Brain Research Bulletin 137 (2018) 140–145

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Comparative of transcranial magnetic stimulation and other treatments in experimental autoimmune encephalomyelitis

Francisco J. Medina-Fernandez[a,b], Begoña M. Escribano[a,c], Evelio Luque[b,d], Javier Caballero-Villarraso[b,e], Jose L. Gomez-Chaparro[a,b], Montserrat Feijoo[a], Fe I. Garcia-Maceira[f], Alvaro Pascual-Leone[g], René Drucker-Colin[h,1], Isaac Tunez[a,b,*]

[a] *Departamento de Bioquimica y Biologia Molecular, Facultad de Medicina y Enfermeria, Universidad de Cordoba Cordoba, Spain*
[b] *Instituto Maimonides de Investigacion Biomedica de Cordoba (IMIBIC), Cordoba, Spain*
[c] *Departamento de Biologia Celular, Fisiología e Inmunología, Universidad de Cordoba, Cordoba, Spain*
[d] *Departmento de Ciencias Morfologicas, Seccion de Histologia, Facultad de Medicina y Enfermeria, Universidad de Cordoba, Spain*
[e] *Unidad de Gestion Clinica de Analisis Clinicos, Hospital Universitario Reina Sofia de Cordoba, Cordoba, Spain*
[f] *Canvax Biotech S.L., Cordoba, Spain*
[g] *Berenson-Allen Center for Noninvasive Brain Stimulation, Division of Cognitive Neurology, Department of Neurology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, USA*
[h] *Departmento de Neuropatología Molecular, Instituto de Fisiologia Celular, Universidad Nacional Autonoma de Mexico (UNAM), Ciudad de Mexico, D.F., Mexico*

ARTICLE INFO

Keywords:
Antioxidant
Clinical drugs
Experimental autoimmune encephalomyelitis
Oxidative stress
Relapsing-remitting multiple sclerosis
Transcranial magnetic stimulation

ABSTRACT

The effects of transcranial magnetic stimulation (TMS), natalizumab (nata), dimethyl fumarate (DMF) and dexamethasone (DEX) on clinical score and oxidative stress produced by a single dose of myelin oligodendrocyte glycoprotein (MOG) in tail of Dark Agouti rats was studied. TMS (60 Hz and 0.7 mT), nata (5 mg/kg), DMF (15 mg/kg) and DEX (300 μg/kg) was applied for 21 after the administration of MOG (150 μg). We estimated clinical score, as well as lipid peroxides, carbonylated proteins and reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio content in brain, spinal cord and blood. MOG triggered significant increase in clinical score and in the levels of lipid peroxides and carbonylated proteins levels, but reduced GSH/GSSG ratio in brain, spinal cord and blood. Both TMS and clinical treatments, although TMS more significantly, decreased the changes caused by MOG administration. These results support the antioxidant and neuroprotective action of TMS, as well as an activity higher than other clinical treatments.

1. Introduction

Different treatments that modify the natural history of the disease (disease-modifying drugs) are applied to patients with multiple sclerosis (MS).

Drugs in the front line of treatment are: beta interferons, glatiramer acetate (Copaxone®, TEVA) and dimethyl fumarate (DMF, Tecfidera®, Biogen). DMF induces to nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and thereby inhibits the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB). The former triggers the activation of the protein antioxidant system, while the latter brings with it a reduction in levels of proinflammatory molecules (Dargahi et al., 2017).

Those in the second line of treatment are: Fignolimod (Gilenya®, Novartis Farmaceutica), Alemtuzumab (Lemtrada®, Genzyme) and Natalizumab (nata, Tysabri®, Biogen). Nata is a monoclonal antibody against alpha-1 subunits of integrins. This action inhibits its interactions with the vascular cell adhesion molecule 1 (VCAM-1), blocking the transmigration of lymphocytes and monocytes from the blood to the Central Nervous System (CNS) (Dargahi et al., 2017).

Finally, the symptoms appearing during a relapse are often treated with corticosteroids such as Methylprenidsolone, Prednisone or Dexamethasone (DEX). DEX is probably the most potent corticoid. It is a synthetic glucocorticoid with an immunosuppressive and anti-inflammatory capacity (Lattanzi et al., 2017).

All the above have shown their efficacy in both the clinical treatment of MS and in the model, experimental autoimmune encephalomyelitis (EAE). In addition, some studies have reported the beneficial effect of transcranial magnetic stimulation (TMS) on spasticity in patients with relapsing and remitting MS (RRMS) (Centonze et al., 2007; Mori et al., 2010), as well as the neuroprotective effect of

* Corresponding author at: Department Bioquimica y Biologia Molecular, Facultad de Medicina y Enfermeria, Universidad de Cordoba, Av. Menedez Pidal s/n, 14004, Cordoba, Spain.
E-mail address: fm2tufii@uco.es (I. Tunez).

[1] Deceased.

https://doi.org/10.1016/j.brainresbull.2017.11.018
Received 30 September 2017; Received in revised form 19 November 2017; Accepted 28 November 2017
Available online 01 December 2017
0361-9230/

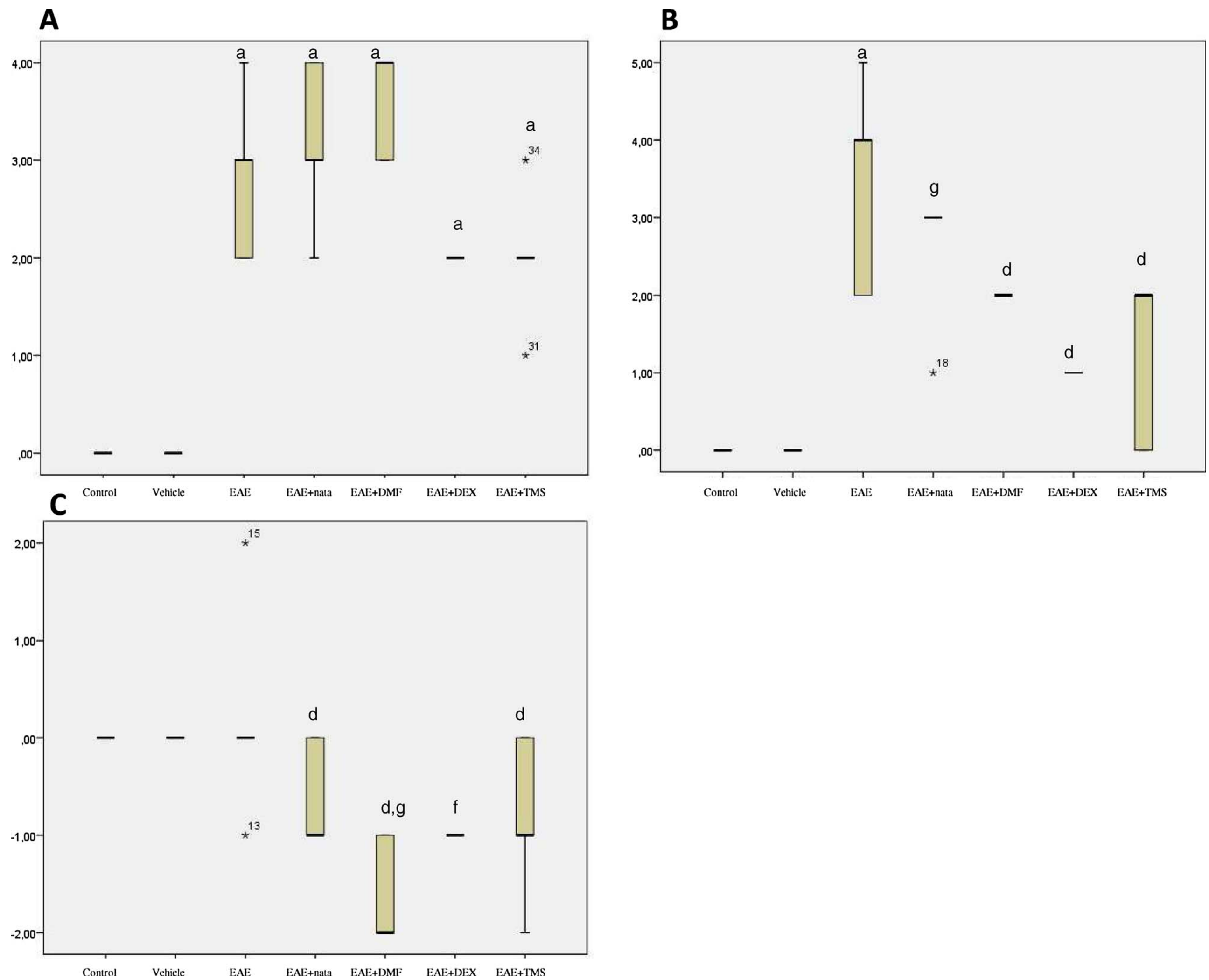

Fig. 1. Changes in clinical score induced by MOG and other treatments.
Clinical scores data are represented as median and box plot with 25th and 75th percentiles. A: Values of clinical score at 14 after MOG administration. ANOVA: dF: 6, F. 33.000, Significance: 0.000; B: Values of clinical score after 35 day after MOG administration and 24 days after beginning treatments. ANOVA: dF: 6, F: 15.000, Significance: 0.000; and C: Data represent the increase occurred between 21st and 35th day (35th – 21st clinical score). ANOVA: dF: 6, F: 5.000, Significance: 0.000.
$^{a}P < 0.001$ vs Control; $^{d}P < 0.001$ vs EAE; $^{f}P < 0.05$ vs EAE; $^{g}P < 0.001$ vs EAE + TMS; $^{h}P < 0.01$ vs EAE + TMS; $^{i}P < 0.05$ vs EAE + TMS.
EAE: Experimental autoimmune encephalomyelitis; nata: Natalizumab; DMF: Dimethyl fumarate; DEX: Dexamethasone; TMS: Transcranial magnetic stimulation

extremely low-frequency electromagnetic fields (EL-EMF), as a paradigm of TMS on EAE model (Sherafat et al., 2012; Zhivolupov et al., 2012; Medina-Fernandez et al., 2017a; Medina-Fernandez et al., 2017b). In addition, the neuroprotective effect of TMS with low intensity may explain interesting therapeutic effects of TMS in clinical activity such as the decreased progression rate with repetitive TMS compared with levodopa in Parkinson's disease (Mály et al., 2004, 2017).

Based on this knowledge, we hypothesized that TMS (EL-EMF) presents the same antioxidant and therapeutic potential as other clinical treatments (nata, DMF and DEX), our aim being to compare the antioxidant effects of TMS to clinical treatments like nata, DMF and DEX, and displaying that: i) the antioxidant effect underlies between mechanisms involved in the effects of therapeutic treatment and TMS; and/or ii) the concept of a molecule or agent with an antioxidant capacity should be reviewed and extended to any molecule and agent capable of reducing oxidative damage and/or inducing antioxidant systems.

2. Material and methods

2.1. Chemicals

Reagents of the highest quality were acquired from Sigma (St. Louis, MO, USA).

2.2. Animals

The rats, young-adult (8-week old) males from Janvier Labs (France) were housed under standard colony conditions: 12:12 light/darkness cycle (lights on at 7:00 a.m.), controlled room temperature (22 ± 2 °C), with free access to food and water. This study was carried out according to the guidelines of the Directive of 24 November 1986 (86/609/ECC) approved by the European Communities Council and RD 53/2013 passed by the Presidency Minister of Spain (BOE 8 February 2013). The protocols were approved by the Bioethics Committee at Cordoba University.

Table 1
Effects of treatments on oxidative damage in experimental autoinmune encephalomyelitis (EAE) models. A. Lipid peroxidation products; B. Carbonylated proteins. Values represent mean ± SD. n = 5 animals/group.

A	*Brain nmol/mg protein*	*Spinal Cord nmol/mg protein*	*Blood nmol/mg Hb*
Control	0.012 ± 0.016	0.055 ± 0.002	283 ± 129
Vehicle	0.061 ± 0.088	0.086 ± 0.029[b]	247 ± 107
EAE	0.277 ± 0.047[a]	0.248 ± 0.022[a]	401 ± 73[b]
EAE + nata	0.173 ± 0.026[a,d]	0.165 ± 0.089[a,d,g]	248 ± 60[d,h]
EAE + DMF	0.190 ± 0.018[a,f]	0.177 ± 0.059[d,g]	Non-data
EAE + DEX	0.005 ± 0.001[c,d,g]	0.007 ± 0.006[b,d]	0.10 ± 0.01[b,d,h]
EAE + TMS	0.170 ± 0.015[a,d]	0.004 ± 0.007[b,d]	13 ± 3[b,d]

B	*Brain pmol/mg protein*	*Spinal Cord pmol/mg protein*	*Blood pmol/mg Hb*
Control	0.038 ± 0.049	0.002 ± 0.001	0.092 ± 0.03
Vehicle	0.018 ± 0.036	0.001 ± 0.001	0.095 ± 0.06
EAE	0.080 ± 0.041[c]	0.227 ± 0.005[a]	0.207 ± 0.04[a]
EAE + nata	0.028 ± 0.032[f]	0.003 ± 0.002[d,h]	0.084 ± 0.02[d,g]
EAE + DMF	0.045 ± 0.031	0.002 ± 0.002[d,h]	Non data
EAE + DEX	0.003 ± 0.001[c,e,g]	0.001 ± 0.001[d]	0.008 ± 0.01[a,d,g]
EAE + TMS	0.055 ± 0.028	0.001 ± 0.001[d]	0.026 ± 0.01[a,d]

ANOVA: A) Brain: dF: 6, F: 22.00, Significance 0.000; Spinal cord: dF: 6, F: 21.000, Significance 0.000; Blood: dF:5, F: 19.000, Significance: 0.000. B) Brain: dF: 6, F: 2.000, Significance: 0.037; Spinal cord: dF: 6, F: 75.000, Significance: 0.001; Blood: dF: 5, F: 2.031, Significance: 0.000.
[a] $P < 0.001$ vs control.
[b] $P < 0.01$ vs control.
[c] $P < 0.05$ vs control.
[d] $P < 0.001$ vs EAE.
[e] $P < 0.01$ vs EAE.
[f] $P < 0.05$ vs EAE.
[g] $P < 0.001$ vs EAE + TMS.
[h] $P < 0.01$ vs EAE + TMS.

2.3. Experimental procedures

A total of 40 Dark Agouti rats weighing 190–220 g were used since this strain bears the closest clinical and pathologic resemblance to MS. The rats were divided into three groups of 5 animals per group: i) Control (the animal was healthy and not manipulated), ii) Vehicle (it was inoculated with complete Freund's adjuvant), iii) EAE (disease-induced with MOG), iv) EAE + Mock, v) EAE + nata, vi) EAE + DMF, vii) EAE + DEX, and viii) EAE + TMS.

2.4. Treatments

2.4.1. EAE induction

EAE induction was performed by injecting subcutaneously, at the dorsal base of the tail, 100 µl of a solution containing 150 µg myelin oligodendrocyte glycoprotein (MOG, fragment 35–55; Sigma–Aldrich, St. Louis, USA) in phosphate buffered saline (PBS) emulsified 1:1 in complete Freund's adjuvant (Sigma–Aldrich, St. Louis, USA). To complete the adjuvant, 400 µg of heat-inactivated *Mycobacterium tuberculosis* (H37Ra, DIFCO, Lawrence, KS, USA) was added. Vehicle induction was performed in control-group (Vehicle) animals by subcutaneous injection of 100 µl of complete Freund's adjuvant.

2.4.2. Natalizumab

Natalizumab (Nata, Tysabris®, Biogen Idec, Inc. and Elan Pharmaceuticals, Inc. Cambridge, MA, USA). Based on the dose of 300 mg delivered to MS patients once every 4 weeks, life equivalence and physiologic features between human and rat, nata was administered at doses of 5 mg/kg weight i.p. every 10 days for 21 days, which means that the animal received two doses of it (Escribano et al., 2017).

2.4.3. Dimethyl fumarate

Dimethyl fumarate (DMF, Sigma–Aldrich, St. Louis, USA). Oral intake makes it be rapidly metabolized originating methyl fumarate, which is a bioactive metabolite. The dose was 15 mg/kg weight by oral administration during a 21 day period (Milenkovic et al., 2008; Escribano et al., 2017).

2.4.4. Dexamethasone

Dexamethasone (DEX, Fortecortin®, Merck Farma and Quimica, Spain) is a glucocorticoid with a wide range of effects on the central nervous system (CNS); it was injected i.p. at 300 µg/kg daily for 21 days (Montilla et al., 2004).

2.4.5. Transcranial magnetic stimulation

Animals were placed in plastic cylindric cages designed to keep them immobile. Each coil consisted of 1000 turns of enameled copper wire (7 cm diameter) contained in plastic boxes (10.5 × 10.5 × 3.5 cm). A pair of Helmholtz coils generated the fields (Magnetoterapia S.A., Mexico). The two coils were positioned dorsally and ventrally to the head. The distance between each coil and the midpoint of the head was approximately 6 cm. The stimulation consisted of an oscillatory magnetic field in the form of a sinusoidal wave with a frequency of 60 Hz and amplitude of 0.7 mT (EL-EMF) applied for two hours in the morning, once a day, five days a week (Monday-Friday), during three weeks (days 14–35), in order to simulate clinical practice (modified from Drucker-Colin et al., 1994; Medina-Fernandez et al., 2017a; Medina-Fernandez et al., 2017b).

To adequately assess the effect of TMS, an appropriate mock group was designed. Animals in the TMS-Mock group were handled in the same way but without receiving real stimulation. The idea of this group was to study the effects of restraint stress caused by plastic cages (Medina-Fernandez et al., 2017a; Medina-Fernandez et al., 2017b).

At 35 days, the animals were sacrificed having been previously anesthetized with an intraperitoneal injection of Ketamine 75 mg/kg (Imalgene® 100 mg/ml, Merial Laboratorios). The blood obtained from neck vascular trunk was collected in tubes with EDTA-K_3. Tubes were centrifuged during 15 for minutes at 3000 rpm at 4 °C, proceeding immediately to the collection of plasma that was frozen and stored in aliquots at −85 °C.

Under controlled temperature conditions, it was proceeded to extract and weigh the brain and spinal cord and to immediately prepare the corresponding homogenates with a mechanical homogenizer (Tempest Virtis). The buffer used for homogenization was Tris (20 mM) at pH 7.4.

2.5. Evaluation of clinical score

The animals were followed at 14 and 35 days and scored in accordance with this severity scale; 0: no signs, 1: tail paralysis, 2: weakness in hind legs, 3: paralysis in hind legs, 4: paralysis in hind legs and weakness in front legs, 5: quadriplegic (Perez-Nievas et al., 2010; Escribano et al., 2017). The increase between score and disease was established (score at 35 days – score at 14 days) (Escribano et al., 2017).

2.6. Biochemical parameters

- Oxidative stress markers in brain, spinal cord and blood: LPO (nmol; lipid peroxides) and carbonylated proteins were measured (nmol).
- Redox glutathione system as antioxidant indicator: total glutathione (nmol; total glutathione), GSH (nmol; reduced glutathione), GSSG (nmol; oxidized glutathione) and ratio GSH/GSSG.
- All parameters were analyzed by spectrophotometry using a Shimadzu spectrophotometer (UV 1603; Kyoto, Japan) in the Departamento de Bioquimica y Biologia Molecular, Facultad de Medicina y Enfermeria, Universidad de Cordoba. The reagent kits

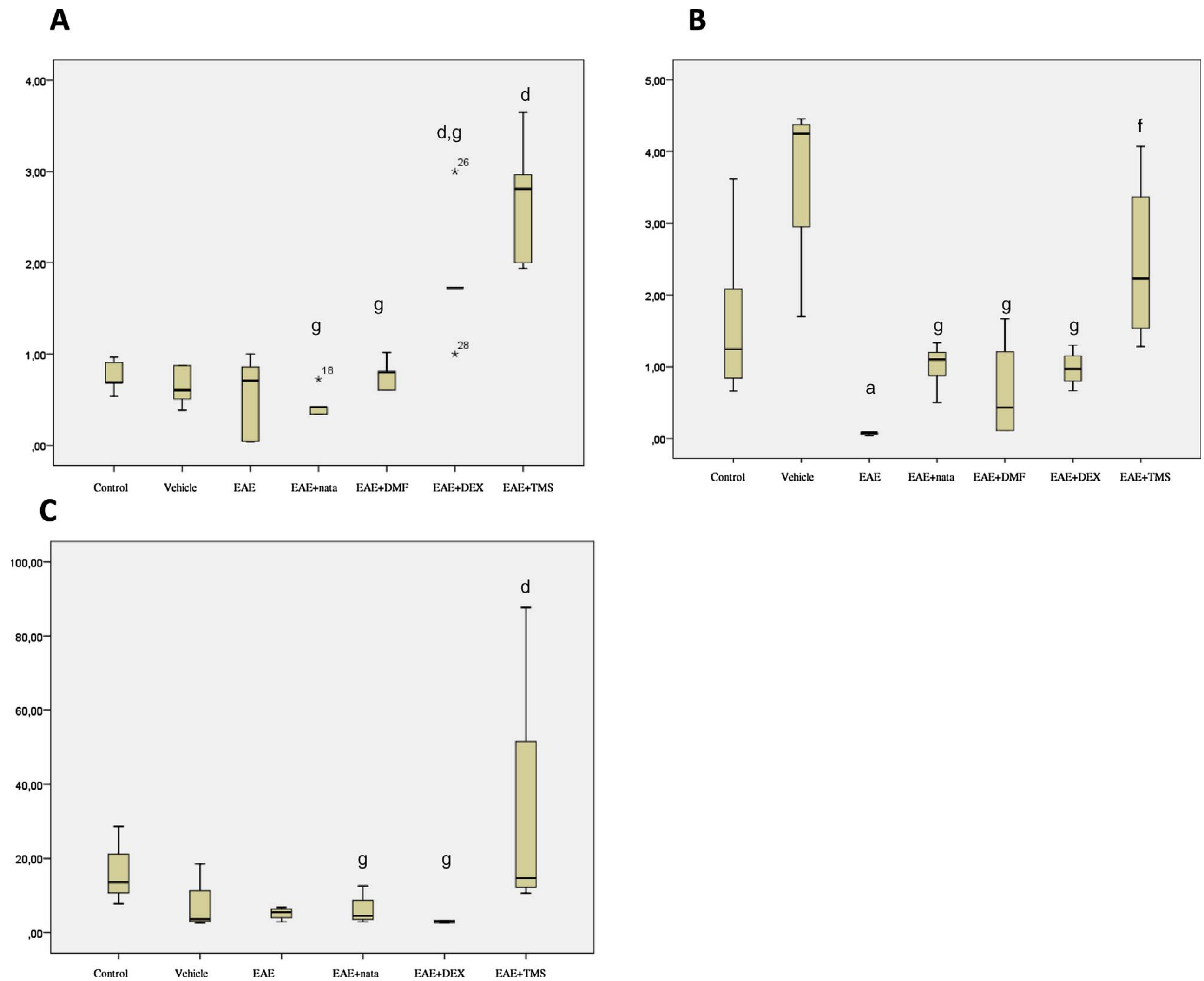

Fig. 2. Effects of nata, DMF, DEX and TMS on changes in levels of glutathione ratio induced by MOG. Values are represented as median and box plot with 25th and 75th percentiles. A: Brain. ANOVA: dF: 6, F. 17.000, Significance: 0.000; B: Spinal cord. ANOVA: dF: 6, F: 7.000, Significance: 0.000; and C: Blood. ANOVA: dF: 5, F: 1.000, Significance: 0.000.
[a]P < 0.001 vs Control; [d]P < 0.001 vs EAE; [f]P < 0.05 vs EAE; [g]P < 0.001 vs EAE + TMS; [h]P < 0.01 vs EAE + TMS.
EAE: Experimental autoimmune encephalomyelitis; nata: Natalizumab; DMF: Dimethyl fumarate; DEX: Dexamethasone; TMS: Transcranial magnetic stimulation

were: LPO 586 (LPO), GSH 420 (total glutathione) and GSH 400 (GSH), whereas the GSSG levels were calculated by subtracting GSH from total glutathione and carbonyl content was evaluated using the Levine et al. method (Levine et al., 1990). The data are expressed in mg of protein (brain and spinal cord) or g hemoglobin (blood).

Protein levels were measured by the Bradford method, using a B6916 assay kit supplied by Sigma-Aldrich (Madrid, Spain), while hemoglobin concentration was determined by Hemoglobin Drabkin Colorimetric methods purchased from Spinreact (Gerona, Spain).

2.7. Statistics

The statistical study was performed with the SPSS application (SPSS INC. Version 15 for Windows). The normality distribution of variables was analyzed by using the Shapiro Wilk test for $n < 40$. Once proven that the values had gone back to a normal distribution, a one-way analysis of variance (ANOVA) was conducted. To determine the concrete differences between the groups, a Bonferroni test was performed. The minimum significance level was 95% ($p < 0.05$). The results were expressed as arithmetic mean ± standard deviation (SD).

3. Results

The present data show that MOG induced a significant increase of paralysis in tail and limb of rats at 14 days (2.0 ± 0.00 in the EAE group vs 0.0 ± 0.0 in the Control group, $P < 0.001$) (Fig. 1). This clinical sign was reversed after 21 days of treatments with both TMS and nata, DMF and DEX (3.0 ± 1.0 in the EAE group vs 2.0 ± 0.001 in the EAE + nata group, 2.0 ± 0.001 in the EAE + DMF group, 2.0 ± 0.001 in the EAE + DEX group, 1.0 ± 1.095 in the EAE + TMS; non-significance, $P < 0.001$, $P < 0.001$, $P < 0.001$, respectively) (Fig. 1). This drop was more intense in TMS than in the other treatments (Fig. 1).

EAE led to increases in the levels of lipid peroxidation products and carbonylated proteins (Table 1), together with an important decline in the glutathione redox ratio in the same tissues (Brain: 0.59 ± 0.10 in the EAE group vs 0.76 ± 0.14 in the control group; Spinal cord: 0.0061 ± 0.024 in the EAE group vs 1.0 ± 1.01 in the Control group; Blood: 5.00 ± 1.00 in the EAE group vs 16.0 ± 10.0 in the Control group. Non-significant, $P < 0.001$, non-significant; respectively) (Fig. 2). Both TMS and nata, DMF and DEX were effective in restoring glutathione redox ratio (Fig. 2) and they inhibited lipid peroxidation and carbonylated proteins in brain, spinal cord and blood (Table 1).

Additionally, the data shows that lipid peroxidation products and carbonylated proteins levels presented by EAE are reduced by TMS and DEX by below those of healthy animals (control group) (Table 1).

4. Discussion

To our knowledge this is the first study that compares the effect of TMS versus other clinical treatments for patients with MS. The present study reveals for the first time that the therapeutic and antioxidant effects of TMS are superior to those of other clinical drugs used in the present work.

Although, previous data of our group found that TMS application to EAE model caused a therapeutic effect characterized by a reduction in: i) tail paralysis and hind limb paresis, ii) number of pyknotic nuclei, iii) astrocytes activation, iv) tarnishing of the fur, and v) edema (Medina-Fernandez et al., 2017a; Medina-Fernandez et al., 2017b).

MS is a chronic neuroinflammatory disease in which oxidative damage plays an important role (Lassmann, 2014). Due to its unclear etiology, a vicious circle is established between inflammation and oxidative stress that increases the numbers of relapses and permanent lesions. It mainly affects young adults, and it is the main cause of non-traumatic neurologic disability (Pugliatti et al., 2006). These facts have a very important impact on socio-economic, health and production systems, as well as on family dynamics (Grima et al., 2000; Naci et al., 2010; Ma et al., 2014). All this supports the design of strategies aimed at slowing down, improving its prognosis, or reversing this disease. Currently, neurologists treating patients with MS use a wide variety of drugs that are focused on changing the natural development of the disease.

As in previous studies, in the present work the administration of MOG induced an experimental model of MS, characterized by an increase in clinical score correlated with an oxidative damage (Escribano et al., 2017; Medina-Fernandez et al., 2017a). Similar findings were found in patients with MS (Tasset et al., 2012; Adamczyk and Adamczyk-Sowa, 2016).

On the other hand, TMS has shown its beneficial action on spasticity in MS patients (Centonze et al., 2007), as well as on a neuroprotective effect in its experimental model of EAE (Medina-Fernandez et al., 2017a; Medina-Fernandez et al., 2017b). This, coupled with the lack of significant side effects beyond headaches and some seizures in patients with epileptogenic foci, makes TMS a potentially useful tool in the treatment of MS (although further clinical studies would be necessary) as a treatment *per se* or as an adjuvant to others currently administered.

In line with previous studies made by our group (Medina-Fernandez et al., 2017a; Medina-Fernandez et al., 2017b), this research showed that the application of TMS causes an improvement in the clinical score presented by the rats with EAE. Also, the other treatments (nata, DMF and DEX) showed their effectiveness in the same way as in the work done by Begoña et al. (Escribano et al., 2017) and other authors in the clinical treatment (Tasset et al., 2013a; Saguil et al., 2014; Filippini et al., 2017).

The clinical score correlated with oxidative stress biomarkers and changes in the redox glutathione system. Thus, in the case of the EAE group, a higher clinical score was in concordance with oxidative damage characterized by increases in lipid peroxidation products and carbonylated proteins, and a reduction in the GSH/GSSG ratio. Similarly, the animals' clinical improvement was associated with decreases in oxidative stress markers and the glutathione ratio.

In this context, the greater effectiveness of TMS over any other treatment in treating symptoms and changes in the biochemical parameters analyzed was manifested. Based on the data obtained and those present in the scientific literature, it can be deduced that, at least partially, the effects appreciated in the TMS application are due to an important antioxidant action that enables it to be proposed as a new therapeutic strategy and an antioxidant agent.

The effects of TMS on oxidative stress biomarkers and antioxidant systems are in agreement with previous data published by our group in EAE model (Medina-Fernandez et al., 2017a), as well as in other experimental models of neuro-psychiatric pathologies such as Huntington's disease and the major depression induced by 3-nitropropionic acid or olfactory bulbectomy, respectively (Tunez et al., 2006; Tasset et al., 2010). In these models, TMS triggered a therapeutic action associated with decrease in lipid peroxides and carbonylated proteins, glutathione redox balance and the recovery of antioxidant enzyme activity. Interestingly, the results reveal that TMS reduces the levels of biomarkers of oxidative stress in spinal cord and blood below the control animals. Nevertheless, the explanation for this finding requires further studies.

The studies carried out on 3-nitropropionic acid model evidenced show TMS induced an augmentation of Nrf2, characterized by a higher concentration in cytoplasm and its translocation into the nucleus (Tasset et al., 2013b). This caused an increase in the detoxifying and antioxidant enzyme expression (Esteras et al., 2016). Taking all of this into account, and analyzing the effect of DMF on Nrf2 and the evolution of the disease, we could deduce that the effect of TMS is partially mediated by its effects on Nrf2 (Gopal et al., 2017).

Additionally, we have analyzed and contextualized the effect triggered by DEX and nata. DEX induces an immunosuppressive and antioxidant activity (Goodin, 2014; Wang et al., 2014), whereas the monoclonal antibody prevents the transmigration of leukocytes into CNS, with this effect inducing a significant oxidative stress reduction (Chataway and Miller, 2013).

All the above led us to hypothesize that a secondary immunomodulatory effect is developed by TMS in its neuroprotective effects. This action enhances the antioxidant and neurotherapeutic effect found. This idea would be endorsed by: i) TMS inducing Nrf2 and an antioxidant effect (Tasset et al., 2013b; Medina-Fernandez et al., 2017a); ii) Molecular cross-talk between Nrf2 and NFκB (Liu et al., 2008; Cuadrado et al., 2014; Wardyn et al., 2015); and iii) previous studies of our group and others showing how the application of TMS provokes an immunomodulation (Medina-Fernandez et al., 2017b).

The present study undoubtedly has some limitations such as: i) reduced number of animals per group, affecting the statistical power; and ii) the variables analyzed, focusing on oxidative damage. However, it opens up many new questions and future possibilities such as: i) Could the biological effects of TMS be associated with conformational changes in the proteins?; and ii) what roles do intra and intercellular communications play?, among others.

5. Conclusions

In brief, our data clearly show that, EL-EMF (60 Hz and 0.7 mT) as a new paradigm of TMS:

- It acts as an antioxidant
- At least partially, its effects are due to this antioxidant capacity
- It could be a novel therapeutic strategy in neurodegenerative processes, especially demyelinating illnesses with an oxidative and inflammatory etio-pathogenesis base such as MS

Finally, more preclinical and clinical studies are needed to clarify its therapeutic potential and action mechanisms.

Author contribution

IT and RDC designed research; BME, EL, FJMF and MF performed research; BME, FGM, FJMF, JCV and JLGCh analyzed data; APL, IT and RDC wrote the paper.

Acknowledgements

Authors thank Ms. Diana Badder for helping us with English editing and revision.

References

Adamczyk, B., Adamczyk-Sowa, M., 2016. New insights into the role of oxidative stress mechanisms in the pathophysiology and treatment of multiple sclerosis. Oxid. Med. Cell Longev. 2016 (1973834).

Centonze, D., Koch, G., Versace, V., Mori, F., Rossi, S., Brusa, L., Grossi, K., Torelli, F., Prosperetti, C., Cervellino, A., Marfia, G.A., Stanzione, P., Marciani, M.G., Boffa, L., Bernardi, G., 2007. Repetitive transcranial magnetic stimulation of the motor cortex ameliorates spasticity in multiple sclerosis. Neurology 68, 1045–1050.

Chataway, J., Miller, D.H., 2013. Natalizumab therapy for multiple sclerosis. Neurotherapeutics 10, 19–28.

Cuadrado, A., Martin-Moldes, Z., Ye, J., Lastres-Becker, I., 2014. Transcription factors NRF2 and NF-kappaB are coordinated effectors of the Rho family, GTP-binding protein RAC1 during inflammation. J. Biol. Chem. 289, 15244–15258.

Dargahi, N., Katsara, M., Tselios, T., Androutsou, M.E., de Courten, M., Matsoukas, J., Apostolopoulos, V., 2017. Multiple sclerosis: immunopathology and treatment update. Brain Sci. 7.

Drucker-Colin, R., Verdugo-Diaz, L., Mendez, M., Carrillo-Ruiz, J., Morgado-Valle, C., Hernandez-Cruz, A., Corkidi, G., 1994. Comparison between low frequency magnetic field stimulation and nerve growth factor treatment of cultured chromaffin cells, on neurite growth, noradrenaline release, excitable properties, and grafting in nigrostriatal lesioned rats. Mol. Cell. Neurosci. 5, 485–498.

Escribano, B.M., Medina-Fernandez, F.J., Aguilar-Luque, M., Aguera, E., Feijoo, M., Garcia-Maceira, F.I., Lillo, R., Vieyra-Reyes, P., Giraldo, A.I., Luque, E., Drucker-Colin, R., Tunez, I., 2017. Lipopolysaccharide binding protein and oxidative stress in a multiple sclerosis model. Neurotherapeutics 14, 199–211.

Esteras, N., Dinkova-Kostova, A.T., Abramov, A.Y., 2016. Nrf2 activation in the treatment of neurodegenerative diseases: a focus on its role in mitochondrial bioenergetics and function. Biol. Chem. 397, 383–400.

Filippini, G., Del Giovane, C., Clerico, M., Beiki, O., Mattoscio, M., Piazza, F., Fredrikson, S., Tramacere, I., Scalfari, A., Salanti, G., 2017. Treatment with disease-modifying drugs for people with a first clinical attack suggestive of multiple sclerosis. Cochrane Database Syst. Rev. 4.

Goodin, D.S., 2014. Glucocorticoid treatment of multiple sclerosis. Handb. Clin. Neurol. 122, 455–464.

Gopal, S., Mikulskis, A., Gold, R., Fox, R.J., Dawson, K.T., Amaravadi, L., 2017. Evidence of activation of the Nrf2 pathway in multiple sclerosis patients treated with delayed-release dimethyl fumarate in the Phase 3 DEFINE and CONFIRM studies. Mult. Scler., 1352458517690617.

Grima, D.T., Torrance, G.W., Francis, G., Rice, G., Rosner, A.J., Lafortune, L., 2000. Cost and health related quality of life consequences of multiple sclerosis. Mult. Scler. 6, 91–98.

Lassmann, H., 2014. Multiple sclerosis: lessons from molecular neuropathology. Exp. Neurol. 262 (Pt. A), 2–7.

Lattanzi, S., Cagnetti, C., Danni, M., Provinciali, L., Silvestrini, M., 2017. Oral and intravenous steroids for multiple sclerosis relapse: a systematic review and meta-analysis. J. Neurol.

Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G., Ahn, B.W., Shaltiel, S., Stadtman, E.R., 1990. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 186, 464–478.

Liu, G.H., Qu, J., Shen, X., 2008. NF-kappaB/p65 antagonizes Nrf2-ARE pathway by depriving CBP from Nrf2 and facilitating recruitment of HDAC3 to MafK. Biochim. Biophys. Acta 1783, 713–727.

Málly, J., Farkas, R., Tóthfalusi, L., Stone, T.W., 2004. Long-term follow-up study with repetitive transcranial magnetic stimulation (rTMS) in Parkinson's disease. Brain Res. Bull. 64, 259–263.

Málly, J., Geisz, N., Dinya, E., 2017. Follow up study: the influence of rTMS with a high and low frequency stimulation on motor and executive functionn in Parkinson's disease. Brain Res. Bull. 135, 98–104.

Ma, V.Y., Chan, L., Carruthers, K.J., 2014. Incidence, prevalence, costs, and impact on disability of common conditions requiring rehabilitation in the United States: stroke, spinal cord injury, traumatic brain injury, multiple sclerosis, osteoarthritis, rheumatoid arthritis, limb loss, and back pain. Arch. Phys. Med. Rehabil. 995, e981.

Medina-Fernandez, F.J., Escribano, B.M., Aguera, E., Aguilar-Luque, M., Feijoo, M., Luque, E., Garcia-Maceira, F.I., Pascual-Leone, A., Drucker-Colin, R., Tunez, I., 2017a. Effects of transcranial magnetic stimulation on oxidative stress in experimental autoimmune encephalomyelitis. Free Radic. Res. 51, 460–469.

Medina-Fernandez, F.J., Luque, E., Aguilar-Luque, M., Aguera, E., Feijoo, M., Garcia-Maceira, F.I., Escribano, B.M., Pascual-Leone, A., Drucker-Colin, R., Tunez, I., 2017b. Transcranial magnetic stimulation modifies astrocytosis, cell density and lipopolysaccharide levels in experimental autoimmune encephalomyelitis. Life Sci. 169, 20–26.

Milenkovic, M., Arsenovic-Ranin, N., Vucicevic, D., Bufan, B., Jancic, I., Stojic-Vukanic, Z., 2008. Beneficial effects of dimethyl fumarate on experimental autoimmune myocarditis. Arch. Med. Res. 39, 639–646.

Montilla, P., Tunez, I., Munoz, M.C., Salcedo, M., Feijoo, M., Munoz-Castaneda, J.R., Bujalance, I., 2004. Effect of glucocorticoids on 3-nitropropionic acid-induced oxidative stress in synaptosomes. Eur. J. Pharmacol. 488, 19–25.

Mori, F., Codeca, C., Kusayanagi, H., Monteleone, F., Boffa, L., Rimano, A., Bernardi, G., Koch, G., Centonze, D., 2010. Effects of intermittent theta burst stimulation on spasticity in patients with multiple sclerosis. Eur. J. Neurol. 17, 295–300.

Naci, H., Fleurence, R., Birt, J., Duhig, A., 2010. The impact of increasing neurological disability of multiple sclerosis on health utilities: a systematic review of the literature. J. Med. Econ. 13, 78–89.

Perez-Nievas, B.G., Garcia-Bueno, B., Madrigal, J.L., Leza, J.C., 2010. Chronic immobilisation stress ameliorates clinical score and neuroinflammation in a MOG-induced EAE in Dark Agouti rats: mechanisms implicated. J. Neuroinflammation. 7 (60).

Pugliatti, M., Rosati, G., Carton, H., Riise, T., Drulovic, J., Vecsei, L., Milanov, I., 2006. The epidemiology of multiple sclerosis in Europe. Eur. J. Neurol. 13, 700–722.

Saguil, A., Kane, S., Farnell, E., 2014. Multiple sclerosis: a primary care perspective. Am. Fam. Physician. 90, 644–652.

Sherafat, M.A., Heibatollahi, M., Mongabadi, S., Moradi, F., Javan, M., Ahmadiani, A., 2012. Electromagnetic field stimulation potentiates endogenous myelin repair by recruiting subventricular neural stem cells in an experimental model of white matter demyelination. J. Mol. Neurosci. 48, 144–153.

Tasset, I., Drucker-Colin, R., Pena, J., Jimena, I., Montilla, P., Medina, F.J., Tunez, I., 2010. Antioxidant-like effects and protective action of transcranial magnetic stimulation in depression caused by olfactory bulbectomy. Neurochem. Res. 35, 1182–1187.

Tasset, I., Aguera, E., Sanchez-Lopez, F., Feijoo, M., Giraldo, A.I., Cruz, A.H., Gascon, F., Tunez, I., 2012. Peripheral oxidative stress in relapsing-remitting multiple sclerosis. Clin. Biochem. 45, 440–444.

Tasset, I., Bahamonde, C., Aguera, E., Conde, C., Cruz, A.H., Perez-Herrera, A., Gascon, F., Giraldo, A.I., Ruiz, M.C., Lillo, R., Sanchez-Lopez, F., Tunez, I., 2013a. Effect of natalizumab on oxidative damage biomarkers in relapsing-remitting multiple sclerosis. Pharmacol. Rep. 65, 624–631.

Tasset, I., Perez-Herrera, A., Medina, F.J., Arias-Carrion, O., Drucker-Colin, R., Tunez, I., 2013b. Extremely low-frequency electromagnetic fields activate the antioxidant pathway Nrf2 in a Huntington's disease-like rat model. Brain Stimul. 6, 84–86.

Tunez, I., Drucker-Colin, R., Jimena, I., Medina, F.J., Munoz Mdel, C., Pena, J., Montilla, P., 2006. Transcranial magnetic stimulation attenuates cell loss and oxidative damage in the striatum induced in the 3-nitropropionic model of Huntington's disease. J. Neurochem. 97, 619–630.

Wang, P., Xie, K., Wang, C., Bi, J., 2014. Oxidative stress induced by lipid peroxidation is related with inflammation of demyelination and neurodegeneration in multiple sclerosis. Eur. Neurol. 72, 249–254.

Wardyn, J.D., Ponsford, A.H., Sanderson, C.M., 2015. Dissecting molecular cross-talk between Nrf2 and NF-kappaB response pathways. Biochem. Soc. Trans. 43, 621–626.

Zhivolupov, S.A., Odinak, M.M., Rashidov, N.A., Onischenko, L.S., Samartsev, I.N., Jurin, A.A., 2012. Impulse magnetic stimulation facilitates synaptic regeneration in rats following sciatic nerve injury. Neural Regen. Res. 7, 1299–1303.