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Short Communication

GSK-3β expression in cerebrospinal fluid in Parkinson's disease

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ABSTRACT

We examined the expression of glycogen synthase kinase-3beta (GSK-3β) in the cerebrospinal fluid (CSF) of Parkinson's disease (PD) patients. GSK-3β is a critical intermediate in pro-apoptotic signaling cascades that are associated with neuro-degenerative diseases, including PD. Because CSF contains proteins and metabolites of brain origin, and GSK-3β levels are increased in brains of PD patients, we decided to measure GSK-3β levels in CSF samples from PD patients. We observed that neither GSK-3β nor pGSK-3β (Ser9) was altered in PD patients. Our results suggest that, although GSK-3β plays a role in the pathogenesis of PD, its CSF levels were unchanged.

KEYWORDS: cerebrospinal fluid, Parkinson's disease, glycogen synthase kinase-3beta, risk factor

INTRODUCTION

Parkinson's disease (PD), a progressive neuro-degenerative disease of unknown etiology [1], is the second most common neurological disorder characterized by motor and behavioral disturbances caused by the degeneration of dopaminergic neurons [2, 3]. Alpha-synuclein (α-syn), a presynaptic protein, is causal in the genesis of PD [2, 4], and gene duplication and triplication of α-Syn are found in sporadic and early onset forms of PD [5]. α-Syn becomes insoluble, self-aggregates and accumulates into intra-neuronal inclusion bodies [4, 6, 7, 8]. A recent study indicates that α-Syn induces hyperphosphorylated tau through specific activation and recruitment of glycogen synthase kinase 3β (GSK-3β), a kinase known to hyper-phosphorylate tau at distinct sites in Alzheimer's disease, which itself becomes activated through autophosphorylation at Tyr216 [9].

GSK-3β is a ubiquitous serine/threonine protein kinase highly abundant in brain tissue, which plays a key role in neural development and neuron survival, and an increase in GSK-3β activity precedes the induction of apoptosis [10]. Elevated levels of GSK-3β were seen in the striatum [9, 11], and peripheral blood lymphocytes [12] from PD patients. These findings were also underscored by a report in which two functional single nucleotide polymorphisms have been reported in PD brains, resulting in increased phosphorylation of tau and interaction of GSK-3β with tau [13]. All these observations suggest that this kinase may be involved in the pathogenic progress of the human PD. We further tested whether the increase in GSK-3β levels is also reflected in the cerebrospinal fluid (CSF) of PD patients.

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MATERIAL AND METHODS

Human samples

CSF samples from PD patients [n = 29 (12 females and 17 males), age = 62.97 ± 2.06 years (data are expressed as mean ± s.e.m.)] and healthy control subjects [(n = 22 (12 females and 10 males), age = 58.33 ± 3.8 years)] were obtained by lumbar puncture, collected in a glass tube, and immediately frozen and stored at -80ºC. All the samples were obtained from the Neurology Service of the Hospital Universitario 12 de Octubre (Madrid, Spain). All PD cases were diagnosed under the criteria of probable PD [14]. The control group was formed of family members or caregivers of the PD patients, who all had a clinical interview with a senior neurologist that showed a completely normal cognitive and functional level. Half ml of each CSF sample was concentrated by lyophylization in a Speed-Vac to about 0.1 ml.

Immunoblot analysis

CSF protein immunoblotting was performed using previously describe methods [15]. In brief, all samples were resolved by 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Bio-Rad) by electroblotting. Primary antibodies were: mouse monoclonal anti-GSK-3β (Santa Cruz Biotechnology, Inc), and rabbit polyclonal anti-pSer9 GSK-3β (Cell Signaling Technology, Inc.). Secondary antibodies were: goat anti-mouse, and goat anti-rabbit HRP-conjugated (Biorad Laboratories). Densitometric analysis was performed using ImageJ software (NIH Image).

Statistical analysis

Data were analysed with SPSS for Windows (version 15.0). Results were expressed as mean ± s.e.m. and statistically analyzed by ANOVA followed by a Tukey-Kramer test, and Mann-Whitney *U*-test analysis when appropriate. The differences were considered to be significant at $p<0.05$. The Spearman rank correlation was used for correlation analyses.

RESULTS AND DISCUSSION

Using Western-blot assays, we observed typical pattern of GSK-3β in human CSF. Neither GSK-3β nor pSer9 GSK-3β (Figure 1A, and B) expression was altered in CSF from PD patients compared with control subjects.

We analyzed whether there was an association between GSK-3β and pSer9 GSK-3β expression and sex, age and onset. We found that both GSK-3β and pSer9 GSK-3β expression correlate with age (Spearman rank correlation R=-0.631, p = 0.002, and R = -0.497, p = 0.019, respectively) but only in the control group.

We here describe novel findings describing GSK-3β status in CSF from PD patients. Despite the fact that the cause of PD remains unknown, α-syn has been classically linked to PD, as the main component of Lewy bodies [16]. Phosphorylated GSK-3β, α-syn, tau, and other proteins are involved in the pathogenesis of several neuro-degenerative diseases [14]. It has been demonstrated that p-GSK-3β is central to the development of the tauopathic process in PD models, and that blockade of the kinase with specific inhibitors

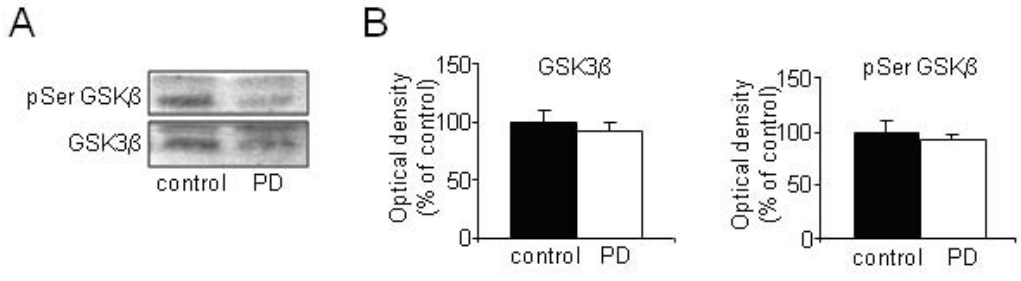

Figure 1. (**A**) Expression of pSer GSK-3β and GSK-3β are unchanged in CSF of PD patients compared with healthy controls. Representative western blots are shown. (**B**) Densitometric measurements confirm these data. (Data are expressed as mean ± s.e.m., n = 22 for control group, n = 29 for PD group. PD = Parkinson's disease).

prevented tauopathic changes and cell death in mesencephalic neurons and cultured cells [17].

To our knowledge, no studies about the assessment of GSK-3β levels in CSF from PD patients have been published. While we have not found significant differences between PD patients and controls, the role of GSK-3β in the cellular damage in PD is well established [9, 11]. Despite the fact that GSK-3β is up-regulated in cellular populations, including neurons [9] and blood lymphocytes [12] from PD patients, we hypothesized that its secretion to peripheral fluids, including CSF, could be reduced. However, we found that in CSF, GSK-3β levels in PD patients were similar to those observed in healthy subjects.

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FINANCIAL DISCLOSURE/CONFLICT OF INTEREST

The authors report no conflicts of interest.

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