

# Follow-up of multiple sclerosis patients treated with Endotherapia (GEMSP)

MICHEL GEFFARD<sup>1</sup>, ARTURO MANGAS<sup>2</sup> and RAFAEL COVEÑAS<sup>3</sup>

<sup>1</sup>Institut pour le Développement de la Recherche en Pathologie Humaine et Thérapeutique, 33400 Talence;

<sup>2</sup>Gemacbio, Research Department, Lieu dit Berganton, 33127 Saint Jean d'Illac, France; <sup>3</sup>Institute of Neurosciences of Castilla y León, Laboratory of Neuroanatomy of the Peptidergic Systems, University of Salamanca, 37007 Salamanca, Spain

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**Abstract.** Endotherapia (GEMSP) is a novel therapeutic approach for multiple sclerosis (MS). The aim of the present study was to demonstrate the efficiency of Endotherapia in the follow-up of 193 patients with MS. The efficiency coefficient that was evaluated was the Expanded Disability Status Scale (EDSS) score, which is a functional scale of MS progression. The evaluated score of each patient during follow-up visits was compared with the theoretical score of the disease progression without GEMSP. The evolution of the EDSS score was evaluated according to the inclusion score. The quantitative global study of the EDSS score highlighted a statistically significant difference between the final average scores of the treatment with GEMSP (M) and worldwide reference (R) groups. The improvement of the M group compared with the R group was 24.5%. According to the final EDSS scores, the study highlighted a difference in favor of the M group with 62.0% for scores  $\leq 3$ , 7.8% for scores between 3 and 6 and 19.6% for scores  $\geq 6$ . According to the qualitative evolution of the EDSS scores, the improvements in favor of group M were 49.3% for scores  $\leq 3$ , 79.1% for scores between 3 and 6 and 19.5% for scores  $\geq 6$ . The qualitative study of the EDSS score showed a statistically significant success percentage; the success percentages were between 59.1 and 90.0%. In a larger population of MS patients, the data confirm the beneficial effects of GEMSP that were previously reported in pre-clinical and clinical studies. In addition, 78% of patients showed an improvement or deceleration of the disease.

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*Correspondence to:* Dr Michel Geffard, Institut pour le Développement de la Recherche en Pathologie Humaine et Thérapeutique, 200 Avenue de Thouars, 33400 Talence, France  
E-mail: mangasam@usal.es; mg.idrpht@orange.fr

**Key words:** multiple sclerosis, Expanded Disability Status Scale score, endotherapia, GEMSP, poly-L-lysine

## Introduction

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating and neurodegenerative disease that predominantly affects people aged between 20 and 40 years (1). The damage appears to be due to inflammatory processes in which lymphocytes become activated at the periphery, disrupt the intracellular matrix of the blood-brain barrier and invade the central nervous system. Environmental (including toxic substances, metabolic stress and possible pathogen infection) and genetic factors may facilitate the movement of auto-reactive T cells and demyelinating antibodies into the central nervous system (1-4).

To date, MS has no cure and only partially efficient therapeutic strategies are available [for example beta interferons, glatiramer acetate (Copaxone), Tysabri, fingolimod and laquinimod]. None of these therapeutic agents reduce the disability experienced by patients suffering from MS. Thus, novel therapeutic strategies and agents are required for the treatment of MS (5). One such therapeutic strategy/agent could be Endotherapia (GEMSP) (6,7). Endotherapia is a novel therapeutic approach against chronic conditions (for example neurodegenerative and auto-immune diseases) that accounts for genetic predisposition, and environmental, bacterial and immunological factors. This includes the identification of specific circulating antibodies in the serum of patients and the use of therapeutic tools, such as small compounds linked to the carrier poly-L-lysine (PLL) and the physiological actions of such compounds being known (e.g., GEMSP) (6,7). PLL is edible, water-soluble and non-toxic for humans (8). Depending on the circulating antibodies directed against the antigens found during the course of MS, the chemical composition and doses of the therapeutic agent (e.g., GEMSP) can be established for each patient (9).

GEMSP was originally conceived for the treatment of the secondary progressive form of MS and it is a combination of amino acids, fatty acids, free radical scavengers and antioxidants linked to PLL (6,7,9). The beneficial effects of GEMSP have been demonstrated in acute and chronic experimental autoimmune encephalomyelitis (EAE) models (10,11). Subsequently, an open clinical trial stated that following treatment with GEMSP for 6 months, the Expanded Disability Status Scale (EDSS) improved in 18% of patients and remained unchanged in 55% (1). Due to the promising results found in

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preclinical and clinical studies, another study with a larger number of MS patients (n=102) was conducted (6) and again, treatment with GEMSP was beneficial for 72% of patients. The present study follows up the evolution of the disease in a larger population of MS patients (n=193) that received GEMSP treatment.

## Materials and methods

**Population description.** The present study was performed in 193 volunteer patients [152 women (78.8%) and 41 men (21.2%)]. The patients provided written informed consent and treatment subsequently commenced. The study was national, multicentre, non-blinded and non-randomized, and the experimental unit was the patient. The EDSS score (9) was evaluated, and treatment with GEMSP (M) and worldwide reference (R) scores were established and compared. The M score was evaluated in each patient following clinical evaluation, whereas the R score was the estimated theoretical international score evolution (0.25 points/year/patient). The period of treatment with GEMSP was from 150 to 7,709 days (21.1 years) and the median was 1,289 days (3.5 years). The following data for each patient affected by MS and treated with GEMSP were collected: Age, gender, date of diagnosis, start/termination of GEMSP treatment and the MS Assessment Questionnaire follow-up for each patient over time. In addition, the evolution of the EDSS score was tracked during the study. A plot of the evolution of the EDSS score (R) over time was conducted. The slope (P=0.25) corresponds to the mean R of the speed of EDSS evolution (0.25 point/year), allowing the evolution of individual scores to be compared with the worldwide mean trend of progression. Finally, the duration of the GEMSP treatment, the number of medical consultations and the interval between these consultations were also taken into consideration.

**Score.** The individual EDSS introduced fluctuations along time. In order to assess the total effect of treatment with GEMSP for each patient, the individual mean of EDSS evolution was determined over time as follows: i) When the individual mean rate of EDSS evolution with GEMSP was greater or equal to the mean speed of the worldwide EDSS reference, this indicated disease progression (a worsening of the state of the patient); ii) when the individual mean rate of EDSS evolution with GEMSP was recorded to be between the mean speed of the worldwide EDSS reference and 0, this indicated a decrease in the progression of the disease; iii) when the individual mean rate of EDSS evolution with GEMSP was equal to 0, this indicated a stabilization of the disease; and iv) when the individual mean rate of EDSS evolution with GEMSP was below 0, the evolution of the disease was reversed (an improvement of the disease and a recovery from lesions occurred).

**Statistical analysis.** In order to overcome the high variability of the MS cohort included in the current study, a method of adapted valuation was applied as previously described (6). Thus, the Mann-Whitney U test was used to compare the current population with the population of reference [where the score evolution of each patient was compared with the worldwide reference score (R)], allowing the comparison of patients with each other and to assess the efficacy of treatment

with GEMSP. P<0.05 was considered to indicate a statistically significant difference and analyses were performed using the SAS Institute Inc., version 9 software (Cary, NC, USA); the conditions of the test application were verified in order to confirm that the statistics were correctly conducted. A comprehensive study of the EDSS score and the EDSS evolution was conducted. In the descriptive analysis, the following parameters were investigated: Strength, percentage, distribution, minimum, 1<sup>st</sup> quartile, median, 3<sup>rd</sup> quartile, maximum (Max), average, standard deviation and 95% confidence interval. In the comparative analysis, a t-test between the M group and R group final scores was performed. Equality of the variance was verified using the Folded F test. In the descriptive analysis the following parameters were evaluated: Strength, percentage and distribution. Regarding the analysis of the evolution of the data by a linear regression model, the following parameters were analyzed for the M and R scores: Coefficient of correlation, R<sup>2</sup> and the associated P-value, intercept and slope (ax + b). For comparison of the success/failure rates, the  $\chi^2$  test or Fisher's exact test were applied according to the theoretical strength obtained by unilateral assumption (% success > % failure).

Furthermore, a qualitative study of the EDSS score (global study) was conducted. In the descriptive analysis the following parameters were evaluated: Strength, percentage and distribution. For comparison of the success/failures rates: The  $\chi^2$  test or Fisher's exact test were performed according to the theoretical strength obtained by unilateral assumption (% success > % failure).

**Dose and synthesis of GEMSP.** GEMSP (15 mg per day) was administered via the sublingual route. GEMSP was synthesized according to patent numbers 792167 (EU) and 6114388 (USA) (6,9). GEMSP is a 'tailor-made' mixture of functional polypeptides: Fatty acids linked to PLL (e.g., thioctic acid, oleic acid and linoleic acid), antioxidants linked to PLL (e.g., ascorbic acid), free radical scavengers and amino acids linked to PLL (e.g., taurine, cysteine and methionine).

## Results

**General considerations.** Patients included in the current study were born between the 1<sup>st</sup> September 1937 and the 1<sup>st</sup> June 1994 (59% of patients were born between 1951 and 1970). The diagnosis of MS was performed between the 1<sup>st</sup> October 1962 and the 28<sup>th</sup> April 2011 (44% of cases were diagnosed between 1996 and 2005). The diagnosis of the disease was performed when patients were aged between 12 and 62 years (median, 36 years). The beginning of the treatment with GEMSP was between the 1<sup>st</sup> September 1994 and the 1<sup>st</sup> August 2011 (median, 1<sup>st</sup> November 2006). The majority of patients began the treatment after the year 2005. The delay between the date of diagnosis and the beginning of the treatment with GEMSP was between 0 and 500 months (median, 106 months). Upon inclusion in the study, global EDSS scores were between 0 and 8.5 (median, 5); the mean score was 4.4. The median of the delay between the date of the EDSS score evaluation and the date of the treatment with GEMSP was 31 days (mean delay, 301 days). The follow-up period was between 150 and 7,709 days [21.1 years; median, 1,289 days (3.5 years)]. The number of

Table I. Expanded Disability Status Scale global study (n=193).

Parameter	Score at inclusion	Final M score	Final R score
Minimum	0.00	0.00	0.25
Quartile, 25%	2.50	2.00	3.50
Median	5.00	5.50	5.82
Quartile, 75%	6.50	6.50	7.23
Maximum	8.50	8.50	10.00
Mean ± SD	4.36±2.30	4.44±2.48 <sup>a</sup>	5.43±2.43
95% CI	4.03-4.68	4.08, 4.79	5.08, 5.77

<sup>a</sup>P<0.0001 vs. Final R score. M, treatment with GEMSP; R, worldwide reference; SD, standard deviation; CI, confidence interval.

Table II. Success/failure global evolution (n=193).

Evolution	Distribution, n (%)	Success, n (%)
Improvement	51 (26.4)	150/193 (77.7) <sup>a</sup>
Deceleration	99 (51.3)	
Degradation	43 (22.3)	

Where failure means degradation, and success is equal to the sum of deceleration and improvement. <sup>a</sup>P<0.0001: Improvement + deceleration vs. degradation.

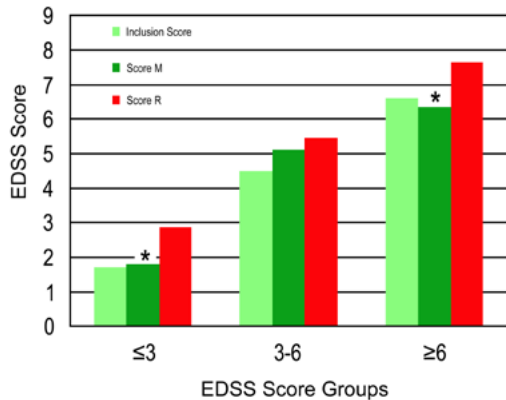


Figure 1. Comparison between the EDSS mean scores. <sup>a</sup>P<0.0001 vs. R group. M, treatment with GEMSP; R, worldwide reference. EDSS, Expanded Disability Status Scale.

visits was between 1 and 29 (median, 7). Finally, the median interval between visits was 121 days.

**EDSS global study.** A statistically significant difference (P<0.0001) was observed between the EDSS mean scores of the M and R groups (Fig. 1 and Table I). The EDSS mean score of the M group was 4.44 and that of the R group was 5.43. Regarding the EDSS mean score upon inclusion in the study, an increase of 1.8% was observed in the M group, whereas in

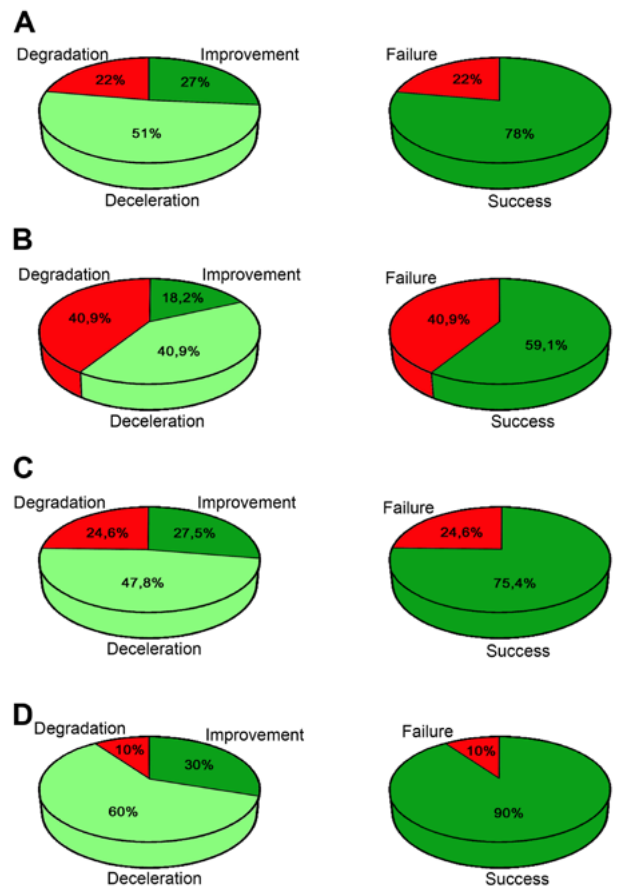


Figure 2. (A) Global evolution of patients. Evolution of patients with inclusion score (B) ≤3, (C) 3-6 and (D) ≥6. Where degradation refers to patients that did not respond to the therapy and evolution follows the R score. Deceleration refers to an M score evolution that is lower than the R score evolution. Improvement indicates scores of patients that were lower at the end of the study than upon inclusion. Failure means degradation, and success is equal to the sum of deceleration and improvement. M, treatment with GEMSP; R, worldwide reference.

the R group the increase was 24.5%. In 193 patients treated with GEMSP, 22% showed a worsening of their state (degradation); 51% showed a decrease in the progression of the disease (deceleration) and 27% showed a reversal of the disease evolution, which is considered to be an improvement in their status (Fig. 2A; Table II). Thus, 78% of patients showed an improvement or deceleration of the disease (Fig. 2A). The difference between the percentages of success and failure was identified to be significantly different (P≤0.0001).

**EDSS score depending on the score upon inclusion in the study.** A statistically significant difference was identified between the EDSS mean scores of the M and R groups for the EDSS score ≤3 (P=0.0001) and ≥6 (P<0.0001), although this difference was not significant for the EDSS score 3-6 (Fig. 1 and Table III; P=0.20). According to the final EDSS scores and in comparison with the EDSS inclusion scores, the percentages of improvement for the M group were as follows: 62% (score ≤3), 7.8% (score 3-6) and 19.6% (score ≥6). Regarding the qualitative evolution of the EDSS scores, a statistically significant difference was identified when comparing the M and R group mean evolutions (for the scores of ≤3 and ≥6), but not in the case of score 3-6. The percentages of improvement

Table III. Expanded Disability Status Scale score depending on the score at inclusion.

A, Score  $\leq 3$ 

Parameter	Inclusion score	Final M score	Final R score
Patients, n	69	69	69
Minimum	0	0	0.25
Quartile, 25%	1.00	1.00	1.75
Median	2.00	1.50	2.96
Quartile, 75%	3.00	2.50	3.81
Maximum	3.00	7.00	7.53
Mean $\pm$ SD	1.71 $\pm$ 1.13	1.80 $\pm$ 1.47 <sup>a</sup>	2.86 $\pm$ 1.63
95% CI	1.44, 1.98	1.45, 2.16	2.47, 3.25

## B, Score 3-6

Parameter	Inclusion score	Final M score	Final R score
Patients, n	44	44	44
Minimum	3.50	1.00	3.66
Quartile, 25%	4.00	4.50	4.84
Median	4.50	5.50	5.60
Quartile, 75%	5.00	6.00	5.90
Maximum	5.50	7.00	8.52
Mean $\pm$ SD	4.48 $\pm$ 0.68	5.11 $\pm$ 1.49 <sup>b</sup>	5.46 $\pm$ 0.92
95% CI	4.27, 4.69	4.66, 5.57	5.18, 5.74

C, Score  $\geq 6$ 

Parameter	Inclusion score	Final M score	Final R score
Patients, n	80	80	80
Minimum	6.00	1.00	6.24
Quartile, 25%	6.00	6.00	6.83
Median	6.50	6.50	7.50
Quartile, 75%	7.00	7.00	8.20
Maximum	8.50	8.50	10.0
Mean $\pm$ SD	6.58 $\pm$ 0.59	6.33 $\pm$ 1.38	7.62 $\pm$ 0.99
95% CI	6.44, 6.71	6.02, 6.64	7.40, 7.84
P-value		<0.0001	

<sup>a</sup>P<0.0001 vs. Final R score; <sup>b</sup>P<0.2 vs. Final R score. M, treatment with GEMSP; R, worldwide reference; SD, standard deviation; CI, confidence interval.

for the M group were as follows: 49.3% (score  $\leq 3$ ), 79.1% (score 3-6) and 19.3% (score  $\geq 6$ ).

*Evolution depending on the score upon inclusion.* A statistically significant difference was observed between the percentages of success and failure for the EDSS scores 3-6 (P<0.0001) and  $\geq 6$  (P<0.0001). However, the EDSS score  $\leq 3$  was at the limit of significance (P=0.088; Table IV). In the case of those patients with an EDSS score  $\leq 3$ , the following

Table IV. Evolution depending on the score at inclusion.

Score	Evolution	Distribution, n (%)	Success, n (%)
$\leq 3$	Improvement	8 (18.2)	26/44 (59.1)
	Deceleration	18 (40.9)	
	Degradation	18 (40.9)	
3-6	Improvement	19 (27.5)	52/69 (75.4) <sup>a</sup>
	Deceleration	33 (47.8)	
	Degradation	17 (24.6)	
$\geq 6$	Improvement	24 (30.0)	72/80 (90.0) <sup>a</sup>
	Deceleration	48 (60.0)	
	Degradation	8 (10.0)	

Where failure means degradation, and success is equal to the sum of deceleration and improvement. <sup>a</sup>P<0.0001: Improvement + deceleration vs. degradation.

results were observed (Fig. 2B and Table IV): Improvement (18.2%), deceleration (40.9%) and degradation (40.9%); that is, success (59.1%) and failure (40.9%). For patients with an EDSS score 3-6, the following results were observed (Fig. 2C and Table IV): Improvement (27.5%), deceleration (47.8%) and degradation (24.6%); that is, success (75.4%) and failure (24.6%). For patients with an EDSS score  $\geq 6$ , the following results were observed (Fig. 2D and Table IV): Improvement (30%), deceleration (60%) and degradation (10%); that is, success (90%) and failure (10%). Thus, the three groups exhibited a percentage of success between 59.1 and 90.0% (Fig. 2B-D).

## Discussion

Approved MS treatments are focused towards the relapsing-remitting phases, but not the progressive phases. Inflammation is important during the relapsing-remitting phase of MS, but not during the secondary progressive phase (6). The majority of the therapeutic agents currently tested for the treatment of MS act against inflammatory processes. However, existing data demonstrate that the inflammatory processes are not exclusive in the pathogenesis of MS, as there are other mechanisms involved (e.g., oxidative stress, axonal injury and neuronal loss) (5,12). A global treatment for MS, including the progressive phases, is required (5). Endotherapy takes into consideration environmental, immunological and bacterial factors, and is proposed as a treatment for chronic incurable diseases exhibiting a multifactorial etiology (e.g., MS and amyotrophic lateral sclerosis). Therefore, GEMSP was developed as an anti-inflammatory therapeutic strategy (the therapeutic agent contains fatty acids), as well as for myelin/neuron protection (1,12). GEMSP contains compounds (e.g., vitamins), which are effective against nitrosative and oxidative stress (1).

The linkage of heterogeneous molecules (e.g., amino acids, vitamins and fatty acids) to PLL offers various advantages as follows (6): Molecule stability; prevention of metabolic degradation of the linked molecules; improvement of the kinetics, 120

1 and increased half-life and permeability of membranes.  
 2 Furthermore, in GEMSP, the different molecules are linked to  
 3 PLL by reduced glutaraldehyde linkages resulting in particu-  
 4 larly flexible bonds (free conformation in space improves  
 5 interactions and facilitates access to the lesion site) (13). The  
 6 beneficial effects of GEMSP only occur when the molecules  
 7 are linked to PLL, since free constituents (not linked to PLL)  
 8 are less active against MS symptoms and it seems that these  
 9 free molecules either degrade or are rapidly incorporated into  
 10 the metabolism (1).

11 In preclinical studies (acute model of EAE) (10), it has been  
 12 reported that GEMSP (0.75 mg/day) inhibited brain leukocyte  
 13 infiltration (10). Later, in a chronic model of EAE, it was demon-  
 14 strated that GEMSP (7.5 mg/day) abolished EAE episodes, the  
 15 clinical score and brain leukocyte infiltration. In addition, one  
 16 compound of the GEMSP (methionine) was located in the  
 17 motoneurons of the spinal cord (11). This finding indicates that  
 18 GEMSP may be involved in the neutralization of free radi-  
 19 cals, exerting a neuroprotective action by inhibiting apoptotic  
 20 mechanisms (11). Furthermore, in a chronic EAE model, it was  
 21 demonstrated that GEMSP protected and enhanced forma-  
 22 tion of the myelin sheath (14). In a phase IIa trial, 22 patients  
 23 were treated with GEMSP (0.75 mg/day, sublingual route) for  
 24 six months. In 55% of patients, the EDSS was stable and in  
 25 18% the EDSS decreased (instead of a normal progression of  
 26 0.25 points on the mean EDSS scale) (1). Furthermore, 28% of  
 27 patients did not react to the GEMSP treatment. No side effects  
 28 (biological, hematological or hepatic) were observed and hence  
 29 GEMSP was demonstrated to be safe. In addition, no toxicity  
 30 was observed in experimental animals (10 mg/kg, single  
 31 intravenous injection) or in humans (11). Another study was  
 32 conducted where the MS patients (n=102) received 15 mg/day  
 33 GEMSP (sublingual route) for ranging from 3 months to  
 34 15 years and the results were as follows: 28% of patients  
 35 showed a worsening of their state; 20% showed a decrease in  
 36 the progression of the disease; 35% showed an improvement  
 37 of their state; and 17% showed stabilization. That is, 72% of  
 38 patients showed a positive evolution of the disease (6). The  
 39 present study (n=193) validates the favorable previous clinical  
 40 study results, with 22 and 102 patients, that were previously  
 41 published (1,6). However, it is important to remark that the  
 42 positive effect of treatment with GEMSP differs depending  
 43 on the initial EDSS score of the patients. Thus, with a higher  
 44 score, a greater positive effect of the treatment is observed:  
 45 59.1% of patients with initial EDSS scores  $\leq 3$  showed a posi-  
 46 tive evolution; when the initial EDSS scores were between 3-6,  
 47 the positive effect was found in 75.3% of patients, increasing  
 48 to 90.0% when the initial EDSS scores were  $\geq 6$ . In the  
 49 previous study performed in 102 patients (6), the percentages  
 50 for the three groups (scores  $\leq 3$ , 3-6 and  $\geq 6$ ) were as follows:  
 51 74, 62 and 73%, respectively. In order to compare the results  
 52 found in the current study (n=193) with those published in the  
 53 previous work (n=102) (6), it is important to remark that the  
 54 MS patients of the latter study are included in the present work  
 55 with an extended follow-up period. Regarding the group in  
 56 which the initial EDSS scores at inclusion were  $\leq 3$  it was found  
 57 that, in the present study, the absence of efficacy of GEMSP  
 58 treatment increased from 26 to 40.9% and that the improve-  
 59 ment of the treatment decreased from 33 to 18.2% when  
 60 compared with the previous study (n=102). The deceleration in

61 the progression of the disability was similar in the two studies  
 62 (40.9 and 41%). Regarding the group in which the initial EDSS  
 63 scores at the inclusion were 3-6, the absence of effectiveness  
 64 of GEMSP treatment decreased from 38 to 24.6%; decelera-  
 65 tion in disability progression increased from 19 to 47.8% and  
 66 the improvement decreased from 43 to 27.5%. Regarding the  
 67 group in which the initial EDSS scores at inclusion were  $\geq 6$ ,  
 68 the absence of efficacy of GEMSP treatment decreased from  
 69 26 to 10%; deceleration in disability progression increased  
 70 from 40 to 60% and the improvement was similar in the two  
 71 studies (33 and 30%).

72 Compared with other therapeutic agents, treatment with  
 73 beta-interferon was demonstrated to be less efficient than  
 74 treatment with GEMSP in the present study, as the former  
 75 decelerates the progression of disability, whereas GEMSP  
 76 improves the disability score. This finding is consistent with  
 77 the results published in a previous study (n=102) (6). Approved  
 78 therapeutic agents for the treatment of MS exert marked side  
 79 effects. Alemtuzumab is more effective than GEMSP, but the  
 80 undesirable effects of alemtuzumab are important (e.g., infec-  
 81 tion, immune thrombocytopenic purpura and thyroid disorder)  
 82 as are those that are caused by the use of beta-interferon (e.g.,  
 83 elevated liver enzymes, leukopenia, influenza-like syndrome,  
 84 formation of neutralizing antibodies). However, no side effects  
 85 were experienced when GEMSP was administered (1,6,9).  
 86 GEMSP contains compounds that are not foreign, but are  
 87 endogenous; these compounds are known by the organism and,  
 88 for this reason, the clinical trials performed to date have shown  
 89 a good safety profile for GEMSP (neither adverse effects nor  
 90 toxicity have been reported). Furthermore, other therapeutic  
 91 agents, such as linomide, mitoxantrone and natalizumab are  
 92 associated with considerable side effects (1). Conventional  
 93 therapeutic strategies (e.g.,  $\beta$ -interferon and glatiramer  
 94 acetate) are based on immunomodulatory drugs modifying  
 95 the number of immunological cells (1,15), whereas GEMSP  
 96 counteracts the inflammatory mechanisms, as well as other  
 97 pathogenic mechanisms (e.g., oxidative stress, demyelination  
 98 and neurotoxicity) (12).

99 In the pathogenesis of MS, oxidative stress is impor-  
 100 tant (16). In GEMSP, the presence of vitamins and certain  
 101 amino acids and their derivatives (alpha-tocopherol-succinate,  
 102 ascorbic acid, taurine and 5-methoxy-tryptamine) are impor-  
 103 tant neuroprotective components and radical scavengers,  
 104 exerting a crucial role against nitrosative and oxidative  
 105 stress (1,11). Cysteine and methionine also act as antioxi-  
 106 dants and scavengers contributing to reduced apoptosis and  
 107 neuronal death induced by reactive oxygen species (17).  
 108 Alpha-tocopherol and neurotransmitters, such as histamine  
 109 and 5-methoxy-tryptamine and amino acids, such as histidine  
 110 act as neuroprotective agents (1,18). Polyunsaturated fatty  
 111 acids exert a neuroprotective effect and act as free radical  
 112 scavengers preventing oxidation of cell membrane unsaturated  
 113 fatty acids (19,20). GEMSP protects and enhances the forma-  
 114 tion of myelin sheath (11,14). Deficiencies in essential fatty  
 115 acids impair myelin synthesis (21) and hence it is possible that  
 116 the fatty acids present in GEMSP are involved in the process  
 117 of remyelination, as observed in a chronic EAE model (14).  
 118 Additionally, it is known that the oleic acid, present in GEMSP,  
 119 acts as a neurotrophic factor in neurons (22) and that fatty  
 120 acids exert anti-inflammatory activity (7).

Previous studies have proposed the involvement of bacteria in the pathogenesis of MS (23-25). Commensal bacteria usually protect the organism, however, may cause autoimmune processes when bacteria pass through the mucosal epithelium to the submucosal tissues. Certain bacterial components act as super-antigens, and induce the proliferation of autoreactive lymphocyte clones and the raising of autoantibodies. In response to lipopolysaccharides (LPS), immune cells produce reactive oxygen species and pro-inflammatory mediators, which are involved in oxidative stress, the demyelination processes and axonal damage (26). Systemic injection of LPS leads to the invasion of the brain by granulocytes and a breakdown of the blood-brain barrier (27). This breakdown may allow the toxins produced by the bacteria to enter the central nervous system. Inside the central nervous system, LPS may exert direct neurotoxic action on microglial cells and LPS is associated with extensive oligodendrocyte death due to LPS-induced neurotoxicity (28). LPS may bind to the blood-brain barrier through their lipid A (gram-negative bacteria) and cross the barrier. Lipid A provides the hydrophobic anchor that secures the molecule within the membrane, while the polysaccharide component interacts with the external environment, including the defences of the host species (29). Thus, an important goal would be to inhibit this induction process by preventing the interaction of bacteria with target cells. In this sense, an amide linkage to fatty acid is preferable to an unstable ester linkage to achieve the same fatty acid presentation as the lipid A component of the LPS of gram-negative bacteria. The similar structure of lipid A and fatty acid, linked to PLL, leads to the neutralization of LPS through the onset of lipid bilayer formation. This approach may prevent LPS from linking to target cells and may allow the inhibition of chronicity factors (6).

In conclusion, preclinical and clinical data suggest that GEMSP presents a novel therapeutic agent/approach for the treatment of MS. GEMSP targets the different aspects of MS rather than the inflammatory aspect alone. GEMSP decreases inflammatory mechanisms, controls oxidative stress, counteracts demyelination, acts as a neuroprotector and fights chronic factors. The present data, using a larger population of MS patients than previous clinical studies (1,6), confirms the beneficial effects of GEMSP that were previously reported.

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