Beyond the Hypothesis of Serum Anticholinergic Activity in Alzheimer's Disease: Acetylcholine Neuronal Activity Modulates Brain-Derived Neurotrophic Factor Production and Inflammation in the Brain

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Key Words

Alzheimer's disease · Serum anticholinergic activity ·
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Abstract

The brain of Alzheimer's disease (AD) patients is characterized by neurodegeneration, especially an acetylcholine (ACh) neuronal deficit with accumulation of β -amyloid protein, which leads to oxygen stress and inflammation. The active oxygen directly damages the neuron by increasing intracellular Ca^{2+} . The inflammation is due to activation of the microglia, thereby producing cytokines which inhibit the production of brain-derived neurotrophic factor (BDNF). As the BDNF acts by neuronal protection, synaptogenesis and neurogenesis, the reduction of BDNF in the brain of AD patients worsens the symptoms of AD. On the other hand, treatment of AD patients with a cholinesterase inhibitor enhances ACh activity and inhibits inflammation. Then the expression of BDNF is restored and neuroprotection reestab-

lished. However, there are several reports which showed controversial results concerning the relationship between BDNF and AD. We speculate that BDNF is related to some neurocognitive process and reflects neuronal activity in other neurodegenerative and neuropsychiatric disorders and that in the mild cognitive impairment stage, BDNF and choline acetyltransferase (ChAT) activities are hyperactivated because of a compensatory mechanism of AD pathology. In contrast, in the mild stage of AD, BDNF and ChAT activity are downregulated.

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Introduction

Alzheimer's disease (AD) is well characterized as an accumulation of amyloid protein and formation of neurofibrillary tangles in the brain seen in histopathology [1]. Neurological changes are also characterized with a cholinergic deficit in the nucleus basalis of Meynert which

projects to the cerebral cortex and hippocampus [2]. As the cerebral cortex including the prefrontal cortex contributes to cognition and the hippocampus contributes to short-term memory, degeneration of the cholinergic neuron is related to the deterioration of memory and cognitive function characterized in AD [3]. The accumulation of β -amyloid ($A\beta$) protein and the formation of neurofibrillary tangles lead to synaptic dysfunction, cell death and brain shrinkage in AD due to inflammation with activation of microglial cells [4]. On the other hand, activation of acetylcholine (ACh) inhibits inflammation in the brain by the inhibition of microglia with activation of α -nicotinic receptors [5]. Moreover, brain-derived neurotrophic factor (BDNF) was found by Barde et al. [6] to promote neuronal survival, neurite growth, synthesis of neurotransmitters and neurogenesis. The expression of BDNF is influenced by neuroinflammation via activation of the microglia [7].

In this review, we will discuss the neurodegenerative disease of AD in the context of a participation and interaction of cholinergic neuronal activity, neuroinflammation and production of BDNF.

Discovery of BDNF and the Expression in AD

In 1982, BDNF was isolated and purified by Barde et al. [6], with the molecular weight of 12,300 Da, consisting of 119 amino acids. BDNF and its receptor, tropomyosin-related receptor kinase B (TrkB), are present in the cortex, hippocampus and hypothalamus, and participate in promoting neuronal survival, neurite outgrowth, synthesis of neurotransmitters and neurogenesis [8]. Sala et al. [9] reported that depolarization-evoked release of ACh is enhanced by BDNF in the visual cortex of rats *in vivo*, and it is blocked by a TrkB kinase inhibitor, k252a. Moreover, human embryonic stem cell-derived neurons are differentiated to preferentially cholinergic neurons by BDNF [10].

BDNF accelerates the synaptic plasticity of neurons in the hippocampus, which plays a role in learning and memory. Indeed, in the BDNF mRNA knockout mouse, expression of long-term potentiation in the hippocampus and spatial learning are reduced [11]. As described above, BDNF regulates synaptic plasticity and leads to memory formation and storage [12]. The main symptoms of AD are deficit of memory and cognition and pathological neurodegeneration; therefore, the loss of BDNF is easily suspected. There are many reports that the mRNA [13–16] and protein [17–20] levels of BDNF

are decreased in the hippocampus and cortex in the AD brain. Moreover, not only BDNF, but also the receptor TrkB is analogously reduced in the hippocampus and frontal cortex in AD [21, 22]. In contrast, a few studies report that the BDNF and the receptor TrkB were not reduced in AD [23–25].

BDNF and Inflammation

An AD model of $A\beta$ protein-treated rats showed memory deficit and decrease in BDNF levels and, simultaneously, an increase in mitochondrial oxidative damage and inflammatory cytokines [4]. Similarly, Ciaramella et al. [26] observed that *in vitro* expression of BDNF in dendritic cells derived from AD patients treated with $A\beta_{1-42}$ protein was significantly decreased, while this was not found in the cells from control subjects.

In an *in vivo* experiment to treat BDNF in amyloid precursor protein transgenic mice, a significant neuroprotective effect was observed without affecting amyloid plaque numbers [27]. It is reported that the serum BDNF level is lowered in AD patients compared with age-matched healthy controls [28]. We observed a significant and dose-dependent reduction of serum BDNF concentration in lipopolysaccharide-administered rats, and the rats showed depression-like behavior in the forced swimming test. Moreover, a significant increase in serum tumor necrosis factor α levels (TNF α) was found in the same rats [29]. In an *in vitro* study, Neumann et al. [30] reported that TNF α inhibits neurite outgrowth and branching in cultured hippocampal neurons. On the other hand, it is reported that ω -3 fatty acids inhibit inflammation through inhibiting microglia phagocytosis of $A\beta_{1-42}$ protein and enhanced production of BDNF [31]. The AD brain features accumulation of $A\beta$ protein and advanced neurodegeneration due to oxidative stress and inflammation. The inflammation reduces the expression of BDNF in the brain. Therefore, the mechanism of neuronal degeneration in AD is possibly direct degeneration by the active oxygen in oxidative stress, and indirectly reduction of BDNF expression by the inflammation.

Cholinergic Activity and BDNF

The deterioration of ACh neurons in the nucleus basalis of Meynert is well characterized in the AD brain. Therefore, cholinesterase inhibitor (ChEI) therapy is successfully established for AD, and the progression of symp-

Fig.1. a MCI stage: AD pathology causes a burden in the brains of patients with AD; however, ChAT activity and BDNF are upregulated and ACh is maintained at a normal level. **b** Mild stage of AD: ACh gradually decreases because hyperactivity of presynaptic neurons may cause early and rapid neuronal degeneration with a consequent downregulation of ChAT activity and BDNF.

toms is delayed. In Japan, clinically available ChEIs are donepezil, galantamine and rivastigmine. Jin et al. [32] reported in an in vitro study of mouse cortical culture that tacrine, a reversible ChEI, and galantamine, a similar ChEI which allosterically enhances nicotinic cholinergic transmission, increased neurogenesis. In vivo animal studies revealed that the ChEIs tacrine and galantamine enhance neurogenesis in the forebrain subventricular zone and hippocampal dentate gyrus. Similarly, Kotani et al. [33, 34] reported that donepezil enhances adult rat hippocampal neurogenesis and is antagonized by scopolamine, a muscarinic ACh receptor antagonist. Leyhe et al. [28] reported that high-dose (10 mg/day) donepezil ameliorated serum BDNF by a 15-month treatment of patients with mild cognitive impairment (MCI) to mild AD. A similar result is reported in an animal study by Wang et al. [35] that BDNF mRNA and protein levels are increased by huperzine A, a ChEI used in China. On the other hand, a decrease in hippocampal BDNF mRNA was observed by fimbria transections, lesion of cholinergic innervations to the hippocampus and atropine, a muscarinic antagonist [36], which is suggested to be involved in the cholinergic regulation of hippocampal BDNF expression.

The neurogenesis caused by ChEIs and ACh is possibly due to activation of muscarinic and nicotinic recep-

tors, and inhibition of inflammation, thereby increasing BDNF production. Kotani et al. [33, 34] reported that donepezil enhances neurogenesis in the hippocampal dentate gyrus and is antagonized by scopolamine, a muscarinic antagonist. They also reported donepezil enhances and scopolamine suppresses phosphorylation of cAMP response element binding protein which is involved in cell survival, while they did not obtain an increase in BDNF protein levels in the hippocampus by donepezil but a decrease by scopolamine. Therefore, they suggested that enhancement of neurogenesis by activating ACh is involved in the activation of muscarinic receptors [33, 34]. On the other hand, Autio et al. [37] argued about a possibility that the neurotrophic effect of ChEI directly activates neurotrophin receptors of TrkA and TrkB without producing nerve growth factor or BDNF by the evidence of no inductions of hippocampal nerve growth factor and BDNF mRNA and protein levels with a chronic 14-day treatment of galantamine administered to mice.

Stimulation of ACh of the vagal nerve has been known to inhibit inflammation for a long time. In 2003, Wang et al. [38] demonstrated the presence of α_7 -nicotinic ACh receptors on macrophages, and the TNF production by lipopolysaccharide is inhibited by nicotine through activating the receptor. Activation of the α_7 -nicotinic ACh

receptor by ACh increases Ca^{2+} flux into cells and activates intracellular signals of Jak-2 and phosphorylates STAT3, then finally inhibits nuclear factor κB and thereby inhibits production of proinflammatory cytokines. Similarly, the α_7 -nicotinic ACh receptor is found on the immune cells, such as mast cells, microglia and Kupffer cells. In the central nervous system, it is demonstrated that regulation of inflammation by ACh is brought about by inhibition of cytokine production by activating α_7 -nicotinic ACh receptors on the microglia [39]. So, there is one possibility that the increase in BDNF expression by ACh is due to inhibition of cytokine production from microglia, and shows e.g. neuroprotection, synaptogenesis and neurogenesis.

Other mechanisms of neuroprotection with activation of α_7 -nicotinic ACh receptors on microglia are reported as follows: one is that excitatory amino acid transporter 1 is expressed via the activation of α_7 -nicotinic ACh receptors on microglia and that it takes up glutamate as the excessive glutamate induces neuronal cell death through increasing intracellular Ca^{2+} [40]; another is that activation of the α_7 -nicotinic ACh receptor prevents the production of reactive oxygen species in fibrillary $\text{A}\beta_{1-42}$ protein-stimulated microglia as oxygen radicals induce neuronal cell damage [41].

Concepts of MCI and Mild Stage in AD Based on BDNF

As reported above, expression of BDNF is beneficial for AD, because BDNF is related to cognitive functions in AD and influenced by inflammation and ACh. BDNF might be a useful biological marker of AD. However, actually, there are several reports which showed controversial results [42]. Lee et al. [43] demonstrated that BDNF was significantly lower in MCI and AD compared with healthy controls and commented that this might be involved in the pathophysiology of cognitive decline in AD. In contrast, Angelucci et al. [44] reported that BDNF serum levels increase in MCI and AD patients, supporting the hypothesis of an upregulation of BDNF in MCI and AD. Moreover, Nagata et al. [42] commented that the plasma BDNF level was correlated with aggressiveness in patients with AD or amnesic MCI, and Diniz and Teixeira [45] reported that BDNF seems to be an unspecific biomarker of neuropsychiatric disorders marked by neurodegenerative changes because altered circulating BDNF levels have also been reported in other neurodegenerative and neuropsychiatric disorders.

From these reports, we speculate that BDNF is related to some neurocognitive process and reflects neuronal activity in other neurodegenerative and neuropsychiatric disorders. As regards AD, we also speculate that there might be a hyperactivity of BDNF in the mild stage of AD because of a high activity of choline acetyltransferase (ChAT) in this stage [46, 47], hyperactivity of ChAT as compensatory mechanism for AD [46, 47]. In MCI, BDNF and ChAT activities were hyperactivated because of a compensatory mechanism of AD pathology when there might not be prominent neuronal loss. In contrast, in mild AD, BDNF and ChAT activities were downregulated because there might be neuronal loss to some degree (fig. 1).

Conclusions

Accumulation of $\text{A}\beta$ protein leads to oxygen stress and inflammation in the AD brain and thereby to neurodegeneration. The inflammation produces cytokines by activation of the microglia and inhibits production of BDNF which exerts neuronal protection, synaptogenesis and neurogenesis. Treatment with ChEI to AD patients enhances ACh activity and inhibits inflammation, then the expression of BDNF is restored and shows neuronal protection. However, there are several reports which showed controversial results between BDNF and AD. We speculate that BDNF is related to some neurocognitive process and reflects neuronal activity in other neurodegenerative and neuropsychiatric disorders and that in MCI, BDNF and ChAT activities are hyperactivated because of a compensatory mechanism of AD pathology. In contrast, in mild AD, BDNF and ChAT activities were downregulated.

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