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Glycogen-synthase kinase-3β is decreased in peripheral blood mononuclear cells of patients with mild cognitive impairment

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Abstract

Glycogen-synthase kinase-3 (GSK-3) plays a central role in Alzheimer's disease (AD). It is involved in the hyper-phosphorylation of Tau and the increased production of β-amyloid. Despite its eminent role, only one study has been published so far in AD blood samples, reporting an increase of GSK-3α and −3β levels in white blood cells. In this study, we measured GSK-3α and −3β by quantitative ELISA in peripheral blood mononuclear cells of patients with mild cognitive impairment (MCI), AD and depression in comparison to healthy subjects. In contrast to the previous study, we observed a significant reduction of GSK-3β levels in MCI patients and less pronounced in AD but not in depression. The data indicate that high GSK-3 brain activity is not reflected in peripheral blood mononuclear cells. Therefore, we conclude that more longitudinal studies have to be performed to clarify whether GSK-3 blood levels may qualify as disease specific biological markers.

Keywords

Alzheimer; GSK-3; Monocytes; Tau-protein

The enzyme glycogen-synthase kinase-3 (GSK-3) plays an important role in Alzheimers disease (AD) due to its role of hyper-phosphorylation of Tau (Hanger et al., 1992; Mandelkow et al., 1992). GSK-3 occurs in two forms, α and β (Phiel et al., 2003; Bhat and Budd, 2002; Eldar-Finkelman, 2002) and both forms are widely distributed and found in circulating lymphocytes. GSK-3 is regulated by phosphorylation at serine 9 in GSK-3β and serine 21 in GSK-3α. GSK-3 has been shown to co-localize with dystrophic neurites and neurofilbrillary tangles (Yamaguchi et al., 1996; Hooper et al., 2008), and active GSK-3 is contained in neurons with pre-tangle changes (Pei et al., 1999). GSK-3 expression is up-regulated in circulating peripheral lymphocytes in both AD and MCI patients (Hye et al., 2005). The aim of the present study was to investigate GSK-3β by quantitative ELISA in our patient samples, in order to further examine whether GSK-3β could be established as a biological marker in peripheral blood samples.

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Healthy subjects (n = 12) and patients with AD (n = 44), with MCI (n = 57) and with depression (n = 25) were assessed by the same diagnostic procedure. Psychiatrists clinically examined all subjects, performed a standardized neurological examination, reviewed medical records. Furthermore, neuropsychological assessment and magnetic resonance neuroimaging was performed as reported previously (Marksteiner et al., in press). EDTA blood (10 ml) was collected during normal routine clinical treatments and processed within 90 min. Peripheral blood mononuclear cells (PBMC) were separated from whole blood on a continuous Biocoll density gradient (1.077 g/ml) after centrifugation (400g, 30 min). PBMC were visible as a white stratum between plasma phase and Biocoll-Paque. The interphase with the PBMC was carefully removed, washed in 50 ml PBS, and the pellet was immediately frozen at −80 °C until use. The pellet was dissolved in 400 µl PBS + protease inhibitor cocktail (Sigma), sonicated on ice (30 s, 125 W/cm^2, 140 µm amplitude, 100%), centrifuged 10 min 13,000g at 4 °C, and the supernatant used for the ELISA. Total protein was determined by Bradford assay using bovine serum albumin as a standard. GSK-3β was determined by a commercial enzyme-linked immunoassay (ELISA) from TiterZyme (catNo. 900–144). The ELISA detects GSK-3β with 100% but with less than 0.01% crossreactivity to GSK-3α. The standard curve was linear up to 5 ng/ml and had a sensitivity of 74 pg/ml. Briefly, PBMC lysates were diluted 1 + 1 with assay puffer, then applied to the pre-coated wells, incubated for 1hr at room temperature washed and then the capture antibody was added. After incubation for 1 h at room temperature wells were washed, the conjugate was added, wells incubated for 30 min at room temperature washed and then the substrate was added and the color measured in an ELISA reader at 450 nm for 30 min. Analysis was determined in duplicates and correlated to a standard curve. Statistical analysis was performed by one-way ANOVA with a subsequent Fisher-PLSD posthoc test where $p < 0.05$ was significant.

The patients' characteristics are shown in Table 1. AD patients were significantly older (Table 1). The MMSE score was not statistically different between control subjects, patients with MCI and with depression, but significantly lower in AD patients (Table 1). GSK-3β levels were significantly decreased in MCI patients, but not in patients with depression. The numerical reduction of GSK-3 in AD was not statistically significant (Table 1).

GSK-3 has been subsequently shown to function in a wide range of cellular processes, including differentiation, growth, motility and apoptosis (Forde and Dale, 2007; Manoukian and Woodgett, 2002). GSK-3 involves several different signaling pathways, such as. e.g., the Akt, Zak-1, Wnt, RCN or glucocorticoid receptor pathways (Forde and Dale, 2007; Manoukian and Woodgett, 2002). Aberrant regulation of GSK-3 has been implicated in several human pathologies, including Alzheimer's disease, non-insulin-dependent diabetes mellitus or cancer (Forde and Dale, 2007). The functional role of GSK-3 in PBMCs has to be eludicated. So far, only one paper has reported Tau expression in human blood lymphocytes at very low levels in healthy subjects (Kvetnoy et al., 2000). The same group reported enhanced Tau levels in blood lymphocytes of AD patients (Kvetnoy et al., 2000). While Hye et al. (2005) showed enhanced expression of GSK-3α/β in white blood cells, the present data indicate a decrease of GSK-3β in PBMC of MCI patients as measured by quantitative ELISA. We can not offer an explanation for these contradictory results. Some of

the differences may be explained by differences in the methodological procedures. Interestingly, Castri et al. (2007) reported that the enzyme phosphatidylinositol-3-kinase (PI3K) is reduced in PBMC in AD patients. This enzyme is directly linked to GSK-3, because the insulin-induced PI3K controls GSK-3 activity. Thus it may be possible that both enzymes, GSK-3 and PI3K are regulated the same way in PBMC in dementia. In conclusion, further experiments are necessary to clarify whether GSK-3 is a suitable biological marker for MCI or AD.

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References

Bhat RV, Budd SL. GSK3beta signalling: casting a wide net in Alzheimer's disease. Neurosignals. 2002; 11(5):251–261. [PubMed: 12566926]

Castri P, Iacovelli L, De Blasi A, Giubilei F, Moretti A, Capone FT, Nicoletti F, Orzi F. Reduced insulin-induced phosphatidylinositol-3-kinase activation in peripheral blood mononuclear leucocytes from patients with Alzheimer's disease. Eur. J. Neurosci. 2007; 26(9):2469–2472. [PubMed: 17986027]

Eldar-Finkelman H. Glycogen synthase kinase 3: an emerging therapeutic target. Trends Mol. Med. 2002; 8(3):126–132. [PubMed: 11879773]

Forde JE, Dale TC. Glycogen synthase kinase 3: a key regulator of cellular fate. Cell. Mol. Life Sci. 2007; 64(15):1930–1944. [PubMed: 17530463]

Hanger DP, Hughes K, Woodgett JR, Brion JP, Anderton BH. Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase. Neurosci. Lett. 1992; 147(1):58–62. [PubMed: 1336152]

Hooper C, Killick R, Lovestone S. The GSK3 hypothesis of Alzheimer's disease. J. Neurochem. 2008; 104:1433–1439. [PubMed: 18088381]

Hye A, Kerr F, Archer N, Foy C, Poppe M, Brown RC, Hamilton G, Powell J, Anderton B, Lovestone S. Glycogen synthase kinase-3 is increased in whote cells early in Alzheimer's disease. Neurosci. Lett. 2005; 373:1–4. [PubMed: 15555766]

Kvetnoy IM, Hernandez-Yago J, Kvetnaia TV, Khavinson VK, Malinin VV, Yarilin AA, Sharova NI, Blesa JR, Anisimov VN, Lenskaia LV, Sluchevskaia SF, Chekalina SI, Tokarev OY, Yuzhakov VV. Tau-protein expression in human blood lymphocytes: a promising marker and suitable sample for lifetime diagnosis of Alzheimer's disease. Neuro Endocrinol. Lett. 2000; 21(4):313–318. [PubMed: 11455366]

Mandelkow EM, Drewes G, Biernat J, Gustke N, Van Lint J, Vandenheede JR, Mandelkow E. Glycogen synthase kinase-3 and the Alzheimer-like state of microtubule-associated protein tau. FEBS Lett. 1992; 314(3):315–321. [PubMed: 1334849]

Manoukian AS, Woodgett JR. Role of glycogen synthase kinase-3 in cancer: regulation by Wnts and other signaling pathways. Adv. Cancer Res. 2002; 84:203–229. [PubMed: 11883528]

Marksteiner J, Kemmler G, Weiss EM, Knaus G, Ullrich C, Mechteriakov S, Oberbauer H, Auffinger S, Hinterhölzl J, Hinterhuber H, Humpel C. Seven out of 16 plasma signaling proteins are enhanced in plasma of patients with mild cognitive impairment and Alzheimers disease. Neurobiol. Aging. in press.

Pei JJ, Braak E, Braak H, Grundke-Iqbal I, Iqbal K, Winblad B, Cowburn RF. Distribution of active glycogen synthase kinase 3beta (GSK-3beta) in brains staged for Alzheimer disease neurofibrillary changes. J. Neuropathol. Exp. Neurol. 1999; 58(9):1010–1019. [PubMed: 10499443]

Phiel CJ, Wilson CA, Lee VM-Y, Klein PS. GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. Nature. 2003; 423:435–439. [PubMed: 12761548]

Yamaguchi H, Ishiguro K, Uchida T, Takashima A, Lemere CA, Imahori K. Preferential labeling of Alzheimer neurofibrillary tangles with antisera for tau protein kinase (TPK) I/glycogen synthase kinase-3 beta and cyclin-dependent kinase 5, a component of TPK II. Acta Neuropathol. 1996; 92(3):232–241. [PubMed: 8870824]

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Table 1

Glycogen-synthase kinase-3β (GSK-3β) levels in peripheral blood mononuclear cells.

Group	Age	*n*	MMSE score	GSK-3β (ng/mg protein)
Control	68 ± 3	12	28.4 ± 0.3	14.3 ± 2.2
MCI	71 ± 1 ns	57	27.0 ± 0.4 ns	9.0 ± 0.5*
AD	76 ± 1*	44	18.6 ± 1**	10.2 ± 0.9 ns
Depression	68 ± 2 ns	25	28.0 ± 0.2 ns	11.0 ± 1.1 ns

PBMC were isolated from EDTA blood of healthy subjects, patients with mild cognitive impairment (MCI) or with Alzheimer's disease (AD) or with depression. The mini mental score (MMSE) was significantly lower in AD patients. Values are given as mean ± SEM; *n* = number of subjects; statistical analysis was performed by one-way ANOVA with a subsequent Fisher-PLSD posthoc test.

* $p < 0.05$.

** $p < 0.001$; ns not significant.