

Assessment of plasma brain-derived neurotrophic factor (BDNF), activity-dependent neurotrophin protein (ADNP) and vasoactive intestinal peptide (VIP) concentrations in treatment-naïve humans with multiple sclerosis

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Abstract

OBJECTIVE: Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by coexisting processes of inflammation, demyelination, axonal neurodegeneration and gliosis. Autoimmune processes play a pivotal role in the disease. The immune system may be modulated by neurotrophins and neurotrophin factors. Aim of the study was to assess plasma levels of brain-derived neurotrophic factor (BDNF), activity-dependent neurotrophin protein (ADNP) and vasoactive intestinal peptide (VIP) in treatment-naïve humans with newly diagnosed multiple sclerosis. We also elucidated the potential influence of selected inflammatory agents on peripheral concentration of BDNF and ADNP.

MATERIAL AND METHODS: The study population comprised of 31 untreated patients with MS and 36 controls from a single hospital centre. Assessment of BDNF and ADNP was performed with use of ELISA methods. VIP was measured with RIA. Selected cytokine levels (IL 6, IL 10, and TNF α) were evaluated with ELISA tests. Statistical analyses were performed.

RESULTS: We failed to find any significant differences between ADNP, BDNF, VIP, CRP levels and concentration of cytokines between individuals with MS and the controls. No correlation was observed between ADNP, BDNF and VIP as the first parameter and CRP, IL 6, IL 10, TNF α levels and the Expanded Disability Status Scale score in MS.

CONCLUSIONS: Newly diagnosed, treatment-naïve patients with MS have comparable levels of plasma BDNF, ADNP and VIP to those of healthy controls.

Abbreviations:

ADNP	- activity-dependent neuroprotective protein
BDNF	- brain-derived neurotrophic factor
CNS	- central nervous system
CRP	- C-reactive protein
CSF	- cerebrospinal fluid
CV	- coefficient of variation
EAE	- experimental autoimmune encephalomyelitis
EDSS	- Expanded Disability Status Scale
IL10	- interleukin 10
IL6	- interleukin 6
MS	- multiple sclerosis
NAP	- NAPVSIPQ neuroprotective peptide
PACAP	- pituitary adenylate cyclase activating polypeptide
SD	- standard deviation
TNF α	- tumor necrosis factor α
VIP	- vasoactive intestinal peptide

INTRODUCTION

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by coexisting processes of inflammation, demyelination, axonal neurodegeneration and gliosis (Kamm *et al.* 2014). Although up to date the pathogenesis of MS is not clearly explained, some evidence are accumulated that autoimmune processes play a pivotal role in the disease (McFarland & Martin 2007; Rostami & Ciric 2013) The immune system may be modulated, amongst others, by neurotrophins and neurotrophin factors (Luhder *et al.* 2013).

Brain-derived neurotrophic factor (BDNF) was discovered in 1982 by Barde and co-workers (Barde *et al.* 1982). BDNF is translated from a pro-form into mature protein that may play a protective role in the neuronal survival, differentiation and synaptic plasticity (Calabrese *et al.* 2014; Noble *et al.* 2011). BDNF binds to the tyrosine receptor kinase B with specific high affinity while it binds to the p75 neurotrophin receptor which belongs to the tumor necrosis factor superfamily, with low affinity (Numakawa *et al.* 2013). The main sources of BDNF in the brain are neurons. Moreover, BDNF may be also secreted by activated astrocytes under inflammatory conditions (Linker *et al.* 2010). Furthermore, activated immune cells including T-cell, B-cells and monocytes are able to produce BDNF (Kerschensteiner *et al.* 1999; Stadelmann *et al.* 2002). Interestingly, in cases of MS BDNF has been found in inflammatory lesions, neurons, glial cells and immune cell infiltrating the CNS (Kerschensteiner *et al.* 2003). Data from literature suggest the neuroprotective role of BDNF under neuroinflammatory and demyelinating conditions in the animal model (Luhder *et al.* 2013). However, studies concerning neuroprotective activity of BDNF in humans with neurodegenerative disease have not yet confirmed these findings unambiguously (Luhder *et al.* 2013).

Activity-dependent neurotrophin protein (ADNP) was discovered by Gozes and co-workers (Gozes *et al.* 1999). The expression of human ADNP has been

reported in the spleen, peripheral blood leukocytes, macrophages, the central nervous system (cerebellum, hippocampus, and cerebral cortex) and astrocytes (Braitich *et al.* 2010). As neuroprotective and immunomodulatory properties of ADNP were confirmed (Gozes 2012), the role of ADNP in neurodegenerative disease has been studied. Braitich and co-researchers reported a reduced expression of ADNP in peripheral blood cells of MS patients. These authors suggested the possibility of an additional deficit of immunoregulation in the inflammatory demyelinating disease (Braitich *et al.* 2010). It has been revealed that ADNP gene expression in astrocytes is regulated by two members of secretin-glucagon family members, vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating polypeptide (PACAP) as well as vasoactive intestinal peptide receptor type 2 (Zusev & Gozes 2004). However, ADNP activity may also be influenced by other than VIP and PACAP mechanisms (Gozes 2012).

Several studies have demonstrated immunomodulatory and neuroprotective activity of VIP (Waschek 2013). Undoubtedly, results of research conducted on inflammatory disease models, including the MS model, confirmed the modulatory, immune-dependent influence of VIP and additionally PACAP on the course of diseases characterised by inflammatory origin (Abad & Waschek 2011). Study on humans suffering from MS revealed altered expression of VIP receptors in T lymphocytes and aberrant Th1 immunity (Sun *et al.* 2006). Tan and Waschek indicated that VIP and PACAP may modulate symptoms of experimental autoimmune encephalomyelitis (EAE), model of MS, on the central level as well as on the periphery (Tan & Waschek 2011). However, Abad and colleagues reported interesting findings that VIP KO mice were highly resistant to EAE but pretreatment with VIP reversed their susceptibility to encephalomyelitis (Abad *et al.* 2010).

To broaden the knowledge about the role of BDNF and ADNP in pathogenesis of MS we aimed to assess plasma levels of these peptides in treatment-naïve patients with newly diagnosed disease. In addition, we also decide to elucidate the potential influence of VIP and selected inflammatory agents on peripheral concentration of BDNF and ADNP in this group of patients.

MATERIAL AND METHODSSubjects

The study population comprised of 31 untreated previously patients with newly diagnosed MS and 36 individual of control group suffering with different type of headaches in whom MS was excluded. Consecutive study participants were recruited on the volunteer basis from a single hospital centre at Bielanski Hospital (Department of Neurology, Medical University of Warsaw, Warsaw, Poland). The age range varied between 19 and 53 years in MS subset, and from 18 to

51 in the controls, respectively. The diagnosis of MS was established according to the Diagnostic criteria for MS (2010), revision to the McDonald criteria (Polman *et al.* 2010). The diagnosis was confirmed by an MRI scan with gadolinium contrast of the brain and the presence of oligoclonal bands in cerebrospinal fluid (CSF). The evaluation of neurological status was performed with the Expanded Disability Status Scale (EDSS). Exclusion criteria were also established. All the subjects with cardiac, hepatic or renal failure, neoplasms, psychiatric diseases, acute inflammatory processes or history of the systemic glucocorticoid treatment within 8 weeks prior to the study date were excluded from the study.

The study protocol was approved by the Ethical Commission of the Medical Centre of Postgraduate Education in Warsaw. Signed consent was obtained from all participants.

Analytical methods

After overnight fast blood was collected to the tubes containing EDTA and aprotinin (protease inhibitor) and immediately centrifuged at 4°C. Plasma samples were isolated and stored at -70°C for further analytical procedures.

BDNF concentrations were evaluated with use of ELISA kit (Ray-Biotech, Inc., Norcross, GA, USA). The detection limit was less than 80 pg/ml. The intra-assay coefficient of variation (CV) was 10% and inter-assay CV was less than 12%.

ADNP levels in plasma were detected with ELISA method (USCN Life Science Inc., Wuhan, China). The sensitivity of this assay was 0.055 ng/ml. The intra-assay and inter-assay precision were less than 10% and 12%, respectively.

VIP concentrations were measured in extracted plasma using a commercially available radioimmunoassay kits (Phoenix Pharmaceuticals Inc., Burlingame, CA, USA) according to the manufacturer's instructions. The sensitivity of VIP evaluation was <1.25 pg/ml. Intra and inter-assay coefficients were less than 10%.

Interleukin 6 (IL 6), interleukin 10 (IL 10) and tumor necrosis factor α (TNF α) levels were determined by ELISA methods (Pierce Biotechnology Inc., Rockford, IL, USA). The sensitivities for IL 6, IL 10 and TNF α were < 1 pg/ml, < 3 pg/ml and < 2 pg/ml, respectively. The intra-assay and inter-assay coefficients for IL 6 and IL 10 were 10%, and 10%, respectively and for TNF α were 5.9%, and 7.1%, respectively.

C-reactive protein (CRP) was established with standard laboratory procedures.

Statistical analysis

Statistical analyses were performed using STATISTICA 10 (Stat Soft Inc. Tulsa, OK, USA). Data are presented as the mean \pm standard deviation (SD). Nonparametric data were compared between groups using the Mann-Whitney U test. Significance of correlations was determined with use of the Spearman rank correlation

coefficient. All values of less than 0.05 were accepted as statistically significant.

RESULTS

All clinical data and results of analytical analyses were presented in Table 1.

We failed to find any significant differences between ADNP, BDNF, VIP, CRP levels and concentration of selected cytokines when we compared the results of the patients with MS and the controls (Table 1).

We did not observe any significant correlations between concentration of ADNP, BDNF and VIP as the first parameter and CRP, IL 6, IL 10, TNF α levels and EDSS score as the second parameter in the group of individuals with MS (data not shown).

DISCUSSION

Previously published MS studies, including those involving clinical as well as experimental protocol, suggested neuroprotective and regenerative activity of BDNF as well as its supporting role in remyelination (Hohlfeld 2008; Kerschensteiner *et al.* 2003). Interestingly, it has been established that immune cells express BDNF mostly in actively demyelinating areas of MS lesions in contrast to the areas of the CNS without ongoing myelin breakdown (Stadelmann *et al.* 2002). Moreover, the presence of neurotrophins has been shown at the actively demyelinating edge of the lesion early in its development (Kerschensteiner *et al.* 2003). Therefore, we found it reasonable to assess

Tab. 1. Clinical and biochemical data of the patients with multiple sclerosis and the controls.

	Multiple sclerosis n=31	Control group n=36	p-value
Women/Men	20/11	31/5	-
Hypertension	4 (13%)	4 (11%)	-
Diabetes type 2	0	1 (3%)	-
Age	35.35 \pm 7.57	32.78 \pm 8.04	ns
CRP (mg/l)	1.23 \pm 1.12	1.43 \pm 1.29	ns
IL 6 (pg/ml)	1.69 \pm 0.9	1.87 \pm 0.84	ns
TNF α (pg/ml)	4.58 \pm 3.34	5.07 \pm 4.8	ns
IL 10 (pg/ml)	3.0 \pm 2.64	2.48 \pm 1.54	ns
ADNP (ng/ml)	4.02 \pm 1.82	4.0 \pm 1.72	ns
BDNF (ng/ml)	2.21 \pm 1.19	2.06 \pm 1.18	ns
VIP (pg/ml)	9.58 \pm 8.15	8.76 \pm 4.45	ns
EDSS	1.69 \pm 0.86	0	-

CRP - C-reactive protein; IL 6 - interleukin 6; TNF α - tumor necrosis factor alpha; IL 10 - interleukin 10; ADNP - activity-dependent neuroprotective protein; BDNF - brain-derived neurotrophic factor; VIP - vasoactive intestinal peptide; EDSS - Expanded Disability Status Scale; ns - non-significant

BDNF peripheral concentration in newly diagnosed and untreated patients suffering from MS. Regrettably, we failed to find any significant differences in the plasma levels of BDNF between MS individuals and the healthy controls. Our results are in agreement with recently published findings of Damasceno and coworkers (Damasceno *et al.* 2015). These authors revealed no changes in serum BDNF concentration in MS patients treated with interferon beta when compared to their healthy counterparts. Moreover, similarly to our study, there was no correlation between BDNF levels and EDSS score (Damasceno *et al.* 2015). Our findings are also in concordance with data published by Lalive and co-authors (Lalive *et al.* 2008). These results indicated that serum BDNF concentrations of MS both interferon beta treated and untreated patients and the controls were on the similar level (Lalive *et al.* 2008). However, the results from other studies concerning the levels of BDNF in serum or plasma of MS individuals are equivocal. The group of Azoulay found lower levels of BDNF in serum and in the CSF (Azoulay *et al.* 2005). Frota *et al.* also indicated a decrease in plasma levels of BDNF in MS patients in comparison with their healthy counterparts. Moreover they observed that plasma BDNF concentration increased significantly after MS relapse (Frota *et al.* 2009). Consequently, Comini-Frota and co-workers reported a reduction in serum BDNF concentration in MS patients as compared to the healthy controls (Comini-Frota *et al.* 2012). Investigators from the group of Tongiorgi analyzed BDNF isoforms in sera of treatment-naïve patients with SM. Interestingly, these authors demonstrated that total serum BDNF concentration was lower in MS subjects than in the controls. Furthermore, there was statistically significant difference in BDNF isoforms percentage between MS patients and the healthy subjects. In details, the ratio of serum level of mature BDNF and pro-BDNF concentration to total serum level of BDNF was significantly decreased, while truncated BDNF concentration was increased. Moreover, it has been established that no correlations between BDNF isoform percentage and clinical or demographic features were present (Tongiorgi *et al.* 2012). On the other hand, data from the study conducted by Yoshimura and co-workers indicated higher serum levels of BDNF in MS subjects. Moreover, these authors found that MS individuals with increased BDNF concentration were younger and showed fewer relapse numbers than patients with lower levels of BDNF (Yoshimura *et al.* 2010).

To our knowledge there is a lack of data concerning plasma levels of ADNP or its important neuroprotective active site, a short eight amino acid peptide (NAPV-SIPQ) termed NAP in MS patients. Thus we report, for the first time, an absence of differences between ADNP plasma levels of MS naïve to treatment patients and the controls. However, Braitch and coworkers (2010) revealed a reduced ADNP mRNA levels in peripheral blood mononuclear cells of MS patients in compari-

son to the controls. These authors suggested decreased immunoregulatory capacity via ADNP in MS patients (Braitch *et al.* 2010).

Several studies conducted on animal model of MS indicated the role of VIP in modulating the course of multiple sclerosis. However, data concerning VIP levels in plasma of MS human subjects are very limited. To our best knowledge the only study report evaluating assessment of VIP concentrations in plasma and CSF in naïve to treatment MS patients was published by our team (Baranowska-Bik *et al.* 2013). Briefly, no differences between VIP levels measured in plasma of MS participants and those of the controls were observed. On the contrary, in the previous project we revealed a non-significant tendency towards lower levels of VIP in the CSF of MS individuals when compared with the controls. Thus, the present study in which we failed to find any significant differences in peripheral VIP concentrations between individuals with and without MS confirmed our previous findings.

We conclude that in our material newly diagnosed, treatment-naïve patients with MS had comparable levels of plasma BDNF, ADNP and VIP to those of healthy controls.

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