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Alzheimer's & Dementia: Translational Research & Clinical Interventions ■ (2017) 1-7

Alzheimer's & Dementia

Featured Article

Effect of physical exercise on markers of neuronal dysfunction in cerebrospinal fluid in patients with Alzheimer's disease

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Abstract

Introduction: Physical exercise has gained increasing focus as a potential mean to maintain cognitive function in patients with Alzheimer's disease (AD). Alongside the markers of specific AD pathology (amyloid-β and tau), other pathologies such as neuronal damage and synaptic loss have been proposed as markers of the disease. Here, we study the effect of physical exercise on biomarkers of neuronal and synaptic integrity.

Methods: Cerebrospinal fluid (CSF) from 51 AD subjects who participated in the randomized controlled trial ADEX was analyzed for the concentration of neurofilament light (NFL), neurogranin (Ng), visinin-like protein-1 (VILIP-1), and chitinase-3–like protein 1 (YKL-40). Participants were subjected to either 16 weeks of moderate- to high-intensity exercise ($n = 25$) or treatment as usual (control group, $n = 26$), and CSF was collected before and after intervention.

Results: No significant differences in CSF concentrations of VILIP-1, YKL-40, NFL, and Ng were observed when comparing mean change from baseline between the exercise and control groups. Similarly, when classifying subjects based on their exercise levels, no significant changes were observed for the biomarkers in the control group compared with the high-exercise group (attending 80% of the exercise sessions with an intensity of 70% of maximum heart rate or above).

Discussion: These results are not supportive of a modulatory effect of physical exercise on the selected biomarkers of neuronal and synaptic integrity in patients with AD.

Keywords: Alzheimer's disease; Physical exercise; Biomarkers; Synapses; Neurons; Stability

1. Introduction

The molecular pathological mechanisms behind Alzheimer's disease (AD) have been studied for decades. Although the exact mechanisms are still not quite clear, accumulation of amyloid-β 42 (Aβ42) is an early pathological hallmark that interacts with and/or promotes neurodegeneration without being directly linked to clinical symptoms [1]. One key feature of neurodegeneration in AD is neuronal and synaptic loss that better reflects AD progression and cognitive decline [1–3]. Studies in transgenic animals suggest effects of physical exercise on neurogenesis, cognition, and amyloid deposition [4]. Further, epidemiological studies suggest that higher physical activity may reduce the risk of dementia in late life [5], and some intervention studies in elderly suggest that participation in physical

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http://dx.doi.org/10.1016/j.trci.2017.03.007

activity and cognitive training may reduce future cognitive decline [6]. However, these studies have investigated possible effects on risk of dementia and cognitive decline in general and have not used any biomarkers to diagnose AD or specifically study effects on AD pathophysiology. Furthermore, in an effort to arrest the decline in cognitive function (CF) and loss of activities of daily living, nonpharmacological approaches have been investigated. In the randomized clinical trial "Preserving Cognition, Quality of Life, Physical Health and Functional Ability in Alzheimer's Disease: The Effect of Physical Exercise (ADEX) study," we analyzed the effect of 16 weeks moderate- to high-intensity physical exercise (EXE) in patients with AD on a cognitive [7], functional [8], and molecular level [9]. Despite some effect on clinical symptoms such as processing speed and neuropsychiatric symptoms [7], we found no effect of EXE on levels of Aβ, tau, and phosphorylated tau (p-tau) in cerebrospinal fluid (CSF) [9]. Therefore, we sought to investigate downstream molecular processes that potentially could be influenced by EXE. In this study, we investigated the effect of EXE on molecular markers of AD neurodegeneration in CSF from patients with AD participating in the ADEX study. This is not a study on the potential diagnostic value of these markers as in cross-sectional study, however an explorative study on modulating effects of selected markers on exercise. Neurofilament light (NFL) is a marker of damage to large-caliber myelinated axons, and elevated CSF levels have been associated with cognitive deterioration and structural changes in the white matter and brain atrophy in patients with AD [10]. Elevated CSF NFL is not a specific AD marker, and CSF NFL levels do not correlate with Aβ42 levels, indicating that the changes in NFL are not driven by Aβ42 pathology [10]. Similar to NFL, CSF neurogranin (Ng) levels do not correlate with Aβ42 levels in patients with mild cognitive impairment (MCI) and AD [11,12]. The synaptic protein Ng is released to the CSF from the synapses, which is an early event in the pathogenesis of AD. This also makes Ng a candidate marker for early AD diagnosis. Alongside this, Ng possesses the ability to differentiate patients with MCI who progress to AD (elevated Ng levels) and patients with MCI who remain stable (low levels of Ng) [11,12]. Just as Ng, the biomarker visinin-like protein-1 (VILIP-1) has shown potential as a marker of MCI progression to AD [3]. VILIP-1 is abundantly expressed in the brain and is excessively released because of neuronal degradation [13]. Although increased CSF levels of VILIP-1 are not specific to AD, they have shown to correlate well with AD progression and pathology [3]. The microglia derived protein YKL-40's role in AD is not fully elucidated. It is speculated that YKL-40 increases with neuroinflammation [14]. However, apart from AD, increased levels have been found in CSF after stroke, other neurological disorders, and normal aging [14–17]. This indicates that YKL-40 is more a general marker of neuroinflammation/astroglial activation secondary to many different etiologies, including Aβ pathology [18].

We hypothesized that the concentrations of markers of neuronal and synaptic damage and astroglial activation would decrease in CSF in patients with AD as an effect of moderate- to high-intensity EXE, because of stabilizing effects of EXE on neurons, synapses, and astrocytes, resulting in less neurodegeneration and inflammation and thereby lead to less release of the selected biomarkers in CSF.

2. Methods

2.1. Study population

Two-hundred community-dwelling patients with clinically diagnosed mild AD according to NINCDS-ADRDA criteria [19] and a Mini-Mental State Examination >19 were included and randomized to either a control group with treatment as usual or a 16-week 60-minute three times per week moderate- to high-intensity aerobic physical exercise (treadmill, stationary bike, and cross-trainer) group, in groups of four to six subjects per group with an educated trainer. For detailed description of the intervention used and study participant enrollment, see Hoffmann et al. [7, 20]. All subjects donated a blood sample before and after intervention and were tested at baseline and at 16 week follow-up with a comprehensive battery of tests of CF, activities of daily function, quality of life, physical activity, and neuropsychiatric symptoms. For details of inclusion and exclusion criteria, methods for inclusion, and cognitive and physical tests, see Steen Jensen et al. [9]. A subgroup of 56 subjects recruited from three of eight centers had a lumbar puncture performed to collect CSF samples before and after intervention. Samples where centrifuged on acquisition at 2000 *g* and stored in 250 μL aliquots at −80°C until use. The baseline characteristics of the study population can be seen in Table 1.

2.2. Samples

CSF samples from 51 patients of the 56 possible CSF samples, subjected to 16 weeks of moderate- to high-intensity EXE (exercise group) or treatment as usual (control group), were analyzed for the concentration of Ng (UniProt #: Q92686), VILIP-1 (UniProt #: P62760), NFL (UniProt #: P07196), and YKL-40 (UniProt #: P36222), at baseline and 16 weeks follow-up.

2.3. Assays

CSF Ng concentration was measured using a previously published in-house Meso Scale Discovery assay as published in Kvastberg et al. [21] and De Vos et al. [22]. Commercially available kits were used for measuring the concentrations of VILIP-1 (Human VILIP-1 ELISA; BioVendor GmbH, Heidelberg, Germany), NFL (NF-light ELISA; IBL international, Hamburg, Germany), and YKL-40 (Quantikine ELISA Human Chitinase-3–like 1; R&D systems, MN, USA) by following the manufactures enclosed