BDNF and pro-BDNF in serum and exosomes in major depression: Evolution after antidepressant treatment

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Abstract

Background: The study of clinically related biological indicators in Major Depression (MD) is important. The Brain Derived Neurotrophic Factor (BDNF) appears to play an important role in MD, through its neurotrophic effect, and its levels are significantly decreased. The variation in the serum levels of its precursor proBDNF, which has opposite effects, is not known. Their distribution between serum and exosomes and their evolution during antidepressant treatment is also not known, and may be important in modulating their effects.

The aim of this study is to evaluate whether serum and exosome mBDNF and proBDNF levels are altered in patients with MD during antidepressant treatment compared to controls, and their association with clinical improvement and clinical variables.

Materials and Methods: 42 MD subjects and 40 controls were included. Questionnaires to assess the severity of depression and cognitive impairment and blood samples were collected during the three visits at D0 (inclusion) and 3 and 7 weeks after the start of antidepressant treatment. Assays for mBDNF and proBDNF levels were performed in serum and exosomes by ELISA.

Results: MD subjects had decreased serum and exosomal BDNF levels and increased proBDNF levels at D0 compared to controls. BDNF and pro-BDNF vary in an inverse manner in both serum and exosomes during antidepressant treatment. No relationship of BDNF and proBDNF levels to clinical improvement and depression scales was found.

Conclusion : We demonstrated an evolution of those molecules either in serum or in exosomes after MD treatment. These transport vesicles could have a role in the regulation of BDNF.

Keywords: depression, exosomes, BDNF, pro-BDNF, antidepressants

1. Introduction

Despite the diversity of available therapeutic approaches, a significant proportion of patients with major depression (MD) do not respond to conventional antidepressant treatments. Less than a third of patients experience complete remission of depressive symptoms after treatment (Rush et al., 2006). Almost 50% do not show improvement when trying a given pharmacological antidepressant and 20% do not respond to any intervention (Labermaier et al., 2013, Warden et al., 2007). The optimization of treatment requires an understanding of the mechanisms of the disease and the study of biological indicators that allow its objectivation in relation to its clinical characteristics.

A reservoir of new biomarkers for human diseases is represented by the proteomic profiles of exosomes. Exosomes mediate intercellular communication and have been identified in various bodily fluids. They consist of 30- to 150-nm membrane-bound vesicles derived from almost all types of cells and contain proteins, lipids, and nucleic acids (Kourembanas et al., 2015). They are currently considered to be among the most complex and physiologically relevant of all messengers. Recent studies suggest that exosomes secreted by neurons and glia play a key role in intercellular communication by transporting messenger RNA (mRNA), microRNA (miRNA), proteins, and lipids between cells, thus modifying the functioning of receptor cells (Pegtel et al., 2014). Their role appears to be determinant in central nervous system (CNS) diseases (Shairmadanova et al., 2020). Exosomes are derived from multivesicular cell bodies, allow the distant release of certain molecules (Izadpanah et al., 2018), and bidirectional communication between the periphery and brain. They represent a major source of biomarkers for neurodegenerative and psychiatric disorders, including addiction (Saeedi et al., 2019, Pegtel et al., 2014, Pinet et al., 2016, Wilson et al., 2015).

Exosomes are also recognized to play a role in the pathogenesis of depression, in addition to their potential as biomarkers and therapeutic vehicles (Buzas et al., 2014; Tavakolizadeh et al., 2018).

Data from neuroimaging, neurophysiology, and cellular and molecular biology have associated depression with various neurobiological phenomena, in particular the dysfunction of neuroplasticity (Lecrubier et al., 2007, Zhou et al., 2013), underpinning the neurotrophic hypothesis of depression (Buttenschon et al., 2015; Frank et al., 1999).

Thus, in recent decades, the role of one pivotal molecule involved in such mechanisms, brain-derived neurotrophic factor (BDNF), has received considerable attention, especially in the study of MD. BDNF is an extracellular signaling protein known for its predominant role in the development of the nervous system, such as neurogenesis, neuroprotection, neurodegeneration (Mattson et al., 2004), synaptic plasticity (Murray et al., 2011), and resistance to neuronal stress (Marosi et al., 2014). Its role is essential in learning and memory mechanisms (Bekinschtein et al., 2008). In MD, a decrease in signaling linked to changes in neurotrophin synthesis is observed, leading to alterations in neurogenesis and brain plasticity of limbic and prefrontal structures. Antidepressant treatments that result in clinical improvement act by modulating brain plasticity (Harmer et al., 2017), and BDNF is thought to mediate these effect (Mendez-David et al., 2013; Björkolm and Monteggia, 2016), as it increases its synthesis (Bégni et al., 2017).

BDNF is mainly expressed in the CNS (Binder et al., 2004) but is also present in the periphery (serum, plasma, lymphocytes) (Yamamoto et al, 1990). It is also associated with drug use disorders (Koskela et al., 2017), many systemic diseases (diabetes, cancer, cardiovascular risk, etc.), and homeostatic regulation of the body,.It is synthesized after proteolytic cleavage of its precursor, pro-BDNF (Koshimizu et al., 2009). Several studies

have shown that pro-BDNF and mature BDNF have opposite effects (Binder et al., 2004, De Vincenti et al., 2019) and play an important role in several physiological functions. BDNF binds mainly to tropomyosin kinase B receptors (TrkB) (Noble et al., 2011) (Klein et al., 1991), which have anti-apoptotic properties and inhibit depression in the long-term (Pang et al., 2004) (Teng et al., 2005), whereas pro-BDNF preferentially binds to the $p75^{NTR}$ receptor and promotes neuronal apoptosis and long-term depression (Woo et al., 2005). Reduced pro-BDNF levels in the cerebro-spinal fluid have been associated to MD (Mizui et al., 2019). In addition, the balance between the pro-BDNF/$p75^{NTR}$ and BDNF/TrkB signaling pathways appears to be deregulated in MD (Jiang et al., 2017; Mizui et al., 2019). The BDNF/pro-BDNF ratio thus appears to be perturbed and may play a major role during antidepressant treatments (Diniz et al., 2018).

According to a study carried out on elderly subjects with walking difficulties (Suire et al., 2017), the concentrations of mature BDNF and pro-BDNF differ between serum and exosomes, with pro-BDNF levels being higher in vesicles of neuronal origin (Suire et al. 2017). It has been suggested that BDNF is transported in exosomes to be released in a more prolonged and specific manner in the CNS (Yuan et al., 2017). The BDNF/pro-BDNF ratio at the periphery and CNS is assumed to be determinant in psychiatric disorders, as is the existence of an essential brain-liver axis (Yang et al., 2017). BDNF, the TrkB receptor, and its associated molecule sortilin are present in the free circulating form in serum, but also in exosomes, allowing longer-acting release to more distant targets, even the CNS (Yuan et al., 2018, Wilson et al., 2014, 2015).

BDNF and pro-BDNF could help in monitoring the evolution of MD modulated by their differential presence in the serum and exosomes during treatment. The evolution of their levels during anti-depressant treatment is not well-known, nor their evolution in exosomal

vesicles. A better knowledge of this repartition may be important for modulating the effects of the two molecules between the periphery and brain.

We aimed to evaluate their potential contribution to patient management by determining their levels in the serum and exosomal compartments and their relationship with certain clinical characteristics of MD (depression intensity, cognitive complaints). We therefore measured BDNF and pro-BDNF levels in the serum and exosomes of MD subjects at the start of antidepressant therapy and 3 and 7 weeks later and studied the evolution of the ratio of the two proteins. The basal levels were compared to those of control subjects (without a psychiatric disease). The relationships of the BDNF and pro-BDNF levels with certain clinical characteristics of MD (depression intensity, cognitive complaints) were also studied.

2. Materials and Methods

2.1. Population

2.1.1. MD group

The study received ethical approval by a Committee for the Protection of the Persons in respect to the French regulation. It was proposed to patients hospitalized for MD, as diagnosed by their referent psychiatrist according to the DSM-5 criteria, during the first week of the initiation of their antidepressant treatment (either an antidepressant drug or repeated transcranial magnetic stimulation (rTMS)). The molecules used were escitalopram 10 mg/day (n = 3), sertraline 50 mg/day (n = 9) or 100 mg/day (n = 3), fluoxetine (40 mg/day (n = 1) or 20 mg/day (n = 1), venlafaxine 75 mg/day (n = 5) or 150 mg/day (n = 150), paroxetine 20 mg/day (n = 20), vortioxetine 5 mg/day (n = 1), agomelatine 25 mg/day (n = 1), phenilzine 15 mg/day (n = 1), and clomipramine 75 mg/day (n = 1). Participants were free of any declared

or symptomatic somatic diseases (cancer, diabetes, colds, etc.) and had a Hamilton depression rating scale (HDRS) score > 16.

2.1.2. Control group

Control participants were recruited from blood donors who were free of antidepressant treatment. They agreed to participate in the study and signed an informed consent form. Whole blood (5 mL) was collected just before blood donation and was immediately centrifuged to collect serum. An agreement was made with the French Establishment of Blood Transfusion to test the donor blood samples for pathogens and pathological conditions after the samples were centrifuged.

The participants of both groups were between 18 and 65 years of age. Exclusion criteria included pregnancy, judiciary or social protection, the absence of health insurance, and participation in another clinical assay.

2.2. Follow-up and clinical assessments

At inclusion, socio-demographic data (gender, age, study level, employment status, substance use (tobacco, cannabis, alcohol), and body mass index (BMI)) were collected for both groups. Data on disease duration, potential psychiatric co-morbidities, therapeutic treatment, and previous antidepressant treatment characteristics, if any, were collected for the MD group. The severity of depression was evaluated using the HDRS (Hamilton, 1960, 1967; Guelfi, 1996) hetero-questionnaire and the Beck Depression Inventory BDI (Beck, 1988) auto-questionnaire. Only the BDI was administered to the control group for ethical reasons. A very good correlation between BDI and HDRS scores has already been demonstrated (Milak et al., 2010). The perceived deficits questionnaire was completed by both groups: this auto-questionnaire evaluates the frequency of perceived cognitive difficulties encountered by the participants using five questions (Fehnel et al., 2016). The evaluations were repeated at the

third (W3) and seventh weeks (W7) after the initiation of antidepressant treatment of the participants with MD. A clinical improvement in response to treatment was defined as an HDRS score at follow-up < 50% of the basal HDRS score at inclusion (W0).Serum was separated from total blood after centrifugation (5 minutes, 300g) and stored at -80°C until use.

2.3. Exosome isolation

To obtain comparable measures of markers in exosomes and serum, we resuspended the pellet after exosome extraction with the same volume as the initial serum used for extraction, so that the exosome concentration after extraction was the same as the concentration in serum before extraction. The exosomes extraction was realized in the same manner for all the samples, and for each patient at the same time (W0, W3 and W7), to discard the risk of different yield in extraction and then measurement.. Exosomes were isolated from 120 µL serum using the Total Exosomes Isolation kit (Invitrogen) according to the manufacturer's instructions. The final pellet was resuspended in 100 µL Cell Lysis Buffer (Cell signaling) in the presence of proteases inhibitors and stored at -20°C until use.

Total protein content of the serum was also measured with Micro BCA Protein Assay Kit (Pierce, Thermo Scientific, Rockford, IL, USA) according to manufacturer's instructions with BSA as standard. Exosomes pellets were resuspended after extraction in Phosphate Buffer Saline containing 0.25% Triton X-100 (Fluka, Sigma-Aldrich).

2.4. BDNF and pro-BDNF measurements

BDNF (1/50 dilution) and pro-BDNF levels (1/4 dilution) were measured in exosomes and serum by ELISA (Adipo Bioscience, Santa Clara, California, United-States) according to the manufacturer's instructions. The BDNF/pro-BDNF ratio was then calculated.

2.5. Statistical analyses

The minimal sample size necessary to evidence a difference between clinical improvement groups according to the data already available about variations in serum BDNF levels, according to different studies in depressed patients with or without treatment compared to controls (Shimizu et al., 2014), and especially to our experience with these assays (Nubukpo et al., 2011, 2017, 2020), was evaluated : the basis of an alpha risk of 5%, a beta risk of 20%, a number of 18 evaluable subjects in each comparison group (improvement versus non-improvement) was required. Considering a proportion of 10% of subjects lost to follow-up or whose records will not be usable, a minimum of 21 subjects had to be included in each comparison group (with or without clinical improvement). We thus included and used the data of the first 42 subjects fitting the criteria.

The results are handled as exploratory thus without correction of type I error (Jouan-Flahault et al., 2004). Quantitative variables are described using mediane (± SD) and comparisons between groups were carried out using the Mann-Whitney U test, due to the non normal distribution of the variables. The paired samples Wilcoxon test was used to compare data in the MD group from a follow-up with the previous one (W3 to W0, W7 to W3). However, as the ANOVA tests are generally robust to the hypothesis of normality, considering the Central Limit Theorem, with a correct sample size (n = 30) and may be applied to not exactly normal data (Santiago, 2015; Vaudor, 2015), we used ANOVA in repeated measures to explore the evolution of our data along time.

Qualitative variables are described using frequencies and percentages, and the distribution between groups was analyzed by the Chi^2 test. The Spearman correlation coefficient was used to assess the correlation between BDNF levels and depression scales. ANOVA of repeated measures was used to explore the evolution of the BDNF and pro-BDNF levels and the BDNF/pro-BDNF ratio over time, with clinical improvement as the independent variable.

Statistical analyses were performed using SPSS software (22.0) and $p < 0.05$ was considered statistically significance.

3. Results

3.1. Study population

Forty-two participants were included in each group. The mean age was 42.2 ± 10.8 years but differed between the control and MD groups. The two groups also differed in terms of tobacco use, with more smokers in the MD group.

Table 1. Characteristics of the entire population at inclusion (W0) and of the MD and control groups.

Median [interquartiles] or n (%)	Total	MD group (n = 42)	Control group (n = 42)	p
Age (years)	42 [34;50]	46 [37; 52]	33 [40; 47]	0.017
Male/female	20/65	10/32	10/32	> 0.999
BMI (kg/m²)	25.25 [22.40; 28.68]	25.35 [21.60; 28.04]	25.15 [23.29; 28.70]	0.3768
smokers/non smokers	27 (57)	18 (24)	9 / 33	0.048
Age at first MD diagnosis		36 [26; 49]		
Duration of current MD episode		6 [2; 12]		
Number of previous MD episodes		2 [0; 7]		
Time since first AD		4 [0; 12]		
BDI	3 [0; 8]	18 [15; 27]	0 [0; 1]	< 0.001
QDP	4 [1; 12]	12 [7; 15]	1 [1; 2]	< 0.001

Current treatment:	
Antidepressant	100 (42)
Anxiolytic	83.3 (35)
Antiepileptic	19 (8)
Antalgic	38.1 (16)
Anti-inflammatory drug	11.9 (5)
Neuroleptics	35.7 (15)
Repeated transcranial magnetic stimulation	21.4 (9)

p : p-value for a difference between control and MD groups with the Mann-Whitney-test

The BDI, and QDP scores were significantly lower in the control than MD group (Table 1), validating the specificity of the depression intensity and cognitive complaints in the MD group. We then followed the evolution of the psychometric characteristics of the MD group during the seven weeks of antidepressant treatment (Table 2). The mean HDRS scores decreased markedly during the antidepressant treatment, as did the BDI and QDP scores, showing that the mean depressive state in the MD group improved. Twenty subjects in the MD group (47.6%) showed clinical improvement after seven weeks of treatment.

Table 2. Evolution of the psychometric scale scores at inclusion for the MD group during the seven weeks of treatment.

	MD group (n = 42)	p
BDI		
W0	18.0 [15.0; 27.0]	

W3	14.5 [8.0; 20.0]	<0.0001[a]
W7	10.0 [3.0; 16.0]	0.0030[b]
QDP		
W0	12.0 [7.0; 15.0]	
W3	10.5 [3.0; 14.0]	0.1714[a]
W7	5.0 [3.0; 12.0]	0.0040[b]
HDRS		
W0	22.0 [20.0; 23.0]	
W3	14.5 [9.0; 18.0]	<0.0001[a]
W7	10.5 [5.0; 15.0]	0.0114[b]

p : p-value (Wilcoxon test) for a difference: [a] between W0 and W3, [b] between W3 and W7

3.2. BDNF and pro-BDNF levels at the initiation of antidepressant treatment and their evolution during treatment

We next evaluated the evolution of the BDNF/pro-BNDF levels during treatment and their correlation with clinical improvement (Table 3). BDNF levels were significantly lower in the MD than control group, both in the serum and exosomes, at W0 and W3. They did not differ from those of controls at W7. On the contrary, pro-BDNF levels were higher in the serum and exosomes in the MD than control group and decreased during treatment. The difference between the levels in the MD group and reference levels in the controls was no longer significant at W7. Indeed, during treatment, the BDNF levels increased in parallel in the serum and exosomes, whereas the pro-BDNF levels in the serum and exosomes decreased in a similar manner.

Table 3. BDNF levels, pro-BDNF levels (mg/mL) , and the BDNF/pro BDNF ratio in the serum and exosomes at W0, W3, and W7 of the MD and control groups

(mg/mL)	MD group	Control group	p-value for a difference between MD and control groups*	p-value for a difference with the previous follow-up**
Serum				
BDNF				
W0	30.7 ± 4.9	38.7 ± 2.1	< 0.0001	
W3	34.4 ± 4.4		< 0.0001	<0.0001 [a]
W7	37.7 ± 5.6		0.9408	0.0098 [b]
Pro-BDNF				
W0	11.9 ± 6.1	8.3 ± 2.5	< 0.0008	
W3	10.8 ± 5.1		0.0098	0.0003 [a]
W7	9.2 ± 4.9		0.6494	<0.0001 [b]
Exosomes				
BDNF				
W0	6.0 ± 3.2	7.95 ± 1.5	< 0.0001	
W3	6.6 ± 3.7		0.0018	<0.0001 [a]
W7	8.3 ± 4.4		0.9482	0.0014 [b]
Pro-BDNF				
W0	6.9 ± 2.7	4.4 ± 1.4	< 0.0001	
W3	6.8 ± 4.2		0.0004	0.0003 [a]
W7	6.8 ± 6.7		0.2557	0.0015 [b]

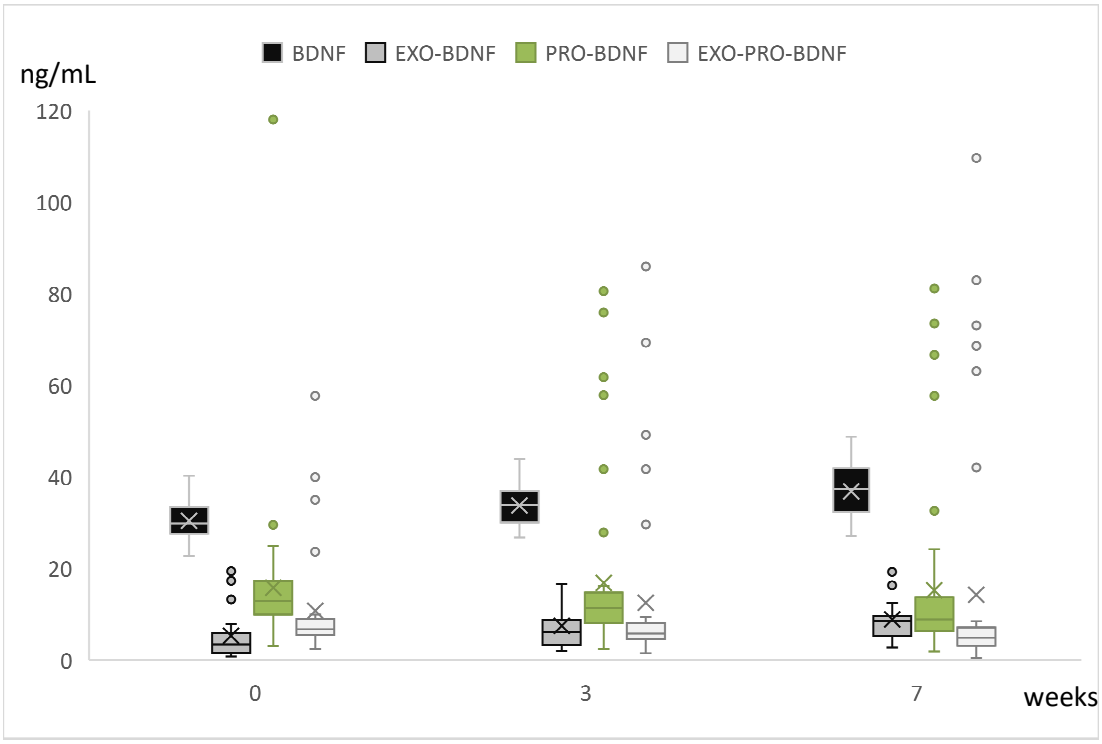
BDNF/pro-BDNF				
In serum	3.2 ± 1.8	6.0 ± 6.0	< 0.0001	
W0	5.1 ± 4.8		0.0009	<0.0001 [a]
W3	6.2 ± 5.3		0.7314	<0.0001 [b]
W7				
In exosomes	0.6 ± 0.5	2.1 ± 1.1	< 0.0001	
W0	1.2 ± 0.80		< 0.0001	<0.0001 [a]
W3	2.1 ± 1.8		0.1169	<0.0001 [b]
W7				

*. Mann-Whitney test

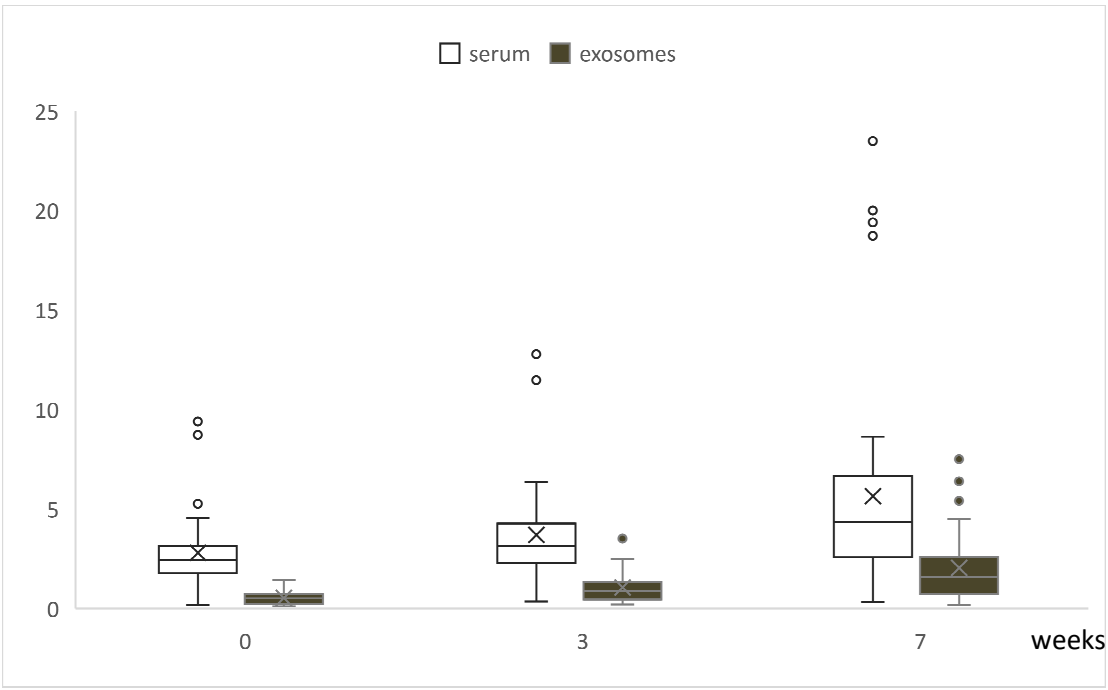
**. Wilcoxon test, [a] between W0 and W3, [b] between W3 and W7

Figure 1. BDNF and pro-BDNF levels (a) and the BDNF/pro-BDNF ratio (b) at W0, W3, and W7 in the serum (BDNF, PRO-BDNF) and exosomes (EXO-BDNF, EXO-PRO-BDNF) from subjects with MD receiving antidepressant treatment at W0.

a.



b.



3.3. BDNF/pro-BDNF ratio in serum and exosomes

At the initiation of antidepressant treatment, the BDNF/pro-BDNF ratio was higher in the control than MD group, both in the serum (2-fold higher) and exosomes (4-fold higher). The ratio was higher in the serum than exosomes at all follow-ups (Table 3).

The BDNF levels were three-fold higher than those of pro-BDNF in the serum at the start of treatment, whereas they were half of those of pro-BDNF in the exosomes. The ratios markedly increased with treatment: they doubled in the serum and tripled in the exosomes between W0 and W7. Indeed, at W7, the ratios in the serum and exosomes in the MD group no longer differed from those in the control groups (Table 3; Figure 1).

3.4. Relationship of BDNF and pro-BDNF levels with psychometric scales and clinical improvement

The overall evolution of BDNF levels over time was significant ($F = 26.522$, $p < 0.0001$) and showed a trend towards varying as a function of clinical improvement ($F = 3.908$, $p = 0.0552$), but there was no significant interaction between time and clinical improvement ($F = 0.493$, $p = 0.6128$).

Pro-BDNF levels changed over time ($F = 10.098$, $p < 0.0001$) but not in relation to clinical improvement ($F = 0.013$, $p = 0.9084$) and the interaction between time and clinical improvement was not significant ($F = 0.058$, $p = 0.9434$).

The BDNF/pro-BDNF ratio in exosomes showed a trend towards varying as a function of clinical improvement ($F = 3.600$, $p = 0.0649$) but the interaction with time was not significant ($F = 1.510$, $p = 0.2279$). There was no association between the BDNF/pro-BDNF ratio in serum and clinical improvement ($F = 0.714$, $p = 0.4032$) and the interaction with time was not significant ($F = 0.721$, $p = 0.4893$).

There were no evident differences in BDNF or pro-BDNF levels depending on the class of drug for treatment, on receiving rTMS or chemicals as antidepressants treatments, tobacco

use, or age at inclusion (Table 4). There were also no correlations between BDNF or pro-BDNF levels and age or psychometric scale scores for any time points (Table 5), except between age and exosomal pro-BDNF, and BDI and serum pro-BDNF at W0, but without relevant correlation coefficients and weak associated p-values.

Table 4. BDNF and pro-BDNF levels (mg/mL) either in exosomes and serum according to the drug used in treatment, rTMS treatment, tobacco use at W0.

(mg/mL)

Drug used in treatment		absence	presence	p
Anxiolytic	BDNF	29.02 [28.03 ; 33.53]	29.9 [27.15 ; 33.31	0.8264
	Exo-BDNF	5.72 [2.7 ; 14.9]	2.99 [1.48 ; 4.99]	0.2582
	Pro-BDNF	13.64 [7.36 ; 20.3]	12.91 [10.41 ;	0.7872
	Exo-Pro-BDNF	8.6 [6.49 ; 20.4]	16.86]	0.1563
			6.68 [5.29 ; 23.64]	
Antalgic	BDNF	30.28 [25.60 ; 33.38]	29.63 [28.15 ;	0.5007
	Exo-BDNF	4.32 [1.95 ; 7.84]	34.00]	0.1780
	Pro-BDNF	14.35 [11.76 ; 18.12]	2.76 [1.25 ; 4.97]	0.0920
	Exo-Pro-BDNF	8.18 [5.60 ; 9.91]	10.45 [8.00 ; 13.72	0.0760
			6.37 [5.57 ; 7.56]	
Antiepileptic	BDNF	29.70 [26.79; 33.09]	32.04 [29.84 ;	0.3054
	Exo-BDNF	2.58 [1.34 ; 5.05]	34.89]	0.1406
	Pro-BDNF	12.94 [9.62 ; 17.22]	4.53 [3.62 ; 7.28]	0.8489
	Exo-Pro-BDNF	7.18 [5.45 ; 9.14]	14.21 [10.66 ;	0.4326
			17.18]	
			6.29 [5.63 ; 8.14]	
Anti-flammatory	BDNF	29.9 [28.37 ; 33.49]	26.95 [26.51 ;	0.4724
	Exo-BDNF	3.32 [1.56 ; 5.22]	31.89]	0.4724

	Pro-BDNF	12.96 [10.30 ; 17.24]	4.13 [2.41 ; 10.02]	0.4967
	Exo-Pro-BDNF	6.75 [5.41 ; 8.95]	12.23 [4.42 ; 17.23	0.9072
			6.68 [5.98 ; 15.57]	
Neuroleptics	BDNF	30.66 [27.51 ; 33.92]	29.34 [27.81 ;	0.4704
	Exo-BDNF	3.32 [1.33 ; 5.55]	32.56]	0.5905
	Pro-BDNF	13.33 [9.62 ; 17.42]	2.85 [1.72 ; 4.75]	0.4310
	Exo-Pro-BDNF	6.690 [5.49 ; 9.26]	11.73 [10.19 ;	0.8234
			13.98]	
			6.48 [4.34 ; 8.03]	
rTMS	BDNF	30.15 [27.77 ; 33.82]	29.84 [27.11 ;	0.7383
	Exo-BDNF	2.70 [1.43 ; 5.72]	31.87]	0.3655
	Pro-BDNF	12.76 [9.62 ; 15.05]	4.52 [2.85 ; 9.11]	0.4194
	Exo-Pro-BDNF	7.18 [5.60 ; 8.88]	15.29 [10.82 ;	0.6163
			17.73]	
			6.254 [5.21 ; 15.62	
Tobacco use	BDNF	29.63 [26.49 ; 32.19]	31.70 [27.84 ;	0.2650
	Exo-BDNF	2.99 [1.21 ; 6.54]	33.85]	0.2542
	Pro-BDNF	13.33 [9.62 ; 16.53]	4.53 [2.17 ; 5.00]	0.9796
	Exo-Pro-BDNF	6.45 [5.52 ; 9.23] [	12.6 [10.3 ; 17.83]	0.6818
			8.14 [5.41 ; 8.73]	

p : p-value for a difference between the presence and the absence of the studied parameter with the Mann-Whitney test.

Table 5. Spearman correlation tests (rho, p-value) between BDNF and pro-BDNF levels, in serum and exosomes, with psychometric scales scores and age at W0, W3 and W7 in the MD group.

		W0				W3			W7		
Rho p-value		age	BDI	QDP	HDRS	BDI	QDP	HDRS	BDI	QDP	HDRS
se ru	BDNF	-0.21	0.094	-0.06	0.10	-0.13	0.11	-0.12	0.01	0.08	-0.14

		0.0531	0.5476	0.6978	0.5240	0.3877	0.4915	0.4442	0.9345	0.5950	0.3716
	Pro-	-0.02	0.340	0.138	-0.00	0.06	0.13	0.2	-0.07	-0.02	0.05
	BDNF	0.8339	0.0297	0.3761	0.9892	0.7188	0.4094	0.1949	0.6722	0.8978	0.7499
exosomes	BDNF	-0.21	0.281	0.96	0.117	-0.02	-0.09	0.1	-0.07	0.00	-0.14
		0.0609	0.0720	0.3393	0.4550	0.8890	0.5453	0.5109	0.6581	0.9988	0.3644
	Pro-	0.25	0.062	0.150	-0.86	-0.06	0.011	0.11	0.03	-0.04	0.02
	BDNF	0.0254	0.6937	0.3376	0.5838	0.6918	0.9435	0.4985	0.8588	0.8093	0.9120

Finally, the total protein levels along the antidepressant treatment was stable in the MD group either in serum ($F = 0.131$, $p = 0.8777$) or exosomes ($F = 0.13$; $p = 0.8761$). Total proteins levels in the MD group did not vary differentially along time with the total protein levels of controls as a reference in serum ($F = 0.145$, $p = 0.8648$) and exosomes ($F = 0.15$; $p = 0.8630$).

4. Discussion

Here, we show that 1) the levels of BDNF and pro-BDNF differ from those of controls at the beginning of an antidepressant treatment but not after seven weeks of treatment, 2) BDNF and pro-BDNF levels change inversely in both serum and exosomes during antidepressant treatment, and 3) these changes are not associated with clinical improvement, defined as a > 50% decrease in basal HDRS scores or depression intensity (Brakemeier et al., 2007) after seven weeks treatment.

The role of the BDNF signaling pathway in the neurophysiological mechanisms of depression is well documented, as is its role as a mechanism of action for antidepressants (Hashimoto, 2010; Zhang et al., 2016). Several studies have shown decreased serum BDNF and pro-BDNF levels in depressed subjects, indicating a modification of the BDNF pathway in depression (Molendjik et al., 2014; Sen et al., 2008) and its restoration with antidepressant treatment. Our results are thus similar to those of Jiang et al. (2017), who reported lower levels of BDNF in the serum of subjects with MD than in controls, as well as a lower BDNF/pro-BDNF ratio.

The levels of BDNF/pro-BDNF increased after seven weeks of antidepressant treatment and were similar to those of controls. Changes in the BDNF/pro-BDNF ratio have also been observed in cerebrospinal fluid (Mizui et al., 2019) in depression. The observed inverse changes also suggest that the neurotrophic effect of BDNF probably becomes more important than the pro-apoptotic effect of pro-BDNF. After secretion, pro-BDNF can be cleaved and locally delivered as mature BDNF, for example, influencing long-term plasticity in the CNS (Pang et al., 2004).

Changes of the levels of the two molecules in opposite directions may represent reactivation of the cleavage pathway with antidepressant treatment. The BDNF/pro-BDNF ratio increases during antidepressant treatment. It was not possible to assess the equivalence between the disappearance of pro-BDNF molecules and the appearance of BDNF in the serum by the techniques used, as the levels of the two proteins cannot be directly compared. However, it is likely that the BDNF measured in serum also came from sources other than serum pro-BDNF (endothelial cells, peripheral blood mononuclear cells, etc.).

The inverse difference between pro-BDNF levels and those of mature BDNF in depression relative to controls probably indicates a change in the pro-BDNF to mature BDNF cleavage pathway in depression (Zhou et al., 2013). Yoshida et al. (2012) did not observe lower pro-BDNF levels in subjects with MD, in contrast to mature BDNF levels. They observed no correlation between the levels of any neurotrophin and identified clinical variables of depressive intensity or cognitive abilities. In contrast, they observed a difference in the levels of matrix metalloproteinase-9 MMP9, which plays a role in the conversion of pro-BDNF to mature BDNF, associated with the intensity of depression. Other molecules associated with the BDNF signaling pathway have also been implicated in the mechanisms of action of antidepressants. Dysfunction in the regulation of the transformation of pro-neurotrophins into

mature neurotrophins by enzymes and regulators may be associated with cell-death mechanisms (Costa et al., 2017).

It has been suggested that BDNF and pro-BDNF, combined with tPA, PAI-1, $p75^{NTR}$, and TrkB, may be a marker of depression (Chen et al., 2017). In mice, the effect of antidepressants has been shown to be associated with an imbalance between the proBDNF/$p75^{NTR}$/sortilin and BDNF/TrkB pathways (Lin et al., 2019).

We did not find any studies reporting a change in exosome contents, nor their cargo of BDNF and pro-BDNF, during antidepressant treatment. Here, we show the same evolution of the levels of BDNF and its precursor in the exosomes as in the serum: lower levels of BDNF in MD subjects, which recovers with antidepressant treatment, and a BDNF/pro-BDNF ratio that reverses to normal values. The value of the BDNF/pro-BDNF ratio in exosomes was lower than that in serum and the change with antidepressant treatment was greater, suggesting different amounts of BDNF/pro-BDNF in the two cell compartments, probably because the sources of pro-BDNF to obtain BDNF are more restricted in exosomes.

We wondered if the changes in the measured BDNF and pro-BDNF levels could be in link with some potential total protein variations. In fact, several conditions may alter the quantity of the measured molecules in relation to the total protein levels, like treatment, but also age, sex, physical activity, circadian rythm… (Lacroix et al., 2012 ; Toth et al., 2007 ; Sossdorf et al., 2011 ; Madden et al., 2008). Thus, activation of cells may result in the activation of protein and micro vesicles secretion (Adhikari et al., 2018 ; Deng et al., 2017 ; Bianco et al., 2009). In fact, the absence of concomitent evolution of total protein content with the one of the measured markers in our results allows to verify that the changes in BDNF and pro-BDNF levels are not only following the changes in the potential protein synthesis induced by the antidepressant treatment. Thus, the consistency of the results is supported by the

consistency in the evolution of the measured BDNF and pro-BDNF levels along time, as of the BDNF/pro-BDNF ratio, whereas the protein levels did not evolve in the same way. The concomitant change in neurotrophin levels in exosomes and serum may indicate that common mechanisms are involved in both compartments. The source of the exosomes was not identified in our study. This may be important and the concentration in total vesicles may not reflect that of the vesicles originating from or destined for the brain: in subjects with cognitive decline and difficulty walking, Suire et al. (2017) did not observe a difference in pro-BDNF levels in plasma and exosomes between subjects with or without cognitive decline, whereas they observed a difference in vesicles of neuronal origin. It is possible that the mechanisms involved in the exosomes mirror those in the brain.

The content of the exosomes would make it possible to perpetuate the action of BDNF in the periphery or facilitate its delivery to the CNS. BDNF passes the blood-meningeal barrier poorly and has a low half-life in serum (Thoenen et al., 2002). It has been shown that exosomes from naïve macrophages may cross the blood-brain barrier to deliver certain cargo proteins to the brain, including BDNF (Yuan et al., 2017). Thus, peripheral exosomes from depressed subjects injected into mice can induce depressive-like behaviors in mice via the regulation of neurogenesis (Wei et al., 2020). Several inflammatory biomarkers of neuronal inflammation have been shown to be higher in neuron-derived exosomes from total blood cells of drug-free MD subjects than those from controls (Kuwano et al., 2018). Interestingly, the levels of sortilin, a molecule that participates in the transfer of exosomes (Wilson et al., 2014), is altered in the serum of MD subjects (Buttenschon et al., 2018; Devader et al., 2017; Mazella et al., 2019), and the levels of its pro-peptide increases upon treatment with electroconvulsive therapy (Borsotto et al., 2018). A marked imbalance between proBDNF/$p75^{NTR}$/sortilin and BDNF/TrkB production was shown in the pathogenesis of depression in a rodent chronic-stress model linked to depression.

The time at which the neurotrophin and pro-neurotrophin levels normalized in our study was seven weeks, consistent with the evaluable clinical expression of improvement in the disorder. We found no relationship between the collected data and the intensity of depression or clinical improvement, defined by an at least 50% decrease in the HDRS score from baseline. However, our study population size was small, and even though half of the subjects significantly improved, it may not have been sufficient to show a difference in clinical improvement.

It is possible that the antidepressant treatment itself may have driven the observed changes. Indeed, a change in neurotrophin and pro-neurotrophin levels and the ratio was observable from W3 and continued over the following four weeks. It is possible that antidepressant treatment influences the physiological mechanisms of the disorder without affecting the clinical signs that we measured. We therefore did not identify the clinical markers that may correlate with the change in the biological markers studied. Modification of the BDNF/pro-BDNF ratio has been observed in other diseases, suggesting the involvement of potential transversal neurobiological mechanisms, without identification of the common clinical correlate: the BDNF/pro-BDNF ratio of alcohol-dependent subjects was shown to be lower than that of controls (Zhou et al., 2017), but the evolution after weaning is not known. It was also shown to be lower in patients with bipolar disorder (Zhao et al., 2017). The question remains as to the significance of changes in peripheral BDNF and pro-BDNF levels, which are not always related to cellular or behavioral indicators of plasticity (Giaccobo et al., 2019). The Val66Met polymorphism has an effect on the pro-domain of BDNF, influencing the stability of the complex that forms between BDNF and pro-BDNF, which may modulate the role of BDNF by attenuating its activity (Uegaki et al., 2017). We did not consider the influence of this polymorphism here, which could contribute to the disparity in the depression and BDNF levels observed in various studies.

A study on a larger sample is needed that considers other symptoms that may improve with antidepressant treatment not directly related to the clinical parameters evaluated by the HDRS scale: sleep, agitation, psychomotor slowing, etc. Neurovegetative symptoms vary highly between patients and could have evolved without being taken into account here (Caroleo et al., 2019). Cognitive complaints were evaluated, and improved in our sample, but were not directly related to the levels of the molecules either. High levels of BDNF have been shown to be associated with good cognitive functioning in bipolar subjects (Mora et al., 2019) evaluated with a fine battery of cognitive tests. Anxiety was also not evaluated in our study: altered levels of BDNF, pro BDNF, and elements of the TrkB pathway have been shown in cases of stress, and probably play a major role in anxiety disorders (Mühlberger et al., 2014).

An other limit is that we used a precipitation technic to isolate exosomes, and not ultracentrifugation, which is the standard technic. The use of a commercial kit is still controversial about the purity of the isolated fraction, and we cannot exclude to have measured extracellular vesicles, and contaminants (Yang et al., 2020; Zhou et al., 2020). However, we directly measured the molecules in the serum, and consequently the measured BDNF and pro-BDNF correspond to the accessible molecules, which do not include those located in the exosomes. In the same manner, the molecules measured in the exosomal part may include those from exosomes, extracellular vesicles, and from contaminants, whose quantity may be insignificant. However, our results should be confirmed by using ultracentrifugation to separate exosomes in the studied samples.

Conclusion

This is the first study to evaluate BDNF and pro-BDNF levels in exosomes of MD patients. We demonstrate changes in the levels of these molecules after MD treatment, not only in serum but also exosomes. These transport nanovesicles may have a role in the regulation of

BDNF. In the CNS, exosomes may facilitate the passage of BDNF through the blood-brain barrier. These vesicles should be considered in future studies to better understand the physiopathology of MD. A better understanding of alterations in the synthesis of BDNF will allow its better characterization as a potential biomarker for neuronal plasticity and its involvement in MD. Another potentially informative approach to better understand the pathophysiological role of BDNF in depression could be the development of another mode of clinical evaluation based on concomitant symptom determination.

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Author contributions

Thibaut Gelle :laboratory measures, data analysis, writing ; Rayhanatou Altine Samey : laboratory measures, data analysis, reviewing ; Brigitte Plansont : investigation, collection of data ; Barbara Bessette : investigation, interpretation of data, writing; Marie-Odile Jauberteau-Marchan : interpretation of data, writing - review ; Fabrice Lalloué : interpretation of data, writing - review ; Murielle Girard : concept of the study, investigation, writing original draft, writing – review.

The two first authors equally contributed to the manuscript.

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