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Original research article

Brain-derived neurotrophic factor levels under chronic natalizumab treatment in multiple sclerosis. A preliminary report

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ARTICLE INFO

Article history:
Received 29 November 2016
Accepted 21 March 2017
Available online 31 March 2017

Keywords:
BDNF
MS
Natalizumab

ABSTRACT

Aim of the study: Our main purpose was to investigate if the chronic treatment with the disease-modifying drug natalizumab shows quantifiable effect on BDNF levels in multiple sclerosis patients.

Materials and Methods: BDNF plasma concentration was evaluated using enzyme-linked immunosorbent assay in healthy individuals, not treated multiple sclerosis patients and patients treated with natalizumab.

Results: Multiple sclerosis patients have a significantly lower amount of peripheral BDNF than healthy individuals. Patients treated with natalizumab have significantly higher BDNF levels than not treated patients.

Conclusions: Chronic natalizumab treatment is associated with significantly increased plasma BDNF concentration in multiple sclerosis.

1. Introduction

Multiple sclerosis (MS) is the most frequent demyelinating disease of the central nervous system (CNS), inducing a considerable disability in sufferers and having an important social impact [1].

The most widely accepted theory regarding the pathophysiology involves an immune attack against the myelin [2]. Following an initial, insufficiently identified trigger, the activated T lymphocites are orchestrating an inflammatory cascade of cytokines [3], disturbing the integrity of the blood–brain barrier (BBB). Further on, disease development requires probably a molecular mimicry-like behavior, a crossed autoreactive injury of the myelin sheath, involving other cytokines and macrophage activation [4]. The aforementioned attack triggers and accompanies neurodegeneration, expressed clinically by progressive brain atrophy [5]. The latter is a good marker for disability progression also [6].

After the attack takes place, several defense pathways are activated [7]. As an effect, migrating oligodendrocytes partially substitute the lost myelin [8]. The mentioned pathways are

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http://dx.doi.org/10.1016/j.pjnns.2017.03.002

activated, among other means, by the production and release of neurotrophic growth factors [9]. They have several beneficial effects in the central nervous system (CNS): stimulating cell differentiation – glia and neuronal cells, neurite growth and plasticity, etc. [10]. Neurotrophins also modulate immune response through activated auto-reactive T-cells [11]. Thus, a neuroprotective treatment, potentially influencing also immune attack, might represent a valuable approach [12].

Available treatment is unsatisfactory to this point. High doses of glucocorticosteroids target relapses, frequently obtaining remission. The problematic part is the disease-modifying therapy, which fails to offer a symptom-free improvement; it only reduces the frequency of relapses, and eventually the severity of individual attacks [13]. Progression is slower, but never stops. For this purpose interferons, glatiramer acetate, natalizumab and other disease modifying drugs are available [14]. We focus here on natalizumab.

Natalizumab is a monoclonal antibody used for the treatment of relapsing-remitting multiple sclerosis (RRMS), proposed already even as first line approach [15] and under evaluation with promising results for secondary progressive multiple sclerosis (SPMS) [16], showing an impact on both progression and relapse frequency [17].

The drug targets the α4 subunit of integrins [18], surface molecules of T lymphocytes, or the integrin very late antigen (VLA-4) [19]. Integrins are playing a role when coupling with vascular endothelial receptors, like the vascular cellular adhesion molecule VCAM-1. The coupling facilitates adhesion of lymphocytes to vessel walls and crossing through the blood–brain barrier (BBB). If the adhesion is interfered, crossing the attack into the CNS compartment is also diminished [20].

A possible proof for the effect, contributing to the overall outcome, is that natalizumab probably induces through a co-stimulatory signaling pathway an increase of effector memory T-cells in the blood, but with no elevation of myelin-reactive cells: a sequestration outside the CNS [21]. Furthermore, natalizumab not only reduces the migration by blocking the integrins, but seemingly also by down-regulation of vascular cell adhesion molecule 1 (VCAM-1) expression [22].

Activated immune cells are capable of producing neurotrophic factors [23]. Early intervention in the cascade of inflammation might have an impact also on neurotrophic factor production [24]. Thus, reducing BBB crossing might have a dual impact.

One of the extensively investigated growth factors is the brain-derived neurotrophic factor (BDNF) [25], a representative of the neurotrophin gene family, along with NGF, NT3 and NT 4/5 [26]. It is produced by several cells, mainly astrocytes, but also immune cells, as it was mentioned before, and acts in both a pro-neurotrophin form, and a mature form [27], both presenting different functional aspects. Even the coupling differs: the immature form binds with the p75 receptor, with low affinity, showing pro-apoptotic effects on neurons, and overall inhibitory effect on regeneration [28]. On the other hand, the tyrosine receptor kinase B, or BDNF/NT-3 growth factors receptor (TrkB) coupling of the mature form induces neuroprotective mechanisms as plasticity, or survival mechanisms [29]. TrkB receptors are expressed on oligodendrocytes and oligodendrocyte progenitor cells [30], influencing myelinization-linked processes. In vitro, for example, BDNF enhances the number of the oligodendrocytes with topographic selectivity in the basal forebrain, not in the cortex [31]. Animal studies reveal similar observations, BDNF knock-out mice showing decrease in myelin proteins in the optic nerve and spinal cord in early development, with recovery during aging [32], and without recovery in the basal forebrain [33]. The effect on myelinization is even more expressed in case of previous injuries producing demyelinization [34].

Growing evidences are available regarding BDNF involvement in different CNS diseases. Among others, schizophrenia [35], Huntington's disease [36] and even Alzheimer's dementia [37] seems to involve alterations in BDNF homeostasis at a pathophysiological level.

Several studies investigated already the expression and different roles of BDNF in MS: on one hand from the point of view of genetic variations, with insufficient proof for association [38], on the other hand regarding the effect during disease course [39]. There are studies presenting that peripheral plasma concentration of BDNF is lowered [40], excepting perhaps transitory elevations during relapses [41]. These observations seem to be applicable for different clinical presentations, like RRMS and SPMS [42]. As presented in the in vitro and preclinical studies, if demyelinization occurs, BDNF has an important role in repair processes. Human studies on this effect reveal that BDNF expression increases around MS lesions and in perivascular spaces, its secretion being assured by microglia, astrocytes, but also by infiltrating immune cells [43].

In relation with the applied treatment, most studies were conducted on glatiramer acetate treated patients [44], results being heterogeneous, some reported increases (Azoulay et al., 2005)[40], and some decreased or no significant effect on plasma concentration [45]. There are also available data regarding BDNF levels under novel treatments, like laquinimod [46].

Our goal was to investigate the possible impact of natalizumab on BDNF plasma concentration, for which, to our knowledge, there are no similar available investigations.

2. Materials and method

Patients with confirmed RRMS were recruited, in accordance with the revised McDonalds criteria. SPMS group was formed from RRMS patients under natalizumab treatment, confirmed at inclusion as having already SPMS, the two diagnostic instances forming a continuum. Even if natalizumab is not an accepted treatment option for SPMS, they formed a new group, as they were already on this treatment. For comparison we have selected an age- and sex-matched group of normal subjects. The study was approved by the ethics committee of our university, being in accordance with the Helsinki principles for biomedical research. All participants signed an informed consent.

We have formed four groups: the control group (CTRL) with 20 healthy age and sex matched individuals, a group of 11 newly diagnosed patients with RRMS, non-treated, (NT), a group of 11 natalizumab-treated RRMS patients (Nat-RRMS) and a group of 9 SPMS (Nat-SPMS), previously RRMS, restaged at inclusion as SPMS, based on EDSS score increase without a

Table 1 – The table presents the demographic data (age, sex) of the participants, including the average EDSS score at inclusion for each group.

	CTRL	NT	Nat-RRMS	Nat-SPMS
Age (Years)	35.83 ± 2.82	37.36 ± 2.05	39.27 ± 1.02	40.89 ± 1.71
Sex				
M	33.33%	28.57%	27.27%	22.23%
F	66.67%	71.43%	72.73%	77.77%
EDSS	/	2.50 ± 0.27	3.37 ± 0.32	5.78 ± 0.34

history of relapse. These patients, after inclusion, continued their treatment until reassigned to another, accepted regimen. Treated MS groups had a history of at least one year of chronic natalizumab treatment. There was no treatment-naïve SPMS group formed, since even if this is statistically desirable, not treating a SPMS patient to this stage (having RRMS for a time period, as this is the natural course of the disease) is not ethical. There was no participant included during relapse, and no participant had glucocorticoid treatment four weeks prior inclusion. Average EDSS scores were higher in the treated groups as in the treatment-naïve group at inclusion, this being understandable, given the much longer disease course in the former groups. The patients recruited in the NT group were at the beginning of the disease, either around the moment of the diagnosis, or waiting to be included on a proper treatment regimen. EDSS score-matched treatment-naïve group cannot be formed, not being ethical to keep a patient without treatment up to a higher disability. Demographic data and Extended Disability Status Scale (EDSS) score for the MS groups are presented in Table 1.

We have collected blood samples in Li-heparinized tubes, 7 ml from each patient, and centrifuged the samples for 7 min at 1200 rpm at 4 °C. The resulting plasma was refrigerated at −80 °C, until tested. Plasma level of BDNF (mature form) was measured using an enzyme-linked immunosorbent assay. The assay procedures were performed in accordance with the manufacturer's instructions (RayBio Human BDNF ELISA Kit, by RayBiotech Inc.). Reading was carried out with a Stat Fax ELISA microplate reader against a blank.

For the statistical analysis SPSS 20 and MS Excel were used, consisting of descriptive statistics, normality testing, non-parametric tests: Kruskal–Wallis (K–W) and Mann–Whitney (MW) tests for independent samples. Threshold for significance was $p < 0.05$.

3. Results

The BDNF plasma concentration of the participants was determined using ELISA. The Kolmogorov–Smirnov test revealed a normal distribution (not shown), but both the number of investigated subjects, and the unequal variances demonstrated by Levene's test (not shown) oriented the statistics toward a non-parametric approach (Fig. 1).

First we have tested between groups differences, four independent samples, using the Kruskal–Wallis (K–W) test, unveiling a highly significant difference ($p_{\text{Nat-RRMS vs. Nat-SPMS vs. NT vs. CTRL}} = 0.00003$, K–W), normal subjects having a higher concentration than the groups of MS patients, but there were differences also among the latter: both natalizumab-treated groups showing a slightly higher plasma concentration than NT.

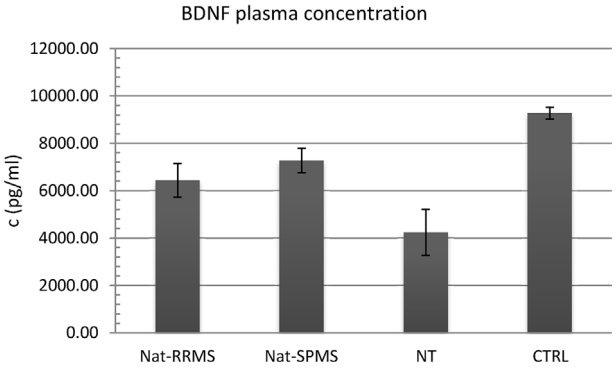

Fig. 1 – The figure shows the plasma concentration of BDNF, expressed in pg/ml, for the four study groups (means and error bars for standard error). One can observe the significantly lower concentration in all MS groups (non-treated (NT) ($p_{\text{NT vs. CTRL}} = 0.0004$) and natalizumab-treated ($p_{\text{Nat-RRMS vs. CTRL}} = 0.0003$, $p_{\text{Nat-SPMS vs. CTRL}} = 0.003$)), and also the significantly increased plasma BDNF for the Natalizumab treated groups ($p_{\text{Nat-RRMS vs. NT}} = 0.04$, $p_{\text{Nat-SPMS vs. NT}} = 0.02$), when compared with the NT group.

Then a pairwise approach was applied, using Mann–Whitney (MW) test. We have compared the non-treated group with healthy controls and found a significant difference ($p_{\text{CTRL vs. NT}} = 0.0004$, MW). The second test was the comparison of the controls with the treated patients, their BDNF plasma concentrations being also significantly lower ($p_{\text{CTRL vs. Nat-RRMS}} = 0.0003$, $p_{\text{CTRL vs. Nat-SPMS}} = 0.003$, MW). Finally we have compared both treated MS groups with the treatment-naïve MS group, having significant differences in all instances, the treated groups showing a higher concentration ($p_{\text{NT vs. Nat-RRMS}} = 0.04$, $p_{\text{NT vs. Nat-SPMS}} = 0.02$, MW). All statistical significances are summarized in Table 2.

4. Discussion

As presented earlier, overall plasma BDNF levels found during our study were in accordance with the data from the literature [41], each MS group showing a significantly lower titer as the normal group. We have previously mentioned also that patients were not presenting relapses at the time of sample taking.

Regarding the treatment with natalizumab, both investigated groups, RRMS and SPMS, showed a significant increase of the plasma concentration of the neurotrophin. Seemingly, the

Table 2 – The table shows the statistical significances found when the study groups were compared, presenting also the statistical method which was used (Nat-RRMS – RRMS patients under natalizumab treatment; Nat-SPMS – SPMS diagnosed at inclusion, under natalizumab treatment; NT – not treated MS patients; CTRL – healthy controls).

Statistical method	Statistical comparison	*p* – Asymp. sig. (2-tailed)
Mann–Whitney *U*	Nat-RRMS vs. Nat-SPMS	0.52
	Nat-RRMS vs. NT	0.04
	Nat-RRMS vs. CTRL	0.0003
	Nat-SPMS vs. NT	0.02
	Nat-SPMS vs. CTRL	0.003
	NT vs. CTRL	0.0004
Kruskal–Wallis	Nat-RRMS vs. Nat-SPMS vs. NT vs. CTRL	0.00003

level in the natalizumab treated SPMS group is even higher than in the treated RRMS group, somehow in opposition with the literature, which states that in SPMS the concentration is usually even lower than in RRMS [47]. The increase was significant compared with our treatment naïve RRMS group (NT). This group had a lower degree of severity and a lower EDSS score, but at this stage we could not correlate the EDSS changes with the treatment, our approach being cross-sectional, not a follow-up study. On the other hand, even if the BDNF concentration was higher in the natalizumab treated groups as in the non-treated patients, still, it was significantly lower than in healthy volunteers.

Blocking the natalizumab-mediated reduction of CNS transfer for activated immune cells reduces the extent of immune attack [18], but also possibly reduces the BDNF production induced by immune cells in the CNS compartment [25]. Still, besides the immune cells, astrocytes and neurons also produce BDNF at the level of the central nervous system [29]. This additional quantity possibly contributes to the overall positive outcome [48]. If this is true, then in case of natalizumab treatment this production might outweigh the loss of CNS immune mediated BDNF production [23]. Otherwise, the loss is probably insignificant when faced with the BDNF gain through the immune-attack reduction. Following the same reasoning, the peripheral increase of activated immune cells, without the possibility to cross into the CNS, but capable of BDNF production [49], might contribute to peripheral BDNF increase.

Aforementioned are suggestions. There is a great amount of uncertainty regarding the possibility to draw a parallelism between CNS and peripheral BDNF levels: amounts uptaken and released by platelets, the relative impermeability of the BBB for BDNF according to some authors [50] or exactly the opposite stated by others [51], etc. High importance is given also by the paracrine-like activity of peripheral BDNF, which leaves only a speculative approach to conclude from peripheral levels on central expression [52]. To eliminate other sources of error, further studies are needed, using higher group sizes, to increase the statistical strength of data. Eventually CSF BDNF levels should also be investigated, along with possible research on models of MS, EAE lineage, where tissue samples might serve the proof for local natalizumab-related BDNF expression and its correlation with plasma concentration, or even with BDNF produced by peripheral blood mononuclear cells (PBMC). Other directions which should be covered in the further research are the correlation of EDSS scores with the changes of the BDNF levels. Research should address also how changes in BDNF serum levels induced by natalizumab treatment influence the progression of RRMS to SPMS – for example if the treatment delays the onset to SPMS – and also, in parallel, quantifying serum/CSF levels of TrkB. All these would strengthen the study and more reliably characterize the effect of natalizumab.

5. Conclusion

Chronic natalizumab treatment is associated with significantly increased plasma BDNF concentration in multiple sclerosis. Further studies are needed to evaluate the extent and impact of the effect.

Conflict of interest

None declared.

Acknowledgement and financial support

This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007–2013, project no. POSDRU/159/1.5/138776.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals.

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