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BRIEF COMMUNICATION

Serum orexin-A levels are associated with disease progression and motor impairment in multiple sclerosis

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Abstract

Objective Diencephalon is frequently affected in multiple sclerosis (MS), and lesions of this region are associated with increased disability. Orexin-A and melatonin, two foremost mediators of diencephalon, modulate cognitive and motor functions through several pathways including the brain-derived neurotrophic factor (BDNF)-cAMP response element-binding protein (CREB) signaling pathway. In this pilot study, our aim was to investigate the prognostic value of these factors in progression of cognitive and physical disability.

Methods Levels of BDNF, melatonin, CREB, and orexin-A were determined by ELISA in sera of 25 relapsing remitting MS (RRMS) patients, 15 secondary progressive MS (SPMS) patients, and 20 healthy controls. Cognitive and motor functions were assessed by a neuropsychological test battery, timed 25-ft walk (T25-FW) and 9-hole peg (9-HP) tests.

Results MS patients had significantly lower serum levels of orexin-A and BDNF than healthy controls, and SPMS patients had significantly lower levels of melatonin and orexin-A than RRMS patients. Serum orexin-A levels were negatively correlated with 9-HP, T25-FW test scores, and progression index in RRMS patients. BDNF, CREB, and melatonin levels did not show any significant correlation with clinical features including EDSS and cognitive/motor performance of the patients.

Conclusion Our results suggest that orexin-A levels are decreased in parallel to disease progression and motor system deterioration in the earlier stages of the disease. Thus, orexin-A might be used as a potential biomarker of physical disability.

Keywords Multiple sclerosis · Melatonin · Orexin · Cognitive · Disability

Introduction

Multiple sclerosis (MS) is a chronic autoimmune and neurodegenerative disease characterized by multifocal destruction of myelin in widespread regions of the central nervous system. Neuroimaging studies have shown that diencephalon is frequently affected in MS, and hypothalamic damage correlates with cognitive and physical disability progression [1]. Animal studies indicate that orexin-A produced by hypothalamus and melatonin produced by the pineal gland are involved in cognitive and motor functions [2, 3]. One of the mechanisms by which these two mediators influence cognition is the brain-derived neurotrophic factor (BDNF)-cAMP response element-binding protein (CREB) signaling pathway, which has been related to neuronal survival, differentiation, and neuroprotection [3, 4]. Thus, we aimed to assess the value of orexin-A, melatonin, CREB, and BDNF as biomarkers for disability progression in MS.

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Material and methods

Subjects

Twenty-five relapsing remitting MS (RRMS) and 15 secondary progressive MS (SPMS) patients fulfilling McDonald's criteria [5] and 20 healthy controls were recruited. All patients underwent standard neurological examination and cranial-

spinal MRI. Patients with acute relapse or steroid treatment during the preceding 3 months, severe disability interfering with neuropsychological testing, coexisting systemic or mental disorders, and a history of alcohol abuse, psychiatric, or antiepileptic drug treatment were excluded. The study was approved by the local ethics committee, and all participants gave written informed consent. Physical disability was scored using the Expanded Disability Status Scale (EDSS), and disease activity was assessed using the annualized relapse rate and progression index (EDSS/disease duration).

Neuropsychological and motor assessment

Participants were evaluated by Brief Repeatable Battery of Neuropsychological Tests (BRB-N) encompassing selective reminding test (SRT), spatial recall test (SPART), paced auditory serial addition test (PASAT), symbol digit modalities test (SDMT), and controlled oral word association test (COWAT). In addition, mood disorders were investigated by Beck depression inventory-2 (BDI-II); motor impairment was assessed by 9-hole peg and timed 25-ft walk tests, and cognitive flexibility and response-inhibition performance were assessed by Stroop test.

Elisa

Sera were collected from all participants between 08:00 and 10:00 a.m. and kept at − 80 °C until use. The levels of BDNF, melatonin, CREB (Abbkine, Wuhan, Hubei, China), and orexin-A (YH Biosearch Laboratory, Shanghai, China) were determined by ELISA as per manufacturer's recommendations. The results were obtained as OD values and were converted to ng/ml or pg/ml under the guidance of the curves generated from the values of standards.

Statistical analysis

ANOVA, chi-square, Student's t test, and Mann-Whitney U tests were used for comparison of study groups, as required. Correlation analysis was done with Spearman's correlation test. $p < 0.05$ was considered statistically significant.

Results

Clinical features

RRMS and SPMS patients had comparable gender features, onset of disease, attack number, progression index values, oligoclonal band positivity rates, and MRI features. SPMS patients had relatively higher age, disease duration, and EDSS values, as expected (Table 1). None of the participants had hypothalamic lesions on standard MRI. MS patients displayed worse scores than healthy controls in all utilized neuropsychological and motor assessment tests (Table 2).

Clinical associations with levels of mediators

MS patients had significantly lower serum levels of BDNF and orexin-A than healthy controls, while CREB and

Table 1 Demographic and clinical features of relapsing remitting multiple sclerosis (RRMS) patients, secondary progressive multiple sclerosis (SPMS) patients, and healthy controls (HC)

	RRMS ($n = 25$)	SPMS ($n = 15$)	HC ($n = 20$)	p value
Age	35.0 (15.5)	45.0 (13.0)	34.5 (11.7)	0.001*
Gender (female/male)	19/6	10/5	12/8	0.512**
Onset of disease	27.0 (13.5)	32.0 (11.0)	–	0.244***
Disease duration	10.0 (10.0)	14.0 (6.0)	–	0.015***
EDSS	2.0 (1.2)	4.5 (2.0)	–	<0.001†
Total number of attacks	5.0 (5.5)	7.0 (6.0)	–	0.144***
Number of annual attacks††	0.6 (0.7)	0.6 (1.0)	–	0.371***
Progression index	0.3 (0.4)	0.4 (0.6)	–	0.232***
Oligoclonal band positive	17	11	–	0.722**
MRI findings				
Number of patients with				
Hemispheric lesions	25	15	–	NA**
Brainstem lesions	16	7	–	0.457
Cerebellar lesions	12	8	–	0.744
Spinal cord lesions	9	6	–	0.800

All numerical values are denoted as median (interquartile range). *EDSS*, expanded disability status scale; *NA*, not applicable

* ANOVA; ** chi-square; *** Student's t test; † Mann-Whitney U test

†† Number of annual attacks = total number of attacks/disease duration

Table 2 Comparison of neuropsychological and motor performance test scores of relapsing remitting multiple sclerosis (RRMS) patients, secondary progressive multiple sclerosis (SPMS) patients, and healthy controls (HC)

	RRMS ($n = 25$)	SPMS ($n = 15$)	HC ($n = 20$)	p value*
Selective Reminding Test	7.3 (2.0)	7.8 (1.7)	9.2 (1.8)	< 0.001
Spatial Recall Test	4.5 (2.8)	4.3 (4.0)	6.0 (1.7)	< 0.001
Paced Auditory Serial Addition Test (PASAT)	33.0 (18.5)	37.0 (23.0)	50.5 (12.2)	< 0.001
Symbol Digit Modalities Test	34.0 (29.5)	31.0 (17.0)	52.5 (17.0)	< 0.001
Controlled Oral Word Association Test	41.5 (30.3)	54.0 (18.0)	74.5 (29.5)	< 0.001
Stroop Test (seconds)	43.2 (48.5)	46.5 (47.1)	36.0 (21.0)	0.031
9-Hole Peg Test (seconds)	22.4 (9.2)	24.1 (5.8)	18.2 (3.5)	0.004
Timed 25 Foot Walk Test (seconds)	7.4 (2.3)	7.9 (5.8)	6.3 (2.4)	0.005
Beck Depression Inventory-2	9.5 (10.0)	17.0 (11.3)	6.0 (8.0)	0.008

All scores are denoted as median (interquartile range). *Evaluated by ANOVA

melatonin levels were comparable among two groups. SPMS patients showed trends towards exhibiting reduced levels of mediators than RRMS patients. However, significant differences among SPMS and RRMS patients were only found in levels of melatonin and orexin-A (Fig. 1). Orexin-A levels did not differ among patients with and without spinal cord, brainstem, or cerebellar MRI lesions ($p = 0.417$, $p = 0.489$, and $p = 0.325$, respectively).

Potential correlations were investigated among EDSS values, neuropsychological-motor performance scores, clinical variables listed in Table 1, and levels of mediators using Spearman's correlation test. Serum orexin-A levels were negatively correlated with 9-hole peg ($p = 0.027$, $R = -0.397$) and timed 25-ft walk ($p = 0.035$, $R = -0.380$) values. When compared separately, orexin-A levels of RRMS patients, but not SPMS patients, showed correlation with 9-hole peg ($p = 0.026$, $R = -0.494$) and timed 25-ft walk test scores ($p = 0.019$, $R = -0.517$). In other words, RRMS patients with lower orexin-A levels required more time to accomplish motor performance tasks. Also, in the RRMS subgroup, orexin-A levels negatively correlated with progression index ($p < 0.001$, -0.712). Other mediators did not show any significant correlation with neuropsychological and clinical variables (Supplementary Table 1).

Discussion

Our study failed to find a biomarker candidate for cognitive deterioration and also failed to display a significant association between serum levels of the melatonin-BDNF-CREB pathway factors and disability in MS patients. Although serum BDNF levels were significantly reduced and showed trends towards further decreasing in SPMS patients, they were not correlated with measures of disability as reported previously [6].

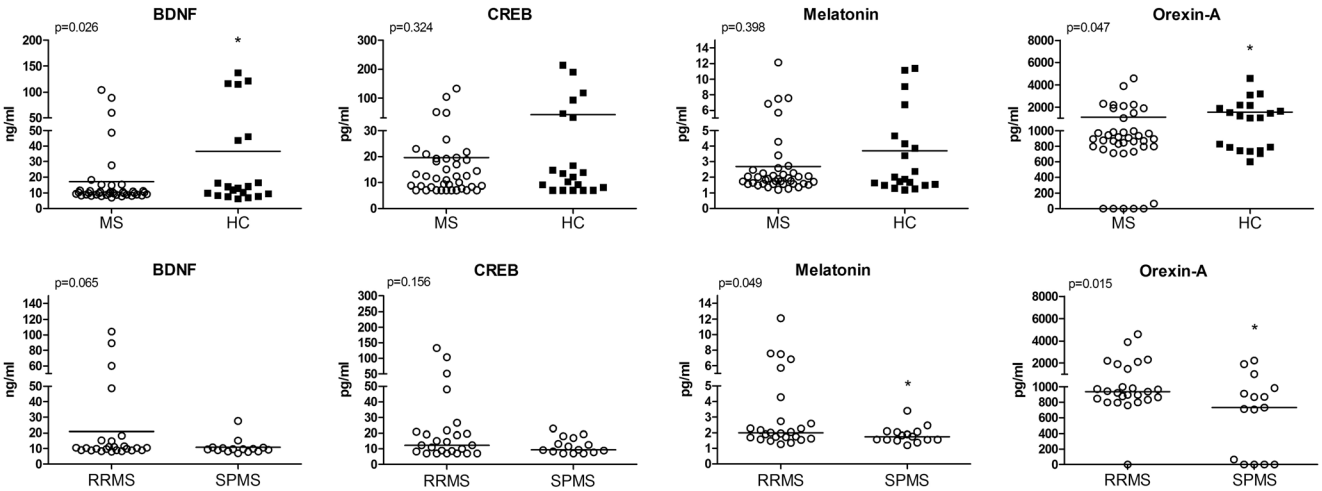

Fig. 1 Comparison of serum levels of brain-derived neurotrophic factor (BDNF), cAMP response element-binding protein (CREB), melatonin, and orexin-A. Upper panels show comparison of multiple sclerosis (MS) patients with healthy controls (HC), and lower panels show comparison of secondary progressive multiple sclerosis (SPMS) patients with relapsing remitting multiple sclerosis (RRMS) patients. *$p < 0.05$ by Mann-Whitney U test; p values are denoted at the upper left quadrant of each panel. Horizontal bars indicate mean values. Mann-Whitney U test was used due to uneven distribution of data

Cerebrospinal fluid orexin levels of MS patients have been reported to slightly albeit insignificantly decrease as compared to controls and several MS patients with reduced CSF orexin-A levels have been reported [7]. To our knowledge, our pilot study showed for the first time that serum orexin-A levels are decreased in MS patients and more significantly reduced in SPMS patients suggesting that hypothalamic orexinergic neurons are gradually lost during the disease course. Moreover, orexin-A levels did not correlate with global EDSS scores as previously reported [7], but showed association with the quality of motor performance only in the earlier RRMS stage of the disease. Whether this association is due to the impact of orexinergic neurons on central motor system control [8] needs to be further studied.

Although attack frequency is known to be reduced in the progressive stage of MS, our RRMS and SPMS patients displayed comparable annual attack numbers. This was probably because our SPMS patients were at a somewhat early stage of progression, as exemplified by a relatively lower EDSS average, and thus had not shown a substantial reduction in attack frequency, as yet. Secondly, the size of the examined cohort was small prompting further validation of the results. Orexin-A production and motor cortex plasticity are reduced by increasing age [9, 10]. Therefore, some of our results might simply be a manifestation of normal aging in relatively older SPMS patients. Thus, larger size cohorts are also required to properly assess the confounding influence of age on orexin-A and MS progression association. Nevertheless, our preliminary results suggest that orexin-A might be utilized as a predictive biomarker of motor system deterioration especially in early stages of MS.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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