regulates pro-apoptotic p53 and Bax, and activates cell survival factors [49].

In summary, we could show for the first time that a short-term treatment with lithium over ten weeks already induces a significant increase of BDNF serum concentrations in AD patients reaching levels of healthy controls. In addition, compared with a placebo-group we found a significant decrease of ADAS-Cog sum scores in AD patients treated with lithium, which was correlated to serum lithium concentration. Upregulation of BDNF might be part of a neuroprotective effect of lithium in AD patients. Thus, the results of our study encourage performing studies with longer treatment phases to observe potential positive long term effects of lithium in AD patients.
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REFERENCES


