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A pilot trial of low-dose naltrexone in primary progressive multiple sclerosis

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A sixth month phase II multicenter-pilot trial with a low dose of the opiate antagonist Naltrexone (LDN) has been carried out in 40 patients with primary progressive multiple sclerosis (PPMS). The primary end points were safety and tolerability. Secondary outcomes were efficacy on spasticity, pain, fatigue, depression, and quality of life. Clinical and biochemical evaluations were serially performed. Protein concentration of β-endorphins (BE) and mRNA levels and allelic variants of the μ-opiod receptor gene (OPRM1) were analyzed. Five dropouts and two major adverse events occurred. The remaining adverse events did not interfere with daily living. Neurological disability progressed in only one patient. A significant reduction of spasticity was measured at the end of the trial. BE concentration increased during the trial, but no association was found between OPRM1 variants and improvement of spasticity. Our data clearly indicate that LDN is safe and well tolerated in patients with PPMS. *Multiple Sclerosis* 2008; **14:** 1076–1083. http://msj.sagepub.com

Key words: β-endorphins; efficacy; low-dose naltrexone; opioid receptors; primary progressive multiple sclerosis; safety

Introduction

Naltrexone is an orally semisynthetic opiate antagonist licensed in 1984 by the Food and Drug Administration (FDA) in a 50–100 mg daily dose as a treatment for heroin and alcohol addiction because it counteracts the effects of opioids by blocking opiate receptors [1]. However, when naltrexone is given at a lower dose, equal to or less than 5 mg/day [low-dose naltrexone (LDN)], its opiate antagonist activity turns into an agonist one so as to trigger a prolonged release of endogenous opioids such as β-endorphins (BE) [2]. BE is a peptide neurotransmitter produced by pituitary and hypothalamic neuronal cells, which has been traditionally considered as a regulator of nociception, mood, food intake, and endocrine secretion. However, recent evidence indicates that such a peptide has a broader activity because when released by lymphocytes it also exerts peripheral anti-nociceptive action [3] and possesses an anti-inflammatory activity [4–6]. All together, these results have led to the widespread off-label use of LDN for the treatment of symptoms such as numbness, spasticity, fatigue, and bladder dysfunction as well as diseases with a dysimmune origin such as HIV, Crohn's disease, lupus arthritis, fibromialgia, and multiple sclerosis (MS) (http://www.LDNers.org). However, most of the evidence so far accumulated is anecdotal, and the first phase II clinical trial assessing safety and efficacy of LDN has been completed only recently in patients with Crohn's disease [7].

MS is an immune-mediated, inflammatory, demyelinating disease of the central nervous system

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in which pain, fatigue, and spasticity are among the more frequent and disabling symptoms [8]. Because these symptoms have a strong impact on occupational functioning, productive activity, and quality of life and could potentially benefit from LDN-induced secretion of endogenous opioids [9,10], we performed a pilot phase II (open label) uncontrolled clinical trial to first assess safety and tolerability of LDN in patients with MS. Symptomatic effect on pain, fatigue, spasticity, and depression was also measured as secondary outcome measures. Only patients with the primary progressive variant of the disease (PPMS) (affecting about 15% of patients with MS) [11] have been selected considering that no disease modifying treatments are available for this variant; spasticity is almost always present, and pain and fatigue are quite frequent. The biological impact on the opioid system of LDN administration [e.g., protein concentrations of BE and mRNA levels of its receptor, the μ-opioid receptor (MOR)] was also evaluated together with the allelic variants of the human MOR gene OPRM1 [12].

Materials and methods

Patients' selection

Forty patients with a definite diagnosis of PPMS according to McDonald criteria [13,14] have been enrolled from December 2006 to March 2007 in four Italian MS clinical centers. At the time of inclusion, patients should be 18–65 years aged, had a disability level measured with the Expanded Disability Status Scale (EDSS) [15] between 3.0 and 6.5, had to have a disease duration longer than 2 years, and had a stable disease course in the 6 months before the enrollment. Patients were enrolled if they were affected by at least one of the following symptoms: spasticity (defined as a score between 2 and 4 in, at least, one limb on the Modified Ashworth Scale) [16], pain [defined as a score >2 at the Visual Analogue Scale (VAS)] [17], fatigue [measured with a score between 36 and 63 at the Fatigue Severity Scale (FSS)] [18], and/or depression [measured with a score >9 at the Beck Depression Inventory] [19]. Patients were excluded if they were treated with concomitant opioid-related drugs at the time of inclusion. Gabaergic and serotoninergic treatments were accepted if the dosage was maintained unmodified in the 2 months preceding and during the trial. Women of childbearing were asked to use contraceptives to prevent pregnancy. A negative pregnancy test was mandatory as well as a written informed consent. The study was approved by the ethical committees and the review boards of each of the participating institutions.

Methods

The design of the study was open, uncontrolled, 6-month duration. Primary outcomes of the study were the safety and tolerability of the drug, measured as the frequency of major and minor adverse events as well as the occurrence of neurological deterioration. The secondary outcome measures were the efficacy of the drug on fatigue, pain, spasticity, and depression measured using validated scales (FSS, VAS, Modified Ashworth Scale, and Beck Depression Inventory, respectively). We also monitored the quality of life, using the Italian-validated version of the SF-36. Four Italian centers participated to the clinical trial: San Raffaele Scientific Institute (Center 1; coordinating centre), Don Gnocchi Scientific Institute (Center 2), Gallarate Hospital (Center 3), and Micone Hospital (Center 4). Nineteen patients have been enrolled in Center 1, whereas seven in each of the other three centers. Once included, patients were given 2 mg as oral dose of naltrexone at bedtime for the first 4 weeks. Using phone call monitoring, the dose was increased up to 4 mg (referred as LDN) within the first 2 weeks of treatment until the end of the study. Physical examination and medical/neurological history were recorded at screening and baseline visits to determine patients' eligibility. Follow-up visits were scheduled after 1, 3, and 6 months (end of the study) after the beginning of the therapy and 1 month after the end of the study. At each safety evaluation visit, assessment of the patients included history and physical/neurological examination, vital signs, adverse event monitoring, and complete biochemical tests [including blood cell count, liver and kidney function, electrolytes, plasma glucose, cholesterol, eritrosedimentation rate (ESR), and urinary analysis]. Adverse events were monitored at each visit according to Common Terminology Criteria for Adverse Events (CTCAE) (v3.0) (http://www.ctep.cancer.gov). Additional visits were performed at the time of adverse event occurrence. Disease progression was measured using EDSS. We considered as progressed any patient showing change in EDSS ≥0.5 between the final and baseline evaluation if the baseline EDSS was ≥5.5 and change in EDSS ≥1.0 and if the baseline EDSS was <5.5. EDSS changes had to be confirmed 1 month after treatment discontinuation.

BE and MOR studies

In all patients, peripheral blood samples were collected in the morning, between 9 and 10 a.m., in a tube containing ethylenediamine tetraacetic acid

(EDTA). BE level was measured in peripheral blood mononuclear cells (PBMC) using a previously described and validated radioimmunoassay procedure [6,20]. Sensitivity of the method was 10 pg per tube, and intra-assay and inter-assay variation coefficients were 8% and 11%, respectively. For the evaluation of mRNA expression levels of MOR, total RNA was purified from PBMC using TRIzol reagent (Invitrogen, Life Technologies, San Giuliano Milanese, Italy), resuspended in 6 μL of diethylpyrocarbonate (DEPC) water, and treated with DNase (DNAfree-Ambion). Then, cDNA was synthesized using Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV RT) (Invitrogen) and subjected to real-time polymerase chain reaction using ABI PRISM 7000 (Applied Biosystems, Forster City, California, USA). Probe/primers specific for human GAPDH (code numberHs99999905_m1) and human MOR (code number Hs00168570_m1) were purchased from Taqman® Assays-on-Demand Gene Expression Products (Applied Biosystems). All PCR assays were performed in triplicate as previously shown [20]. To determine the genotypic variant of MOR, genomic DNA was isolated from peripheral blood of all but one of the patients with PPMS at baseline visit according to standard procedures. The Asn40Asp single nucleotide polymorphism in the human MOR gene OPRM1 was genotyped using the ABI Taqman assay for rs1799971. The primer sequences were forward, 5′-CCTTGGCGTACTCAAGTTGCTC-3′ (fluorescently labeled), and reverse, 5′-TTCGGACCGCATGGGA CGGAC-3′. Participants were grouped by OPRM1 status using the previously reported criteria [12].

Statistical analysis

An intention-to-treat analysis was performed. Data from all enrolled patients, including dropout when available, were included in the statistical analysis. Baseline values and end of the study values were mandatory; only one missing value over the 6-month period was tolerated. BE concentrations were analyzed by means of one-way analysis of variance for repeated measures followed by Bonferroni *t*-test for multiple comparisons. Secondary outcome (efficacy) measures were analyzed by means of nonparametric tests (Fisher's exact test for categorical variables and Wilcoxon signed-rank test for quantitative data). The association between OPRM1 genotype category and clinical responsiveness to treatment was assessed using the Fisher's exact test. SPSS statistical package (Chicago, Illinois, USA) was used for the analysis. No formal measurement of sample size was performed because the primary outcome of the study was the safety and tolerability of the drug.

Results

Patients' demographic

Table 1 shows the baseline clinical and demographic characteristics of the PPMS patients enrolled in the study. No differences have been found between the different clinical centers in terms of gender ratio, age at enrollment, and time from symptom onset at the inclusion. Median baseline EDSS disability level was lower at Center 3 (3.5) than in the other MS centers (5.5, 5.5, and 6.0) ($P < 0.05$). The gender proportion (female:male ratio 1.1) and the age of disease onset (40.8 ± 8.9 years) were comparable to previous population-based studies on PPMS [21]. Subacute or chronic walking impairment was the most common symptom at onset, the so-called spinal cord variant. In most of the cases, it was due to paraparesis and less frequently by hemiparesis. Mean disease duration (12.7 ± 6.8 years) and disability levels (median EDSS 6.0, ranging from 3 to 6.5) of the enrolled patients were comparable to those of larger studies such as PROMISE [22], and MAGNIMS [23], and reflected the epidemiological characteristics of patients with PPMS [11,21]. Table 2 shows baseline levels of fatigue, pain, spasticity, and depression in our cohort of patients with PPMS. No differences have been found across clinical centers as regards to baseline levels of fatigue, pain, spasticity, and depression.

Safety and tolerability results

Thirty-five (87.5%) patients completed the 6 months of therapy. Five patients (12.5%) terminated pre-

Table 1 Baseline demographic and disease-related characteristics of patients with primary progressive multiple sclerosis

Patients characteristics	MS (*n = 40*)
Sex	
Female:male	21:19 (52.5%)
Age at enrollment [mean (SD)]	53.4 (8.0)
Age of onset [mean (SD)]	40.8 (8.9)
Time from symptom onset to enrollment [mean (SD)]	12.7 (6.8)
Symptoms at presentation	
Paraparesis/spinal cord	22 (55%)
Hemiparesis	10 (25%)
Cerebellar syndrome	5 (12.5%)
Other	3 (7.5%)
Cerebrospinal fluid examination	
Oligoclonal bands	28 (70%)
Normal	3 (7.5%)
Unknown	9 (22.5%)
EDSS [median (range)]	6.0 (3–6.5)
Progression index [median (range)][a]	0.43 (0.12–1.65)

EDSS, Expanded Disability Status Scale.
[a]Progression index represents the ratio between EDSS score and disease duration.

Table 2 Neurological status of patients with primary progressive multiple sclerosis as assessed at baseline

Measure[a]	Values
Visual Analogue Scale [median (range)]	2 (0–10)
0–2	23 (57.5%)
>2	17 (42.5%)
Modified Ashworth Scale [median (range)]	0.875 (0–3)
<2 in all limbs	19 (47.5%)
≥2 in at least 1 limb	21 (52.5%)
Fatigue Severity Scale [median (range)]	45 (9–63)
<36	7 (17.5%)
≥36	33 (82.5%)
Beck Depression Inventory [median (range)][b]	5 (1–30)
<9	28 (73.7%)
9–16	7 (18.4%)
>16	3 (7.9%)

[a] When not specified, values represent the number of patients (%).
[b] Two patients were not evaluated at baseline for mood alteration.

maturely the study. One patient (ID_1) decided to interrupt the treatment 48 days after the beginning due to the occurrence of enuresis. ID_13 decided to interrupt the treatment 5 months after the beginning due to a subacute clinical worsening of left upper and lower limb hypostenia. Neurological worsening was not confirmed 1 month after treatment discontinuation. ID_16 exited the study 3 months after the beginning of the treatment because of a >2 fold increase of bilirubin (total 2.32; indirect 1.46). ID_38 exited the study 4 months after the beginning of treatment because of a urinary infection causing renal failure (creatinine 3.9 mg/dL) not requiring dialysis. ID_40 dropped out for a major protocol violation: 15 days after the beginning of the study, the patient admitted the use of an opioid-containing drug (tramadol) to treat pain.

Adverse events experienced during the trial are reported in Table 3. According to The National Cancer Institute CTCAE, we observed only two major adverse events of grade III (severe) or IV (life-threatening or disabling). One patient was the above-mentioned dropped-out patient suffering from renal failure not requiring dialysis (ID_38). Further analysis showed a previously unrecognized polycystic kidney disease. The other patient was diagnosed as having bone metastases presenting lung carcinoma (ID_36). In this latter case, the patient completed the trial because the diagnosis was made only at the end of the treatment.

All the other adverse events recorded during the trial were considered minor being of grade I (non-interfering with function) or II (not interfering with daily living activities).

Four grade II adverse events were recorded in four patients. Two patients (ID_12 and ID_13) had an increase of γ-glutamyl-transpeptidase levels (147 and 121 U/L, respectively) (>2.5–5.0 × Upper Limit of Normal range [ULN]). In both patients, increased enzymatic levels occurred 3 months after the beginning of the treatment and normalized 1 month after the end of treatment. One patient (ID_16) showed increased levels of bilirubin (total 2.32; indirect 1.46) (>1.5–3.0 × ULN) – despite normal levels of liver enzymes [aspartate aminotransferase (AST) 36; alanine aminotransferase (ALT) 50] – 3 months after the beginning of the treatment. The treatment was soon after discontinued, and the patient dropped out of the study. Three months after drug withdrawal, blood levels of bilirubin were still increased (total 1.80; indirect 1.31), hepatic enzymes remained within normal values (AST 27, ALT 40) but a liver ecography showed a liver ecostructure diffusely hyper reflecting as in hepatopathy. Eleven months after drug discontinuation, bilirubin levels and liver function returned to normal values and the liver ecostructure was ameliorated. We attribute this adverse event to a possible mild and transitory "Gilbert like-effect" induced by the investigational drug, as reported for other

Table 3 Number of patients experiencing major and minor adverse events during the trial

Side effects[a]		Center 1	Center 2	Center 3	Center 4	Total
Major (grade III or IV)	Lung carcinoma renal failure	0	0	0	2	2 (5%)
Minor (grade I or II)	Irritability	1	1	3	0	5 (12.5%)
	Hematological abnormalities	10	2	1	1	14 (35%)
	Urinary infection	3	2	2	1	8 (20%)
	Other	4[b]	1[c]	2[d]	1[e]	8 (20%)
Number of patients experiencing at least one adverse event		17/19 (84.2%)	4/7 (57.1%)	4/7 (57.1%)	3/7 (42.9%)	27/40 (67.5%)

[a]Adverse events were monitored according to the Criteria for Adverse Events v3.0 (CTCAE) (http://ctep.cancer.gov). Severe (grade III) or life-threatening (grade IV) events, requiring hospitalization, have been considered as major adverse events. Events not interfering with function (grade I) and events not interfering with activities of daily living (ADL) (grade II) have been considered as minor adverse events.
[b]Joint pain (1 patient), mood alteration (1 patient), asthenia (1 patient), enuresis (1 patient).
[c]Mood alteration (1 patient).
[d]Gastro-intestinal infection (1 patient), decrease of libido (1 patient).
[e]Facial peripheral palsy (1 patient).

treatments, such as rifampim [24]. We cannot exclude that the concomitant use in this patient of low-dose methotrexate – a labeled treatment for MS at 7.5 mg per week – might have contributed to hepatic toxicity. One month after the beginning of the therapy, ID_34 experienced a peripheral facial nerve palsy, which resolved after steroid treatment.

Forty-three grade I adverse events were recorded in 21/40 (52.5%) patients. Nine of 40 (22.5%) patients showed more than one grade I event during the trial. Leucopenia (<Lower Limit of Normal range = 3.0 × 109 /L) was measured in 14 patients. Increased levels of cholesterol (>ULN = 300 mg/dL) were recorded in six patients. Increased levels (≤2.5 × ULN) of liver enzymes were measured in six patients. Such abnormalities were transient and in all cases subsided at the end of the trial. Asymptomatic urinary infections occurred in eight patients and mild irritability in five patients. Mood alteration (two patients), gastrointestinal infection (one patient), decrease of libido (one patient), joint pain (one patient), enuresis (one patient; ID_1, drop out), and asthenia (one patient) were the remaining grade I adverse events recorded during the trial. Such symptoms were transitory, did not interfere with function, and did not lead to any change in the dosage of the treatment.

Neurological disability was evaluated in 39/40 (97.5%) patients using the EDSS at different time points during the study. ID_40 was lost at follow-up. At the end of the study, only one (2.6%) patient experienced progression of the disease as measured by a 1-month confirmed 1.0 EDSS point increase (ID_30, from 3.5 to 4.5). In 35 (90%) patients, EDSS remained unchanged at the end of the trial compared with baseline values. In three patients, a 0.5 point EDSS improvement was measured at the end of the trial (ID_2 and ID_23, from 6.0 to 5.5; ID_26 from 5.5 to 5.0).

Secondary outcome (efficacy) measure results

Data are shown in Table 4. As of intention-to-treat analyses, a statistically significant reduction of spasticity, measured using Modified Ashworth Scale, was observed at 3 ($P < 0.001$) and 6 months ($P < 0.01$) after the beginning of the treatment when compared with baseline. The positive effect persisted up to 1 month after treatment discontinuation ($P < 0.05$).

No significant changes were observed for fatigue at the 3- and 6-month evaluations. One month after treatment discontinuation, FSS data from 21 patients were available. In these patients with PPMS, FSS was significantly decreased compared with baseline value (median value, respectively, 37.0 vs 42.4; $P < 0.05$).

VAS showed a significant increase of pain at 3 (median value 3.0; $P < 0.01$) and 6 months (median value 3.0; $P < 0.05$) after treatment beginning when compared with baseline values (median value 2.0). One month after treatment discontinuation, VAS data from 30 patients were available. Pain showed a significant decrease (median value 1.0; $P < 0.05$) when compared to the 6-month evaluation.

Beck Depression Inventory did not change during the trial at any of the time points analyzed. As regards quality of life measured using SF-36, there was no statistically significant improvement in any of the items measured at the 6-month examination compared with baseline despite a trend of amelioration was observed for some items (Figure 1).

Protein and mRNA concentration of BE and MOR

BE concentrations in PBMC from patients with PPMS at baseline, 3 months, and 6 months after the beginning of treatment and 1 month after the end of treatment are reported in Figure 2. As expected from previous studies [25], PBMC BE concentration in

Table 4 Effect of low-dose naltrexone administration in primary progressive multiple sclerosis patients on spasticity, fatigue, pain, and depression during the 6 months study

Secondary outcome measures	No. of patients[a]	Baseline value (median)	Final value (median)	% improved	% stable	% worsened	*P* value
Spasticity (Modified Ashworth Scale)	38	0.87	0.5	47.4%	42.1%	10.5%	0.008
Fatigue (Fatigue Severity Scale)	39	45	44	33.3%	25.6%	41%	0.51
Pain (Visual Analogue Scale)	39	2	3	28.2%	15.4%	56.4%	0.01
Depression (Beck Depression Inventory scale)	36	5	4.5	55.6%	11.1%	33.3%	0.09

[a] The following patients were not included in the intention-to-treat analysis: ID_40 dropped out of the study for protocol violation 15 days after the beginning of the treatment (he had a baseline mean Ashworth Modified Scale of 2.3, Fatigue Severity Scale of 45, Visual Analogic Scale of 6, and Beck Depression Inventory scale of 5) and was lost at follow-up. ID_38 was evaluated at baseline (Ashworth modified scale of 1.5), at the 3-month visit (Ashworth Modified Scale of 1.5) but not at the end of the study. ID_12 and ID_14 had no baseline evaluation of the Beck Depression Inventory scale. ID_5 had no final evaluation of the Beck Depression Inventory scale.

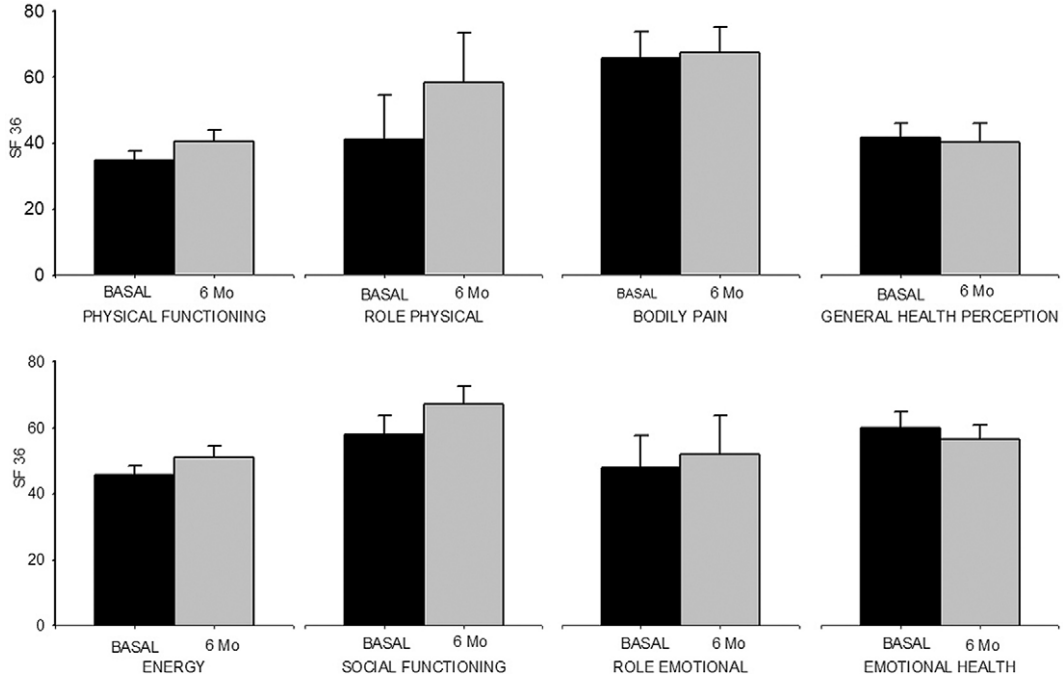

Figure 1 SF-36 health survey in patients with primary progressive multiple sclerosis treated with low-dose naltrexone (LDN). Mean scores (± SEM) are shown at baseline (BASAL) and after 6 months (6 Mo) of LDN therapy. Each item measured by the SF-36 health survey is represented.

patients with PPMS (48.3 ± 1.9 pg/10^6 PBMC) were lower than in healthy controls at baseline. A significant ($P < 0.05$) increase of BE concentration (mean ± SEM) was measured either at 3 months (63.4 ± 4.8) and 6 months (76.9 ± 3.3) after the beginning of LDN treatment. BE concentration remained elevated up to 1 month (104.6 ± 12.4) after the end of treatment.

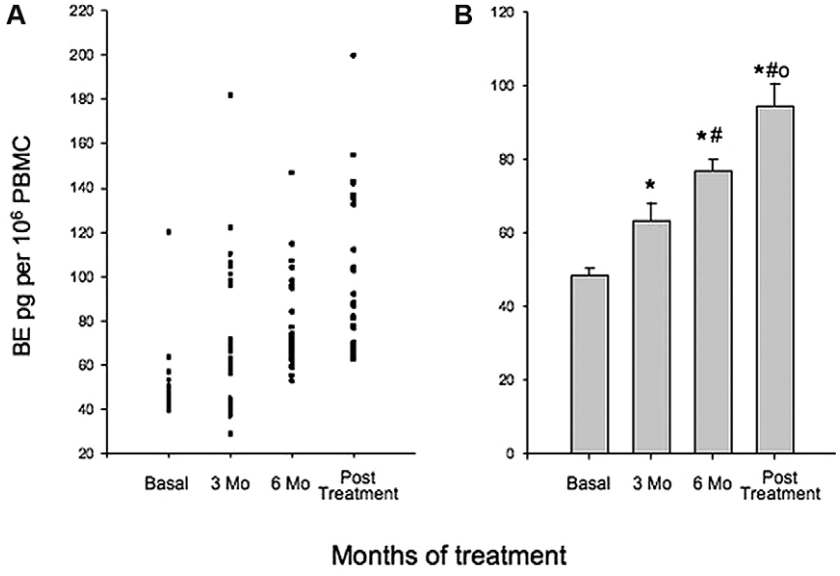

Figure 2 β-endorphin (BE) levels in peripheral blood mononuclear cells (PBMC) from patients with primary progressive multiple sclerosis. In panel A, a scatter plot reporting PBMC BE concentrations for each patient is depicted. In panel B, BE mean values (± SEM) are represented. BE concentrations are expressed as pg/10^6 PBMC. Measurements have been performed at baseline, 1 month, and 3 months (Mo) after beginning of the treatment and 1 month after therapy discontinuation (Post Treatment). *$P < 0.05$ versus basal, #$P < 0.05$ versus 3 months, °$P < 0.05$ versus 6 months.

The mRNA levels of MOR – measured at 3 and 6 months after the beginning of LDN treatment and 1 month after the end of therapy – did not change in any of the patients compared with baseline values (data not shown).

We found 31 patients (79.5%) homozygous and 8 (20.5%) heterozygous for the more common A variant of OPRM1 gene, and no association was found between allelic variants and amelioration of spasticity (P = NS; OR and 95% C.I. of G carriers and efficacy on spasticity: 3.11; 0.6–16.8).

Discussion

To the best of our knowledge, here we report the first therapeutic trial aimed at assessing the safety and tolerability of a 6-month LDN therapy in patients with PPMS. Five patients dropped out during the study. None of the remaining 35 patients asked to discontinue the drug, missed the clinical scheduled visits, or was lost during the follow-up. The compliance was generally acceptable. The only two major adverse events (grade III and IV) reported were unlikely related to the drug. We hardly see any relationship between bone metastasis presenting lung carcinoma in patient ID_36 and the LDN mechanisms of action. The patient was a 40 cigarette/day smoker with a familial risk for cancer, and metastatic disease to bones is a common occurrence in patients with advanced lung carcinomas. We attribute the occurrence of renal failure not requiring dialysis in patient ID_16 to a pre-existing polycystic kidney disease. The most common adverse events documented in this trial were minor (grade I and II), well tolerated, and disappeared after the end of the treatment. Differently from what occurred in the recent trial exploring LDN therapeutic efficacy in Crohn's patients [7], we had not to change LDN schedule of administration to solve irritability problems. Finally, disease progressed in only one patient (2.5%) at the end of the study. This latter finding is reasonably attributable to the natural history of the disease [11,21] and not to the treatment in itself considering the cohort of the patients enrolled in the study. All together, these data indicate that LDN is safe and well tolerated by patients with PPMS.

Assessment of LDN efficacy in our cohort of patients with PPMS was limited owing to the open uncontrolled design of the clinical trial. Nevertheless, we decided to assess LDN efficacy on pain, fatigue, depression, and spasticity because these are typical and frequent symptoms of the MS variant we studied and their responsiveness to LDN has been, although only anecdotically, reported (http://www.LDNers.org). Our preliminary results, based on intention-to-treat analysis, suggest a beneficial effect of LDN on spasticity only. Fatigue significantly ameliorated 1 month after treatment discontinuation but, owing to the uncontrolled nature of the trial, this is only barely attributable to the treatment itself. As regards quality of life, most of the items were improved at the end of the study but none of them at a statistically significant level.

We performed several experiments to evaluate whether or not improvement of spasticity was paralleled by an LDN-induced biological phenomenon. Because LDN is supposed (but never documented) to exert its efficacy by triggering the release of BE, we serially measured BE concentration in the PBMC of our patients. We were able to provide the first in-vivo demonstration that LDN treatment is able to increase the intracellular concentration of BE in PBMC of patients with MS. BE concentration started increasing 3 months after the beginning of the therapy and remained elevated (compared to baseline values) up to 1 month after therapy discontinuation. At this stage, we might only speculate that the symptom improvement is related to the increased circulating concentration of BE. Nevertheless, it is interesting to note that symptom amelioration paralleled increasing BE concentration during the trial and persisted 1 month after treatment discontinuation when BE level peaked. Furthermore, besides its own potential benefit, BE might exert its therapeutic effect in PPMS by interacting with the endocannabinoid system [26]. Although acting on two different receptor pathways, the synergistic activity between BE and cannabinoids can occur at different levels because of cross-tolerance and cross-sensitization as well as receptor co-localization in some brain areas [26–30]. BE release is, for example, critical for peripheral antinociceptive action induced by cannabinoid 2 receptor stimulation [3]. Administration of cannabinoids in patients with MS and experimental animals has been associated to an improvement in bodily pain and mental health as well as to an amelioration of symptoms such as spasticity, mood, sleep disturbances, tremor, and muscle cramps [31–36].

Our data clearly indicate that LDN is a relatively safe and well-tolerated drug in patients with PPMS. However, a randomized, double-blind, placebo-controlled trial needs to be performed to cogently assess the potential efficacy of this drug in patients with MS.

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at 50–100 mg daily dose). Pharmaceutical companies had no involvement in the study design, collection, analysis, and interpretation of data, in the writing of the report, in the decision to submit the paper for publication, neither in study funding. None of the authors have any conflicts of interest to declare. We are indebted with all the physician and nurses operating within the involved MS centers for their help in recruiting and assessing enrolled patients. We thank F. Esposito and S. Lupoli for performing genotype analyses. We thank L. Licciardello and M. Motta from Zambon Group for providing LDN for the trial. We are grateful to all the patients participating to the trial.

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