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ORIGINAL PAPER

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Increase of BDNF serum concentration during donepezil treatment of patients with early Alzheimer's disease

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Abstract Alzheimer's disease (AD) can be treated with inhibitors of the enzyme acetylcholinesterase (AChE). Recent pre-clinical and clinical studies gave evidence that AChE-inhibitors have neuroprotective effects and thereby a disease-modifying potential. The mechanism of this action is still discussed. In an animal model oral administration of an AChE-inhibitor lead to an increase of brain derived neurotrophic factor (BDNF) in hippocampus and cortex. Recent studies have found a decrease of BDNF in the serum and brain of AD patients with potentially consecutive lack of neurotrophic support and contribution to progressive neurodegeneration. BDNF serum concentrations were assessed by ELISA in 19 AD patients and 20 age-matched healthy controls at baseline and in the AD patients after 15 months of treatment with donepezil 10 mg per day (one patient received just 5 mg). Before treatment with donepezil we found in AD significantly decreased BDNF serum concentrations (19.2 ± 3.7 ng/ml) as compared to healthy controls (23.2 ± 6.0 ng/ml, $P = 0.015$). After 15 months of treatment the BDNF serum concentration increased significantly in the AD patients (23.6 ± 7.0 ng/ml, $P = 0.001$) showing no more difference to the healthy controls ($P = 0.882$). The results of the present study confirm data of prior investigations that a down-regulation of BDNF in serum and brain of AD patients seems to begin with the first clinical symptoms and to be persistent. A treatment with the AChE-inhibitor donepezil is accompanied with an increase of BDNF serum concentration in AD patients reaching the level of healthy controls. Thus, up-regulation of BDNF might be part of a neuroprotective effect of AChE-inhibitors. The molecular mechanism of this potentially disease-modifying mechanism of action of donepezil should be clarified.

Key words BDNF · donepezil · acetylcholinesterase-inhibitor · neuroprotection

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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 [3]. A treatment with inhibitors of the enzyme acetylcholinesterase (AChE) is recommended by several guidelines. These drugs enhance the central concentrations of synaptic acetylcholine, thus improving interaction between neurons of the cholinergic system being involved in memory function. Besides this symptomatic mechanism of action there is accumulating evidence that AChE-inhibitors have neuroprotective effects and thereby a disease-modifying potential [28]. Supported by pre-clinical and clinical data several models of neuroprotection by AChE-inhibitors are discussed [35]. A recent study has shown, that oral administration of huperzine A, a reversible and selective inhibitor of AChE, significantly increases mRNA and protein levels of brain-derived neurotrophic factor (BDNF) and markedly attenuates the memory deficits and neuronal damage after transient cerebral ischemia and reperfusion in mice [42]. In addition, chronic donepezil treatment of rats has been demonstrated to increase the phosphorylation of CREB (cAMP response element binding protein), which is an important upstream signalling of BDNF [16].

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). Recent studies showed an increase of BDNF serum concentration during antidepressive treatment [11]. In some subgroups of depressive patients, BDNF may be associated with the response to electroconvulsive therapy [14].

In the last years, there is growing evidence for its involvement in the pathogenesis of AD [23] and the possible role of BDNF as a therapeutic target for AD is discussed [10]. In several postmortem analyses decreased levels of BDNF mRNA or protein could be demonstrated in the hippocampus and cortex of AD brains [12, 13, 25, 31], and Yasutake et al. [41] showed that BDNF serum concentration in late stages of AD (mean Mini-Mental State Examination (MMSE) of 6.88 ± 6.78 SD) is significantly diminished in comparison to patients with vascular dementia and normal controls. In a previous study we found a decreased serum concentration of BDNF already in patients with beginning AD (mean MMSE 23.6 ± 1.6 SD) compared to healthy age matched people [18]. It was hypothesized that this may reflect a lack of trophic support and thus contributes to progressive neurodegeneration in AD brains.

In the present study we investigated the influence of a donepezil treatment on BDNF serum concentrations in patients with early AD.

Materials and methods

Subjects and clinical assessment

Nineteen AD patients and 20 healthy controls were investigated (Table 1). All AD patients met the diagnostic criteria of probable AD according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition [5], the ICD-10 Classification of Mental and Behavioural Disorders [39] and the criteria of the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer's Disaease and Related Disorders Association (NINDS-ADRDA) [24]. The severity of dementia was assessed by MMSE [7] at baseline before treatment with donepezil and after 15 months of treatment with donepezil 10 mg per day (one patient received just 5 mg). The control subjects were without any organic brain disorders and had to reach a MMSE score ≥27 (mean of 28.5 ± 1.5 SD). Patients with manifest depressive or psychotic episodes were excluded from the study. Concomitant medication was not known to interfere with BDNF. Especially no patient received antidepressants, nonsteroidal antiphlogistics or statins.

Table 1 Demographic and clinical parameters of Alzheimer's disease patients (AD, before treatment with donepezil) and the control group. MMSE = Mini-Mental State Examination, *n* = number of subjects, SD = standard deviation

Variables	AD patients *n* = 19	Control group *n* = 20	*P*-value
Male/Female, *n*	4/15	13/7	0.01
Age (years), mean ± SD	70.9 ± 8.7	69.6 ± 11.6	0.7
MMSE, mean ± SD	23.5 ± 1.6	28.5 ± 1.5	<0.0001

The AD patients and the control group underwent a physical, neurological, and psychiatric examination. In addition, an electroencephalography and a computed tomography or magnetic resonance imaging (MRI) was also performed, to validate the diagnosis of AD. Routine laboratory tests included lues serology, analysis of vitamin B 12, folic acid and thyroxine levels, to exclude other causes of dementia. The regional ethical committee approved the study and written informed consent was obtained from each individual.

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 into account a possible circadian rhythm. Tubes were immediately immersed in melting ice. To minimize the source of platelets, serum was centrifuged within 30 min after gaining and stored at −18°C until further analysis. Serum levels of BDNF were measured using an enzyme-linked immuno sorbent 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 AD patients BDNF was measured at baseline before treatment with donepezil, and after 15 months of treatment with donepezil.

Statistical analysis

The data were presented as the mean ± standard deviation (SD). For statistical analysis of gender differences between the AD patients and the healthy controls Exact Fisher's Test was used. BDNF serum concentrations of AD patients at baseline and after 15 months of treatment with donepezil were compared by the two-tailed *t*-test for paired samples. All other statistical analyses were performed using the two-tailed *t*-test for unpaired samples. Significance for the results was set at $P < 0.05$. All statistical analyses were carried out using the statistical analysis software package SPSS 12.0® (Munich, Germany).

Results

AD patients and healthy controls were comparable regarding age but not gender and MMSE scores (Table 1).

Before treatment with donepezil we found in AD significantly decreased BDNF serum concentrations (19.2 ± 3.7 ng/ml) as compared to healthy controls (23.2 ± 6.0 ng/ml, $P = 0.015$). After 15 months of treatment the BDNF serum concentration increased significantly in the AD patients (23.6 ± 7.0 ng/ml, $P = 0.001$) showing no more difference to the healthy controls ($P = 0.882$; Fig. 1).

MMSE scores of AD patients after 15 months of treatment with donepezil were significantly diminished compared to scores before treatment (23.5 ± 1.6 versus 21.1 ± 3.2; $P = 0.005$).

Discussion

In the present investigation we could confirm the results of a previous study showing significant decrease of BDNF serum concentration in patients with

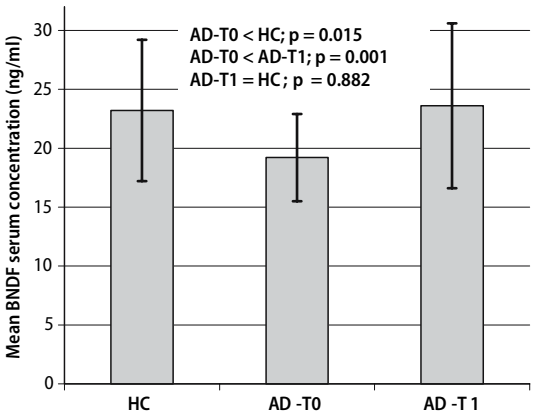

Fig. 1 Mean BDNF serum concentration (standard deviation indicated by error bars) of healthy controls (HC), Alzheimer's disease patients before treatment (AD-T0) and after 15 month of treatment with donepezil (AD-T1)

beginning AD compared to healthy age matched controls [18]. After 15 months of treatment with the AChE-inhibitor donepezil we found a significant increase of BDNF serum level revealing no more difference to the healthy controls. MMSE scores of the AD patients significantly decreased in the same time. To our knowledge this is the first study describing the impact of a treatment with an AChE-inhibitor on BDNF serum concentration in patients with AD.

BDNF protects neurons in CNS against different forms of brain injury caused for example by cerebral ischemia, hypoglycemic coma [20], HIV [26] and bacterial meningitis [2]. Reactive glial cells (microglia and astrocytes) produce neurotrophins such as BDNF [19], that selectively regulates microglial proliferation and function [6, 27], suggesting a possible involvement of these cells in the mechanism of neuronal survival.

The main source of serum BDNF are human platelets [17, 32, 40] being responsible for the 100-fold higher average serum BDNF levels compared to plasma [34]. Individual factors can limit the usefulness of serum BDNF measurements [21]. The protein can cross the blood-brain barrier [29, 33], and an animal study found a positive correlation between serum and cortical BDNF concentrations [15]. Thus, it seems reasonable to assume that there might be an association between BDNF levels in the brain and serum.

Several post mortem analyses have shown decreased cerebral BDNF levels in pre-clinical stages of AD [30] and decreased levels of BDNF mRNA or protein in the hippocampus and cortex in later stages of the disease [12, 13, 25, 31]. We could demonstrate significantly diminished BDNF serum concentration in patients with beginning AD compared to normal controls in this and a previous study [18], while Yasutake et al. [41] could show this for AD patients in late stages compared to vascular dementia and healthy controls. It can be concluded that in AD brains BDNF levels are diminished paralleled by a significant reduction of BDNF serum concentration. This may reflect a lack of trophic support and thus contribute to progressive neurodegeneration.

As a completely new result we found a significant increase of BDNF serum level in AD patients during treatment with the AChE-inhibitor donepezil. After 15 months the concentration showed no more difference to age matched healthy controls. As a limitation, it must be mentioned that BDNF measurement of the control subjects was just performed at baseline. Besides their well known mechanism of action by inhibiting the enzyme acetylcholinesterase recent preclinical and clinical studies gave evidence for a neuroprotective effect of the AChE-inhibitors [9, 35]. The exact mechanism of neuroprotection by AChE-inhibitors is still discussed [1, 28]. A chinese study showed a significant increase of mRNA and protein level of BDNF in mice after oral administration of huperzine A, an AChE-inhibitor used in clinical treatment of AD in China [42]. The animals got the drug 2 days before and 7 days after transient cerebral ischemia and reperfusion initiated by bilateral common carotid occlusion combined with systemic hypotension. The AChE-inhibitor markedly attenuated the memory deficits tested in a water maze task and neuronal degeneration in cerebral cortex and hippocampus of the mice.

Thus, up-regulation of BDNF might be at least part of the neuroprotective effect of AChE-inhibitors. Besides a compensatory repair mechanism this may lead to other neuroprotective mechanism. Accumulation of beta-amyloid (Abeta) and abnormal hyperphosphorylation of tau protein are crucial pathological processes for neuronal cell death (apoptosis) in AD. BDNF has been demonstrated to contribute to increased Abeta degradation by promoting the expression of somatostatin [22, 37]. Somatostatin increases neprilysin activity in primary cortical neurons, which is the key in vivo enzyme degrading Abeta [36]. In addition, BDNF is capable to inactivate glycogen synthase kinase 3 beta (GSK-3beta) [8], which is involved in hyperphosphorylation of tau protein [4].

The up-regulation of serum BDNF was not paralleled by a cognitive stabilization or improvement in the AD patients in our study. MMSE score significantly decreased during donepezil treatment. Generally there is a decline in cognition to below baseline levels after approximately 1 year of treatment with AChE-inhibitors, but the level of cognition remains above that predicted for those not receiving pharmacologic treatment [38]. Thus, it appeared unethically to put patients with AD for 15 months on placebo. We cannot say whether a placebo group would have shown more cognitve decline and no up-regulation of BDNF serum levels. As Yasutake et al. [41] have found decreased BDNF serum concentration also in late stages of AD patients compared to normal controls, it is reasonable to assume that a

persistent decrease of serum BDNF is usual in AD and the increase during donepezil treatment in our study is due to the AChE-inhibitor. The molecular mechanism, how the administration of donepezil leads to an up-regulation of BDNF has to be clarified.

Conclusions

The results of the present study confirm data of prior investigations showing a decrease of BDNF levels in serum and brain of patients with AD. This down-regulation seems to begin with the first clinical symptoms of the disease and to be persistent. It may reflect a lack of trophic support and thus contribute to progressive neurodegeneration. A treatment with the AChE-inhibitor donepezil is paralleled with an increase of BDNF serum concentration in AD patients reaching the levels of healthy controls. Thus, up-regulation of BDNF might be part of a neuroprotective effect of AChE-inhibitors. These results should be confirmed in larger studies, and the molecular mechanism of this potentially disease-modifying mechanism of action of donepezil should be clarified.

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