Effect on cognition of estroprogestins combined with Interferon beta in multiple sclerosis: analysis of secondary outcomes from a randomized controlled trial

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Abstract

Introduction. Cognitive impairment is a disabling symptom in multiple sclerosis (MS). While its management remains challenging, beneficial effects on cognition of disease-modified treatments (DMTs) have been reported and a positive role of estroprogestins has been hypothesised, suggesting that the combination of the two medications in women with MS could offer a promising treatment strategy.

Objectives: We investigated whether the combination of estroprogestins with Interferon beta (IFN beta) can improve cognition in women with MS.

Methods. Women with relapsing remitting (RR) MS were randomly assigned (1:1:1) to take IFN-beta only (Group 1), IFN beta plus ethinylestradiol 20 mcg and desogestrel 150 mcg (Group 2) or IFN beta plus ethinylestradiol 40 mcg and desogestrel 125 mcg (Group 3) in a randomised, controlled trial, of which we report the analysis of secondary outcomes. At baseline and at 24 months all patients underwent MRI and a comprehensive cognitive assessment, including the Rao's Brief Repeatable Battery (RBRB) and the questionnaires for depression, fatigue and quality of life. A failure of at least two tests of the RBRB defined "cognitive impairment".

Results. At baseline, there was no difference in the proportion of cognitively impaired patients. At month 24, Group 3 (34.8%) had a lower proportion of patients with cognitive impairment than Group 1 (47.6%) (p=0.03). The risk of developing cognitive impairment over 24 months was lower in Group 3 (p=0.02). Mood and fatigue scores were comparable across the groups over time at both time points. However, at month 24, Group 3 showed worsening on the sexual function subscale of the 54-item MS quality of life questionnaire (p=0.03).

Conclusions. This study suggests that the combination of high dose estroprogestins with IFN beta may have positive effects on cognition. However, the effect of this treatment on sexual function requires caution to be exercised.

Protocol number NCT00151801, registered in ClinicalTrials.gov

1. Introduction

Cognitive impairment is a common and disabling symptom in multiple sclerosis (MS) that particularly affects domains such as memory, attention and executive functions.[1-2] It is frequently associated with depression and fatigue, resulting in reduced quality of life that is independent of physical disability.[3]

Effective treatments for cognitive impairment in MS are lacking. However, a beneficial effect of Interferon beta (IFN beta) on domains of information processing and learning/memory has been reported, along with a sustained effect in reducing the risk of cognitive deterioration.[4-8] While on IFN beta, women seem to be more protected against the development of cognitive impairment than men. This suggests greater response to therapy in women or the consequence of the better prognosis that, in MS, is associated with the female sex.[8,9]

Gender and sex hormones have a recognised role in MS clinical characteristics and in pathology.[10] The prevalence and expression of symptoms differ between the sexes and these differences can be explained, at least partially, by gender differences in the characteristics of tissue damage.[11,12] Experimental data suggest that sex hormones can exert anti-inflammatory and protective effects on brain tissue. Estrogens can also enhance brain plasticity by increasing neurogenesis, connectivity and synaptic transmission, particularly in areas sub-serving complex cognitive tasks, and by boosting the brain's energy supply and utilization.[13] In the healthy brain, as well as in neurodegenerative diseases, these effects of estrogens result in a reduced risk of cognitive decline with hormonal replacement therapies for menopause.[14]

Previously, we demonstrated that the combination of IFN beta with high-dose estroprogestins in patients with relapsing-remitting MS (RRMS) has a pronounced anti-inflammatory effect.[15] In the present study, we test the hypothesis that, in women with RRMS, the effect of this combination therapy can specifically benefit cognition and aspects of quality of life related to cognition.

2. Material and Methods

2.1 Participants

Data from patients participating in a multicentre trial on the effect of estroprogestins in combination with IFN beta in women with MS (number NCT00151801, ClinicalTrials.gov) are included in this study, where we analyse the secondary outcomes of the trial. [15] Recruitment from five Italian MS centres started in September 2004; the last visit was in November 2009. The investigators obtained approval for the study protocol from the Local Ethical Committee, and each patient provided written informed consent. We enrolled women with RRMS,[16] aged between 18 and 45 years and with an entry score of the Expanded Disability Status Scale (EDSS)[17] equal to or less than 5.0 and at least two relapses in the previous 48 months or one relapse during the previous 12 months. Exclusion criteria included relapses or steroid intake in the previous 60 days, pathologies of the reproductive system, pregnancy or interruption of pregnancy in the previous 12 months, prior immunosuppressive therapy, glatiramer acetate, IFN beta or any experimental drugs before entry into the study, estroprogestins in the previous 3 months, and severe psychiatric illnesses, including severe depression, alcohol or substance abuse.

2.2 Study design

After a screening phase, women with RRMS were randomly assigned in a 1:1:1 ratio to receive IFN beta-1a (Rebif®, Merck Serono, Geneva, Switzerland) 44 mcg 3 times a week (t.i.w.) subcutaneously (s.c.) (*Group 1*) or IFN beta-1a 44 mcg t.i.w. s.c. plus ethinylestradiol 20 mg and desogestrel 150 mg (Mercilon®, MSD Italia SRL, Rome, Italy) (*Group 2*) or IFN beta-1a 44 mcg t.i.w. s.c. plus ethinylestradiol 40 mg and desogestrel 125 mg (Gracial®, Organon Italia S.p.A., Rome, Italy) (*Group 3*). The randomization list was computer generated, with a dynamic allocation provided by an independent national research organization (Istituto Superiore Sanità, Rome, Italy). A two-physician (treating and assessing) model was used to assist with study masking. At each site, the treating physician was responsible for evaluating patient eligibility, supervision of study drug

administration and for recording and managing adverse events and monitoring safety assessments. The treating physician was unblinded to treatment arm. The assessing physician was exclusively responsible for all neurological assessments, beginning with the screening assessment and for cognitive testing. Patients underwent clinical assessments, cognitive evaluations and MRI scanning at baseline, at month 12 and at month 24. At the same time-points they completed the self-reported questionnaires for quality of life, fatigue and depression.

2.3 Behavioural assessments

To assess cognitive functions we used the Rao's Brief Repeatable Battery[18] that includes:

- *the selective Reminding Test-Long Term Storage (SRT-LTS), Selective Reminding Test-Consistent Long Term Retrieval (SRT-CLTR) and SRT-delayed recall (SRT-D)* for verbal memory acquisition and delayed recall;
- *the 10/36 Spatial Recall Test (10/36-SPART) and the 10/36-SPART-delayed recall (10/36-SPART-D)* for visuospatial memory acquisition and delayed recall;
- *the Paced Auditory Serial Addition Test at 3 (PASAT-3) and 2 (PASAT-2)* seconds, and the Symbol Digit Modalities Test (SDMT) for concentration, sustained attention, and information processing speed;
- *the Word List Generation (WLG)* for verbal fluency on semantic stimulus.

A test failure was defined as performance one standard deviation (SD) below the mean Italian normative values.[19] Cognitive impairment was defined as a failure in at least two tests.[20]

Mood, fatigue and quality of life were evaluated with the Hamilton Depression Scale (*HAM-D*),[21] the Fatigue Severity Scale (*FSS*) questionnaire[22] and the 54-item MS quality of life questionnaire (*MSQoL-54*).[23]

2.4 MRI data acquisition and analysis

All MRI scans were performed on a 1.5 Tesla magnet (Philips Gyroscan NT 15, Netherlands) at

baseline, month 12 and month 24. The MRI protocol included proton density- and T2-weighted spin echo images (TR 2000 ms, TE 20/90 ms, matrix size 256×256, field of view 24 cm×24 cm, slice thickness 3 mm, gap 0 mm, 48 axial slices) and T1-weighted images (TR 600 ms, TE 15 ms, matrix size 256×256, field of view 24 cm x 24 cm, slice thickness 3 mm, gap 0, 48 axial slices) obtained before and five minutes after an intravenous injection of 0,1 mmol/kg of gadolinium-diethylenetriamine penta-acetic acid (Gd).

Volumes of hyperintense lesions on T2-weighted images (T2-LL), hypointense lesions on T1-weighted post-contrast images (T1-LL) and hyperintense lesions on T1-weighted post-contrast images (T1-Gd-LL) were quantified using a semi-automated method (Jim 4.0, Xinapse System, Leicester, UK). The number of patients with Gd-enhancing lesions at baseline and at month 24 was calculated for each group. The cumulative number of combined unique active (CUA) lesions, defined as new Gd enhancing lesions or new T2-weighted lesions (non-enhancing on post-contrast T1-weighted acquisitions, in order to avoid double-counting), was also quantified at month 24.

2.5 Statistical analysis

For data analysis, normalized values of cognitive scores were obtained using Italian normative data.[14] Balancing of treatment groups after randomization was tested with the Kruskal-Wallis test or the Chi-square test, as appropriate. Differences between cognitively impaired patients and non-cognitively impaired patients at baseline were tested with Mann-Whitney Test.

The between-group comparison in the proportion of cognitively impaired patients at month 24 was assessed with a proportion logistic-regression model with adjustment for baseline covariates: study group, age, years in formal education and number of impaired cognitive tests at baseline.

Differences in the MSQoL sub-scale scores at different time-points and between the groups were tested with an ANOVA model with repeated measures.

Risk factors for cognitive impairment at two years were identified using a logistic multivariate model: age, disease duration, years of formal education, EDSS, baseline number of impaired tests,

baseline MRI parameter (T1-LL, T2-LL, T1-Gd-LL) and cumulative number of CUA lesions at month 24 entered a univariate model. Variables that were significant in the univariate model were included in the multivariate model that identified risk factors for cognitive impairment at month 24. Between-group comparisons were carried out for the scores of cognitive tests, HAM-D and FSS at different time-points using a *t*-test or the Wilcoxon Signed Rank Test, if the criteria for a dependent samples *t*-test were not met.

All *p*-values less than 0.05 (two-tailed) were considered as significant. All statistical analyses were performed using SAS 9.1 (SAS Institute, Inc., Cary, NC). Data analysis was performed by an independent research organization (TFS: Trial Form Support S.L., Rome, Italy) with no role in the study design and data collection.

3. Results

Demographic, clinical and MRI characteristics at baseline

Out of 150 randomized women 149 took at least one dose of the study drug. Of these 149 patients, 142 had a complete baseline neuropsychological assessment (**Figure 1**).

There was no significant difference among the 3 groups in the baseline demographic, clinical and MRI characteristics (**Table 1**). Mean scores of cognitive tests, Ham-D and FSS were also similar across groups (**Table S1**). We did not find significant differences in the MSQoL-54 domains, except for the subscale of "role limitations due to emotional problems" that was significantly higher in Group 3 (mean ±SD score Group 1: 55±40; Group 2: 43±39; Group 3: 76±36, p=0.014) (**Table S2**).

At baseline, 89 (59.9%) patients met the criteria for cognitive impairment. There was no significant difference between groups in the number of cognitively impaired patients (**Table1**). Compared with cognitively preserved patients, those with cognitive impairment were significantly older (mean±SD age 30.9±6.3 *vs.* 27.5±6.3 years, p=0.007) and had longer disease duration (4.3±4.3 *vs.* 2.6±2.8 years, p=0.03) (**Table S3**).

Clinical and MRI characteristics at follow-up

Out of 142 patients with a baseline assessment, one patient was lost at follow-up and 13 patients did not complete the cognitive battery at month 24. Therefore, follow-up data at month 24 were available in 128 patients: 42 in Group 1, 40 in Group 2 and 46 in Group 3 (**Figure 1**).

Figure 2 shows the number of patients with cognitive impairment in the three groups at different time-points. At month 12, we did not find a significant difference in the proportion of patients with cognitive impairment across groups [23 (55%) in Group 1, 20 (46%) in Group 2 and 21 (45%) in Group 3, p=0.24]. At month 24, cognitive impairment was found in 20 (48%) patients in Group 1, 21 (53%) in Group 2 and 16 (35%) in Group 3, and the proportion of cognitively impaired patients was significantly lower in Group 3 when compared to Group 1 (p=0.03) (**Figure 2**).

The logistic multivariate model revealed that protective factors for cognitive impairment at month 24 were the absence of cognitive impairment at baseline (odds ratio 0.04; 95% CI 0.01-0.14; $p<0.001$) and belonging to Group 3 (odds ratio 0.27; 95% CI 0.08-0.93; $p=0.02$). No other factors (i.e., age, years from disease onset, years of formal education, EDSS score and MRI measures) were associated with the development of cognitive impairment at follow-up.

Testing the specific changes in cognitive domains over time (**Figure 3**), the Wilcoxon Signed Rank test showed significant improvements in the following tests for the following groups: STR-LTS in all the groups; STR-CLTR in Group 1 and Group 3; STRD in Group 2; SPART-D in Group 1; PASAT 3 and 2 in all the groups; SDMT in Group 2. No significant between group differences were found in WLG and SPART-D.

During the follow-up, we did not observe significant differences across groups in relapse rate, EDSS score, Ham-D and FSS. ANOVA with repeated measures showed only a significant difference in "satisfaction with sexual function" subscales at month 24 in the group treated with estroprogestins, with a significant difference between Group 1 and Group 3 ($F= 5.07$, $df=1.0$, $p=0.03$). No significant differences were found in the other MSQoL subscales (**Figure 4**).

4. Discussion

This study suggests that, over 2 years, high doses of oestrogen in combination with IFN beta may exert protective and reparative effects against the development of cognitive decline in women with RRMS**.**

During the follow-up we observed an improvement in cognitive function in all treatment groups, which supports previous findings of a positive effect of IFN beta on cognition**.**[6-8] However, a significant proportion of patients in the group treated with high-dose estrogens improved their cognitive status compared with those treated with IFN beta alone, supporting the hypothesis that estrogens contribute to preserve cognition in MS through specific direct or indirect effects. Indeed, inflammation has a deleterious effect on cognitive function, as demonstrated by the worsening of neuropsychological performance during relapses.[24-25]As previously suggested, high doses of estrogens may enhance the anti-inflammatory effects of IFN beta and thus exert their beneficial effects on cognition.[15] Estrogens are also known as potent and efficacious mediators of synaptic transmission, promoting neural plasticity and neurogenesis and sustaining the energetic demand by increasing glucose transport, aerobic glycolysis and mitochondrial function.[13,26] In experimental models, estrogens help to increase neurogenesis in the dentate gyrus of the hippocampus and promote a rapid increase of dendritic spine numbers or contacts in the hippocampus, the prefrontal cortex, the medial amygdala and the hypothalamus.[27] In these specific regions, neuronal plasticity helps to maintain cognitive function and protects women from cognitive decline with aging.[14,27] Although both IFN beta and estrogens may improve cognitive function in MS,[6,13] in this study, the lack of a group of patients treated only with estroprogestins leaves uncertainty over the individual contributions of these drugs on preserving/restoring cognition, i.e., whether estroprogestins with high dose estrogens enhance the IFN beta effect or if IFN beta predisposes the immune system to the beneficial effects of estroprogestins.

When we investigated the effects of the combination therapy on specific aspects of cognition domains, we could not identify specific cognitive domains that benefitted more from this treatment,

observing instead a global advantage for cognitive function. The size of the sample, as well as the relatively mild cognitive impairment in our population, may have limited our ability to detect specific effects of the combination therapy on cognitive domains.

Consistently with previous reports, which have demonstrated a beneficial effect of IFN beta on quality of life,[28-29] we have also observed some benefit on "Change in health" of MSQoL in all treatment groups. However, we also found a decrease of the "satisfaction with sexual function" in the groups treated with estroprogestins. This effect could be due to a drug-related decrease in the total and free testosterone levels and an increase in the sex hormones binding protein concentrations.[30] The presence of androgens is crucial for the maintenance of sexual desire in women.[31] In MS, estroprogestins could alter a balance that is already precarious due to the presence of increased risk factors for sexual dysfunction.[32,33] In addition to this effect on aspects of quality of life, our study confirmed that other adverse events that are typical of the two experimental drugs and, in combination therapy, have been reported elsewhere, [15] such as flu-like syndrome and deep vein thrombosis, should be taken into account in the case of this combination therapy.

This study is not without its limitations. We previously mentioned the lack of a group taking estroprogestins only. While this may have limited our ability to disentangle the effect on cognition of IFN beta from that of estroprogestins, we could not withhold disease-modifying treatments in eligible patients, this being unethical. Although we did not test specific domains with measures of frontal executive function and interference tests, this study used one of the most diffusely accepted cognitive batteries for cognition in MS, making our results more easily interpretable.[18]

5. Conclusions

Overall, our results, observed over a period of 24 months, suggest that high doses of estrogens may be a viable therapeutic option to preserve or restore cognitive functions in MS patients. However, since estroprogestions carry side effects that may impact on quality of life and are risk factors for cardiovascular disorders and breast cancer, this strategy would require a careful risk-benefit assessment in the individual case.

Contributions

LDG coordinated the study procedures, acted as treating physician and drafted the manuscript. FM and FDE acted as assessing physicians. VTB performed the MRI data collection and analysis. PVA undertook the statistical plan and the data analysis. FF and NP contributed to the discussion of results and writing up of the manuscript. PP supervised MRI data collection and analysis, contributed to the discussion of the results and the revision of the manuscript. VT generated the hypotheses of the study, designed the study with CP and revised the manuscript. CP designed the study with VT, was responsible for recruitment and data analysis, and contributed to writing up and subsequent revision of the manuscript.

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Compliance with Ethical Standards

The local Ethics Committees approved the Protocol. Participants provided written informed consent.

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Conflicts of Interest

Nickolaos Petsas has received a lecture fee from Biogen Idec-Portugal. Patrizia Pantano has received funding for travel from Novartis, Genzyme and Bracco and speaker honoraria from Byogen. Carlo Pozzilli has received consulting and/or lecture fees and/or research funding and travel grants from Almirall, Bayer Schering, Biogen, Genzyme, Merck Serono, Novartis, Roche and Teva. Laura De Giglio, Fabiana Marinelli, Valeria T. Barletta, Veronica A. Pagano, Floriana De Angelis, Fulvia Fanelli and Valentina Tomassini declare that they have no conflicts of interest to disclose

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Figure legends

Figure 1. Study flow-chart. *Abbreviations*: IFN beta, Interferon beta.

Figure 2. Number of patients with cognitive impairment at baseline and at month 24. Only significant *p* values, which refer to logistic-regression model with adjustment for study group, age, years in formal education and number of impaired cognitive test at baseline, are reported.

Figure 3. Mean scores of cognitive tests at baseline and at 24 month. Values for cognitive tests are expressed as mean±SD scores. *p* values refer to Wilcoxon Signed Rank Test (**p*<0.05; ***p*<0.01; ****p*<0.001; *****p*<0.0001; ns= not significant). *Abbreviations:* SRT-LTS, Selective Reminding Test-Long Term Storage; SRT-CLTR, Selective Reminding Test-Consistent Long Term Retrieval; SRT-D, Selective Reminding Test-Delayed; SPART-D, Spatial Recall Test-Delayed; PASAT-3, Paced Auditory Serial Addition Test-3 seconds; PASAT-2, Paced Auditory Serial Addition Test-2 seconds; SDMT, Symbol Digit Modalities Test.

Figure 4. Changes over time (month 24 *vs.* baseline) in MSQoL-54 subscales according to the study groups. Increasing scores indicate improved aspects of quality of life. *p* value refers to repeated measures ANOVA. *Abbreviations*: PH, physical health; RLPP, role limitation due to physical problems; RLEP, role limitation due to emotional problems; EW, emotional well-being; En, Energy; HP, health perception; SF, social function; CF, cognitive function; HD, health distress; SF, sexual function; CH, change in health; SSF, satisfaction with sexual function; OQL, overall quality of life.

Tables

Table 1. Patients' characteristics at baseline. All values are expressed as mean±SD, unless otherwise indicated. None of the between-group comparisons was significant (Kruskal-Wallis Test, all nominal $p>0.05$). ***Abbreviations:*** EDSS= Expanded Disability Status Scale.

	Group 1 (n=46)	**Group 2 (n=48)**	**Group 3 (n=48)**
Age (years)	30.4±7.0	29.1±6.4	30.6±5.9
Education (years)	14.3±5.1	15.1±2.9	15.2±3.1
Disease duration (years)	4.2±4.5	3.3±3.0	3.5±3.9
Annualised relapse rate in the previous 2 years	0.8±0.5	0.8±0.3	0.8±0.4
EDSS score	1.7 (0.7)	1.8 (0.9)	1.6 (1.0)
No. cognitively impaired patients (%)	31 (67)	27 (56)	31 (64)
No. patients with Gd-enhancing lesions (%)	26 (57)	31 (65)	28 (59)
No. Gd-enhancing lesions	29±51	38±78	39±10
T2 hyperintense lesion volume (mm^3), median (range)	4,771 (649-32,724)	4,284 (762-61,428)	4,378 (1,142-51,298)
T1 hypointense lesion volume (mm^3), median (range)	549.5 (0-9,964)	478 (0-15,435)	600 (0-15,501)