INTERFERON-BETA TREATMENT INCREASES BDNF SERUM LEVELS IN RELAPSING-REMITTING MULTIPLE SCLEROSIS FEMALE PATIENTS: A GENDER-SPECIFIC EFFECT?

Arianna Sartori[1], Giuliana Metelli[2], Alessio Polacchini[2], Alessio Bratina[1], Paolo Manganotti[1] and Enrico Tongiorgi[2]

1. Neurology Clinic, Department of Medical, Surgical and Health Sciences, University of Trieste, 34127 Trieste, Italy
2. Department of Life Sciences, University of Trieste, 34127 Trieste, Italy

OBJECTIVES

In the last 20 years many medications for Multiple Sclerosis (MS) have become available, in order to control the inflammatory aspects of the disease. Despite many advances its treatment, neuroprotection is considered a major therapeutic target that still need to be studied. Some of the currently available therapies for MS based on immunomodulation have been revaluated for their ability to stimulate production of neurotrophic factors that may provide support to neural cells against neurodegeneration. In particular, several studies have investigated brain-derived neurotrophic factor (BDNF) as one of the best candidates for such neuroprotective effects. BDNF supports neuronal survival and it regulates neurotransmitters release and neuronal growth. We previously demonstrated a reduction in the serum levels of pro-apoptotic proBDNF and pro-survival mature BDNF and an increase of truncated BDNF levels in drug-naïve relapsing-remitting (RR) MS patients.
The aim of the present study study was to compare the levels of total and mature BDNF of MS patients with no disease modifying drug (DMD) treatment to those of patients treated with interferon-beta (IFN-β) and glatiramer acetate (GA).

MATERIALS and METHODS

We analyzed the routinely collected sera of seventy-one RR-MS patients who accessed our clinic between April 2013 and February 2015. The patients have been grouped as follows: patients on interferon-beta treatment (G1), patients on glatiramer acetate (G2), and patients without DMD treatment (G3).
Inclusion criteria were: diagnosis of RR-MS according to 2010 McDonald Criteria, DMD treatment for at least 2 months (for G1 and G2) and DMD-free condition for at least 6 months (for G3). For all groups, exclusion criteria were: relapse and/or corticosteroid therapy in the last month, concomitant treatment with antidepressants and/or mood stabilizers, antibiotics, antihypertensives, and gastroprotective agents.
Serum samples were prepared as previously described. Serum levels of total and mature BDNF were measured by using BDNF *Rapid*™ ELISA Kit (Human, Mouse, Rat, 2 Plates; Cat #: BEK-2211-2P, Biosensis Pty Ltd., SA, Australia) ELISA kit, and by human BDNF ELISA Kit (Cat #: SK00752-01, Aviscera-Bioscience, Santa Clara, CA, USA), respectively, in duplicate. One-way ANOVA followed by Dunn's correction were applied to asses differences in BDNF concentration among groups. Values of $p<0.05$ were considered statistically significant. All statistical analysis and graphs were performed using SigmaPlot 11.0 (Systat Sofware, Inc.).

Characteristics of patients	G1 Interferon beta (n = 31)	G2 Glatiramer acetate (n = 20)	G3 No therapy (n = 20)	p*
Gender M	9 (29%)	8 (40%)	3 (15%)	0.211
Gender F	22 (71%)	12 (60%)	17 (85%)	
Age (years)	41.8±9.7	40.5±11.6	44.3±10.2	0.753
Disease duration (months)	95 (23-346)	137 (5-305)	131 (6-353)	0.776
Total number of relapses	2 (1-12)	2 (1-28)	2 (1-12)	0.920
Annual Relapse Rates (ARR)	0.3 (0-1.3)	0.3 (0.0-2.4)	0.3 (0.1-3.4)	0.920
Expanded Disability Status Scale (EDSS)	1 (0-3)	1 (0-5.5)	1.3 (0-3.5)	0.828
Family history of MS	2 (6.5%)	3 (15%)	2 (10%)	0.606
No Family history of MS	29 (93.5%)	17 (85%)	18 (90%)	
Family history of autoimmune diseases	4 (12.9%)	5 (25%)	1 (5%)	0.186
No Family history of autoimmune diseases	27 (87.1%)	15 (75%)	19 (85%)	
Symptom at onset				
Optic pathways	7 (22.6%)	3 (15%)	5 (25%)	0.715
Supratentorial	6 (19.4%)	2 (10%)	2 (10%)	0.532
Brainstem/cerebellum	10 (32.2%)	6 (30%)	4 (20%)	0.622
Spinal cord	7 (22.6%)	8 (40%)	8 (40%)	0.298
Polyregional	1 (3.2%)	1 (5%)	1 (5%)	0.934
Complete Recovery from 1st relapse	27 (87.1%)	16 (80%)	17 (85%)	0.789
Incomplete Recovery from 1st relapse	4 (12.9%)	4 (20%)	3 (15%)	
CSF OCBs	17 (54.8%)	11 (55%)	13 (65%)	0.741
Absent CSF OCBs	5 (16.1%)	6 (30%)	2 (10%)	0.241
Not available CSF OCBs	9 (29%)	3 (15%)	5 (25%)	0.514
Time to 2nd relapse (months)	22 (1-162)	36 (2-157)	24 (2-278)	0.966
Time from last relapse (months)	51 (1-329)	42 (5-271)	24 (2-251)	0.264
Number of steroid treatment	1 (0-8)	1 (0-27)	1 (0-20)	0.831
Time from last steroid treatment (months)	45.5 (1-29)	45 (5-203)	27 (2-231)	0.630
Previous DMD	7 (22.6%)	8 (40%)	10 (50%)	0.117
No Previous DMD	24 (77.4%)	12 (60%)	10 (50%)	
Numbers of previous DMD	1 (1-2)	1 (1-3)	1 (1-3)	0.571
Duration of previous DMD (months)	34±31.2	58.5±33.7	77.3±36.9	0.056
Time from last DMD (months)	na	na	31.5 (9-51)	0.062
Current DMD duration	46 (9-175)	34 (2-146)	na	0.120
Number of relapses before last (ongoing) treatment	2 (1-10)	2 (1-26)	na	0.785
ARR before last (ongoing) treatment	0.9 (0.1-9.0)	0.5 (0.1-4.1)	na	0.359
Number of relapses during ongoing treatment	0 (0-3)	0 (0-2)	na	0.210
ARR during ongoing treatment	0.0 (0.0-1.2)	0.0 (0.0-0.3)	na	0.103
ARR worsening during ongoing treatment	2 (6.5%)	0 (0%)	na	0.247
EDSS before last (ongoing) treatment	1.5 (0-3)	1.5 (0-6)	na	0.364
EDSS worsening (≥1) during ongoing treatment	3 (9.7%)	3 (15%)	na	0.565
EDSS or ARR worsening during ongoing treatment	5 (16.1%)	3 (15%)	na	0.914
EDSS improving (≥1) during ongoing treatment	2 (6.5%)	3 (15%)	na	0.139

Table 1. Clinical characteristics of the enrolled patients. Mean ± standard deviation or median (range); * Chi-square test or Kruskal-Wallis test, where appropriate. ARR: annual relapse rate; EDSS: Expanded Disability Status Scale; DMD: disease modifying drug; OCBs: oligoclonal bands; na: not available; ns: not significant.

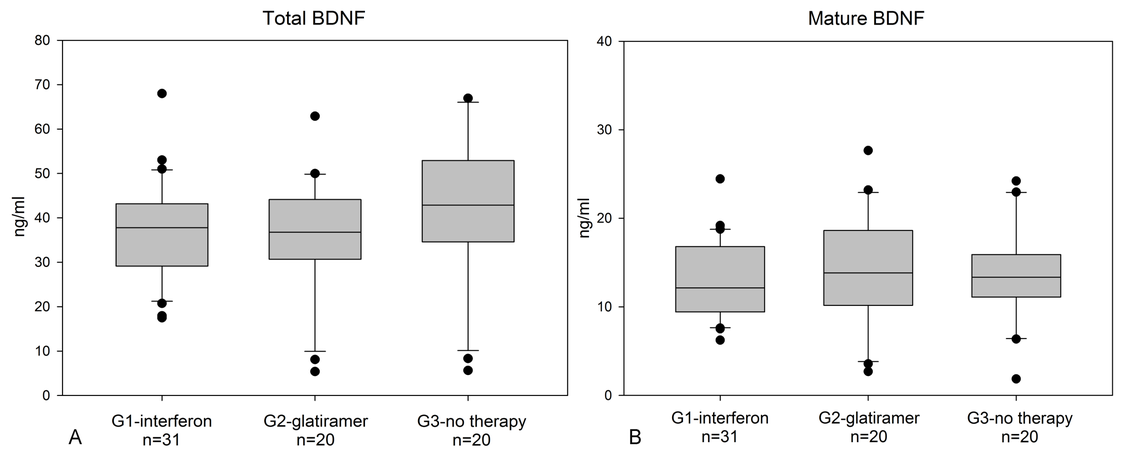

Figure 1. Total and mature BDNF serum level measured by ELISA assay. A) Box plot of total BDNF serum concentration (ng/ml) in healthy controls and RR-MS patients with different therapy: Group 1 (G1) interferon, Group 2 (G2) glatiramer acetate and Group 3 (G3) no-therapy groups. The upper line of the box marks the75th percentile, the middle line is the median value and the lower line specifies the 25th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles, respectively. Dots indicate the outlier values within each group. B) Box plot of mature BDNF serum concentration (ng/ml), as described in panel A. n = number of patients in each group.

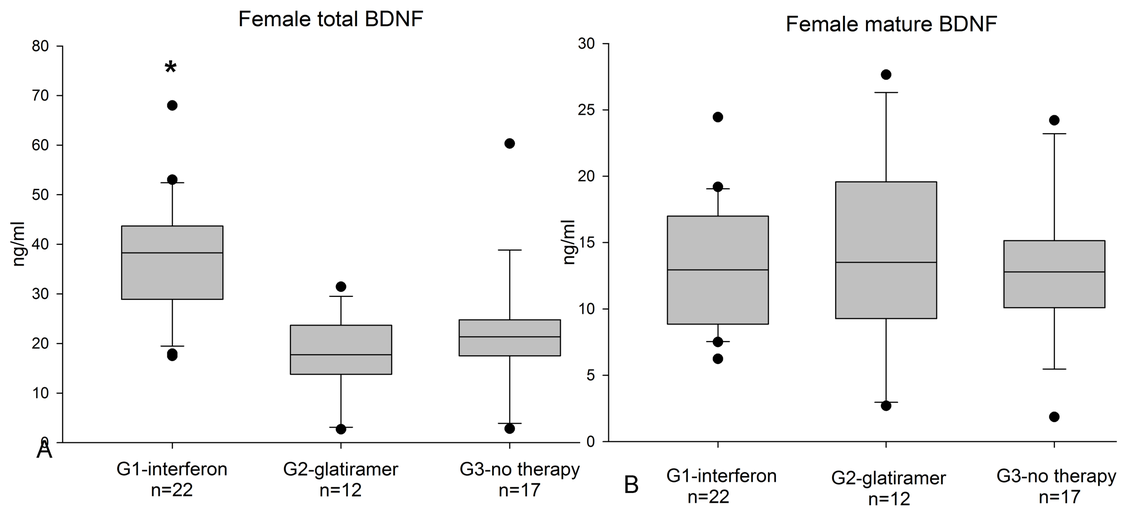

Figure 2. Genders-specific BDNF serum levels measured by ELISA assay in female patients. A) Box plot of total BDNF serum concentration (ng/ml) in healthy controls and RR-MS female patients with different therapy: Group 1 (G1) interferon, Group 2 (G2) glatiramer acetate and Group 3 (G3) no-therapy groups. Box plot are defined as in figure 1. * = $P<0.05$ vs G3 and G2. B) Box plot of mature BDNF serum concentration (ng/ml) in female patients as described in panel A.

RESULTS

71 sera (20 RR-MS patients treated with GA, 31 with IFN-β and 20 patients without any specific treatment) have been analysed. The characteristics of the patients are reported in Table 1. All the clinical and paraclinical variables were well-balanced in the groups, with no significant differences in terms of of disease duration, annual relapse rate (ARR), type of disease onset, CSF profile.
We did non find any difference between groups in terms of total BDNF levels (Median G1=37.79 ng/ml, Q1-Q3=29.42-43.18, Median G2=36.76 ng/ml, Q1-Q3=30.79-43.80, Median G3=42.88 ng/ml, Q1-Q3= 35.02-52.26; p=0.22; see Fig. 1A) and mature BDNF levels (Median G1=12.14 ng/ml, Q1-Q3= 9.52-16.40, Median G2=13.82 ng/ml, Q1-Q3= 10.37-18.58, Median G3=13.35 ng/ml, Q1-Q3= 11.50-15.48; p=0.66; see Fig. 1B).
We observed a statistical difference in female patients between the interferon group with respect to glatiramer acetate and no-therapy groups ($P<0.05$; G1 vs G2 and g1 vs G3; see figure 2A). In particular, we found significantly increased circulating levels of total BDNF in female subjects treated with interferon (Median G1=38.28 ng/ml, Q1-Q3=29.13-43.63, Median G2=17.73 ng/ml, Q1-Q3=14.36-22.98, Median G3=21.32 ng/ml, Q1-Q3= 17.72-24.41; $P<0.05$). This difference was not detectable in mature BDNF levels (Median G1=12.95 ng/ml, Q1-Q3=8.98-16.80, Median G2=13.50 ng/ml, Q1-Q3=9.77-19.21, Median G3=12.78 ng/ml, Q1-Q3= 10.39-14.55; see Fig. 2B).

DISCUSSION and CONCLUSIONS

In this study, we found that chronic treatment with interferon-beta is associated with higher levels of circulating total BDNF in RR-MS female patients. This effect was not seen for mature-BDNF, for male patients, when the entire population of RR-MS patients was analysed (males + females), or in GA treated patients (irrespective of the BDNF form or the sample population considered).
Our results confirm a recent study showing that INF-beta can increase serum BDNF levels and a previous study demonstrating an increased production of BDNF by peripheral blood mononuclear cells following stimulation with INF-beta. GA effect on BDNF levels is discussed, with some studies reporting increased serum levels or no effect. Finally, we are the first to describe a gender specific effect of INF-beta on serum BDNF levels. To interpret this finding, we report a previous observation that in experimental autoimmune encephalomyelitis (EAE) a gender difference in expression of molecules potentially related to myelin damage and repair has been described.
In conclusion, our study offers a new insight in the already explored field of BDNF-mediated neuroprotection, that could be influenced by the currently used MS treatments. Moreover, we observed a gender-specific role of IFN-β in terms of BDNF levels in MS patients. This observation needs additional studies on larger populations and further investigations in order to determine the possible mechanism of such gender difference in drug responsiveness.

REFERENCES

Azoulay D, Vachapova V, Shihman B, et al. Lower brain-derived neurotrophic factor in serum of relapsing remitting MS: reversal by glatiramer acetate. J Neuroimmunol. 2005 Oct;167(1-2):215-8.
Ehling R, Di Pauli F, Lackner P, et al. Impact of glatiramer acetate on paraclinical markers of neuroprotection in multiple sclerosis: A prospective observational clinical trial. J Neuroimmunol. 2015 Oct 15;287:98-105.
Kerschensteiner M, Gallmeier E, Behrens L, et al. Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? J Exp Med. 1999 Mar 1;189(5):865-70.
Lalive PH, Kantengwa S, Benkhoucha M, et al. Interferon-beta induces brain-derived neurotrophic factor in peripheral blood mononuclear cells of multiple sclerosis patients. J Neuroimmunol. 2008 Jul 15;197(2):147-51.
Massella A, D'Intino G, Fernández M, et al. Gender effect on neurodegeneration and myelin markers in an animal model for multiple sclerosis. BMC Neurosci. 2012 Jan 24;13:12.
Mehrpour M, Akhoundi FH, Delgosha M, et al. Increased Serum Brain-derived Neurotrophic Factor in Multiple Sclerosis Patients on Interferon-β and Its Impact on Functional Abilities. Neurologist. 2015 Oct;20(4):57-60.
Polacchini A, Metelli G, Francavilla R, et al. A method for reproducible measurements of serum BDNF: comparison of the performance of six commercial assays. Sci Rep. 2015 Dec 10;5:17989.
Tongiorgi E, Sartori A, Baj G, et al. Altered serum content of brain-derived neurotrophic factor isoforms in multiple sclerosis. J Neurol Sci. 2012 Sep 15;320(1-2):161-5.

DISCLOSURES

A. Sartori has received funding for travel and/or speaker honoraria from Teva, Merck-Serono, Novartis, Genzyme and Biogen. G. Metelli: nothing to disclose. A. Polacchini: nothing to disclose. A. Bratina has received funding for travel and/or speaker honoraria from Teva, Novartis, Almirall and Genzyme. P. Manganotti: nothing to disclose. E. Tongiorgi: nothing to disclose.

XLVII CONGRESSO
SOCIETÀ ITALIANADI NEUROLOGIA
Venezia, 22-25 Ottobre 2016