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Dynamin protein in stroke and vascular dementia

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HIGHLIGHTS

- Patients with vascular dementia had lower concentrations of Dynamin1 in cortical white matter.
- The highest values of Dynamin1 were reported in patients with stroke without dementia.
- The level of Dynamin1 in white matter correlates positively with cognitive scores.

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ABSTRACT

Damage to sub-cortical white matter is a key substrate of vascular dementia (VaD) leading to deficits in executive function and cognitive processing speed. Dynamin1 is a 100 kDa protein, accounting for 0.4% of the total brain protein, and has a central role in many intracellular processes such as synaptic vesicle trafficking and recycling. In this study, we examined the status of Dynamin1 in the white matter from frontal cortex area. In order to measure the levels of Dynamin1, we isolated cortical white matter from a total of 34 post-mortem brains derived from controls ($N = 11$), mixed Alzheimer's disease (AD) and VaD ($N = 8$), VaD ($N = 7$), and stroke no dementia (SND, $N = 8$) subjects. A commercial ELISA kit was then used to determine the level of Dynamin1. In comparison to controls, Dynamin1 was elevated in patients SND (+400%) and reduced in patients with mixed VaD (−50%). Furthermore, levels of Dynamin1 were significantly associated with preserved cognition as indicated by the MMSE and CAMCOG and upregulation of vesicular glutamate transporter 1. This work indicates that Dynamin1 is associated with both preserved cognition and regenerative responses in older people with cerebrovascular disease and may represent a novel treatment target.

1. Introduction

Stroke related dementia and vascular dementia (VaD) account for more than 20% of the 35 million people with dementia worldwide. Stroke is also a key factor in more than 40% of people with Alzheimer's disease (AD). In particular, stroke survivors and individuals with mild cognitive impairment in the context of cerebrovascular disease are at especially high risk of developing dementia [1]. About 20–25% of stroke patients develop dementia within three months after stroke [2,3]. In addition, the risk of delayed dementia is increased 10 fold over the subsequent 5 years (annual incidence of 9% per annum over this period), with an even higher risk in people over the age of 75 [2,4]. Microvascular lesions in the frontal lobe, Brodmann area (BA9) are a key vascular substrate of significant cognitive impairment [5,6] probably related to the disruption of key white matter tracts.

Currently there are no licensed pharmacological therapies for the treatment of VaD. Cholinesterase inhibitors and memantine have been examined in clinical trials in VaD patients, but benefits have been too modest to justify licensing these agents for the treatment of VaD [7]. The main approaches to novel drug discovery have focussed on examining the potential value of treatments for AD and there has been very little effort to identify novel protein changes specific to VaD that may enable the development of specific pharmacological therapies for these individuals.

Dynamin1 is ~100 kDa protein with a GTPase activity that is involved in many intracellular trafficking process including synaptic vesicle recycling, neurotransmitter reuptake and receptor internalization [8,9]. In the brain Dynamin1 accounts for 0.4% of the total brain protein [10]. It is up-regulated during new neurite formation [11] and is down-regulated during neurite retraction. Experimental studies have demonstrated that silencing the initiation codon for dynamin significantly hampers the formation of axon

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like processes. It has also been suggested that dynamin may play a key role in establishing and maintaining mature neuronal structure [12,13]. A role for dynamin has also been postulated in stabilization of the microtubule cytoskeleton [14]. Recent work highlighting increased neurogenesis and receptor up-regulation in patients with cerebrovascular disease, and the relationship of this up-regulation to preserved cognition [15–17]. Dynamin is closely related to several processes that may be integral to improved outcome in people with cerebrovascular insults. We therefore examined Dynamin1 protein in the brain grey and white matter in patients with cerebrovascular disease and correlated concentrations to previously reported neurochemical measures. We hypothesised that changes in Dynamin1 concentration in white matter could reflect differential mechanisms occurring post stroke, with axons degeneration specifically in the VaD group associated with cognitive decline.

2. Materials and methods

2.1. Human post-mortem samples

All participants or their next of kin gave informed consent for use of post-mortem material and for data evaluation for research. The VaD and stroke subjects were originally recruited between 1996 and 2002 from representative hospital-based stroke registers in Tyneside, Wearside and Cleveland (UK). The use of post-mortem human material for this study was supplied by the Newcastle Brain Tissue Resource, part of the Brains for Dementia Research network and its use was approved by the North West Regional Ethics Committee (ref: 08/H1010/5). The longitudinal study "Memory after Stroke" was approved by the Newcastle & North Tyneside Health Authority Joint Ethics Committee (ref: 99/153).

2.2. Diagnoses and histopathology of cases

Pathological diagnosis of VaD [18] was defined by the presence of multiple or cystic infarcts involving both cortical and subcortical structures, borderzone infarcts, lacunae (<15 mm), microinfarcts (visible by microscopy only) and small vessel disease in subcortical structures in the general absence of neurofibrillary pathology (see [19] for full description). None of the "pure" VaD patients exhibited tangle burden above Braak stage III [20]. Clinical evidence of dementia along with the same type or a combination of these lesions at three different coronal levels was considered diagnostic for VaD [21]. Neuropathological features of AD were characterized by the presence of neuritic plaques and neurofibrillary tangles in all cortical lobes and hippocampal formation as defined for probable AD by the Consortium to Establish a Registry for AD (CERAD) [22]. Tangle burden was graded according to the method of Braak and Braak [20] and neuritic plaque by the method of Thal et al. [23]. Patients meeting neuropathological criteria for VaD who also met CERAD criteria for probable or definite AD or who had Braak stage tangle pathology greater than stage III were diagnosed as mixed VaD/AD. Although the main diagnosis of VaD was made neuropathologically, patients also had to meet DSM IV clinical criteria for dementia during life. Patients with a stroke according to World Health Organisation criteria [24], who did not meet NINDS–AIREN criteria for VaD or DSM IV criteria for dementia were diagnosed as 'stroke no dementia' (SND). These brain tissue resources constitute a large collection of frozen and formalin fixed brains from clinically characterized subjects, a substantial proportion of whom (55%) were from prospective clinical cohort studies with serial standardized evaluations. For individuals who participated in longitudinal clinical studies, standardized cognitive evaluations were completed with the mini-mental state examination (MMSE) and with the Cambridge Assessment of Mental Health for the Elderly, Section B (CAMCOG), at baseline and at annual intervals until death, undertaken by trained psychology assistants or research nurses.

2.3. Preparation of tissue

Cortical white matter tissue was dissected from the BA9 region of a total of 34 post-mortem brains derived from subjects; controls ($N = 11$), mixed AD and VaD ($N = 8$), VaD ($N = 7$), and stroke no dementia (SND, $N = 8$). Tissue was homogenized in a buffer solution consist of 50 mM Tris-base, 150 mM NaCl, detergents (1% NP-40, 0.1% SDS, 0.5% Deoxycholate), 0.1 mM EDTA, and a cocktail of protease inhibiters (Complete mini tablet Roche, USA), pH 8.2. Approximately 100 mg tissue was homogenized using manual hand homogenizer under ice in a total volume of 2 ml buffer. Homogenization was performed under ice until no visible 'bits' were left. Tissue debris was then removed by centrifugation maintained at $15{,}000 \times g$ for 15 min at 4 °C. The supernatant was taken and re-spun for 60 min at $40{,}000 \times g$ to ensure soluble proteins are retained. The resultant supernatant was collected and the protein concentrations were determined using Bio-RAD protein assay reagent. Samples were aliquoted in and stored in −80 °C for use for various analyses.

2.4. Dynamin1 level measurement by ELISA

Dynamin1 concentration was measured using a commercial ELISA kit developed by USCNLIFE TM (Wuhan, China). The microtiter plate was pre-coated with biotinylated polyclonal antibody specific to Dynamin1. 100 μl standards or samples (diluted to same final concentration of total proteins for each sample) were added and incubated for two hours at 37 °C. 100 μl detection reagent A (avidin conjugated to horseradish peroxidase (HRP) was added to each micro plate well and incubated for 1 h at 37 °C. After washing 4 times, 100 μl of a TMB (3,3′,5,5′ tetramethyl-benzidine) substrate solution was added to each well and incubated for 1 h at 37 °C. Colour develops in proportion to the amount of bound analyte. Finally determination of presence and quantity of Dynamin1 was achieved by terminating the enzyme-substrate reaction by adding 1N sulphuric acid followed by measuring colour changes using a spectrophotometer at a wavelength of 450 nm. The concentration of Dynamin1 in the samples was determined by comparing the O.D. of the samples to the standard curve. Within-assay precision for a replicated sample on the same plate (%CV of intra-assay variation) for a selected sample (123/03) was <6%. The inter-assay variability for same sample analyzed on three different plates was <15%.

2.5. Statistical evaluation

Statistical analyses were performed with SPSS software package for the Social Sciences for Windows (version 15, SPSS Inc., Chicago, IL, USA). Group means was compared using one-way analysis of variance (ANOVA) following by Bonferroni multiple range test or Kruskall–Wallis ANOVA followed by Mann–Whitney U test as appropriate. Intercorrelations of neurochemical variable and correlations with demographic and clinical features were examined using Pearson product moment (r) or Spearman rank (R_S) correlation as appropriate.

3. Results

We assessed 34 brains from following subjects: VaD $n = 7$, mixed VaD/AD $n = 8$, SND $n = 8$, controls $n = 11$ (see Table 1). The VaD group included patients with infarct dementia (Inf D) or subcortical ischemic vascular dementia (SIVD), with most of the infarct dementia patients also having significant concurrent small blood