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Transcranial magnetic stimulation modifies astrocytosis, cell density and lipopolysaccharide levels in experimental autoimmune encephalomyelitis

Francisco J. Medina-Fernández [a,*], Evelio Luque [b,c], Macarena Aguilar-Luque [a,b], Eduardo Agüera [b,d], Montserrat Feijóo [a], Fe I. García-Maceira [e], Begoña M. Escribano [b,f], Álvaro Pascual-Leone [g], René Drucker-Colín [h], Isaac Túnez [a,b,i]

[a] Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Cordoba, Spain
[b] Maimonides Institute for Research in Biomedicine of Cordoba (IMIBIC), Spain
[c] Department of Morphological Sciences, Histology Section, Faculty of Medicine, University of Cordoba, Spain
[d] Department of Neurology, Reina Sofia University Hospital, Spain
[e] Canvax Biotech S.L., Cordoba, Spain
[f] Department of Cell Biology, Physiology and Immunology, Faculty of Veterinary Medicine, University of 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, USA
[h] Department of Molecular Neuropathology, Institute of Cell Physiology, National Autonomous University of Mexico (UNAM), Mexico
[i] Cooperative Research Thematic Network on Aging and Frailty (RETICEF), Spain

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ABSTRACT

Aims: Experimental autoimmune encephalomyelitis (EAE) is considered a valid experimental model for multiple sclerosis, a chronic neuroinflammatory condition of the central nervous system. Additionally, some evidence has shown that some microbial products such as the bacterial lipopolysaccharide could lead to the activation of reactive immune cells, triggering neuroinflammation. Several studies have found that transcranial magnetic stimulation (TMS) may exert a neuroprotective effect. Therefore, we aimed to assess the effect of TMS on the neuroinflammation occurring in EAE.

Materials and methods: A total of 44 male Dark Agouti rats were used. EAE induction was performed administering subcutaneously at the dorsal base of the tail a single dose of myelin oligodendrocyte glycoprotein. Clinical evaluation of motor symptoms was performed. Brain and spinal cord were collected and analyzed for nitric oxide, bacterial lipopolysaccharide and lipopolysaccharide-binding protein. We also carried out a histologic exam, which included an astrocyte immunostaining and Nissl staining for the assessment of brain cell density and pyknotic nuclei.

Key findings: TMS effectively ameliorated motor impairment secondary to EAE. This form of magnetic field was capable of decreasing the proliferation of astrocytes as a response to the autoimmune attack, reducing the content of nitric oxide, bacterial lipopolysaccharide and lipopolysaccharide-binding protein in central nervous system. Moreover, in treated animals, brain cell density was improved and the number of pyknotic nuclei was decreased.

Significance: Transcranial magnetic stimulation modifies astrocytosis, cell density and lipopolysaccharide levels in EAE. These results suggest that TMS could be a promising treatment for neuroinflammatory conditions such as multiple sclerosis.

1. Introduction

Experimental autoimmune encephalomyelitis (EAE) is considered a valid experimental model for multiple sclerosis (MS), a chronic debilitating neuroinflammatory condition of the central nervous system (CNS), in which demyelination, gliosis and axonal loss are encountered [1]. During neuroinflammatory processes like EAE or MS, astrocytes, i.e. cells belonging to the neuroglia which participate in neuronal homeostasis, become highly active and may exert functions such as phagocytosis, inflammatory mediator production (like nitric oxide (NO)), and antigen presentation, being responsible for oxidative/nitrosative damage that leads to cellular dysfunction, progressive axonal loss and

* Corresponding author at: Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Cordoba, Avda. Menéndez Pidal s/n, CP: 14004, Spain.
E-mail address: mefef.dr@gmail.com (F.J. Medina-Fernández).

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neuronal degeneration. Additionally, in relation to the pathogenesis of CNS disorders, there is growing evidence highlighting the role of commensal gut flora as contributive to the development of these conditions, especially in those closely linked to autoimmunity. In certain scenarios, both metabolites and microbial products such as the bacterial lipopolysaccharide (LPS), could lead to the activation of CNS-reactive immune cells, triggering neuroinflammatory phenomena [2].

Recently, Elzamarany et al. have shown that transcranial magnetic stimulation (TMS) may improve dexterity in MS patients [3]. In this line, some experimental studies have pointed out that extremely low-frequency electromagnetic fields (EL-EMF), a paradigm of TMS, may exert a neuroprotective effect in models of Huntington's disease, Alzheimer's disease and depression by attenuating cell loss and oxidative stress [4–8], and may induce neurogenesis via brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF) [9–12]. This non-invasive therapy applies a magnetic field through the skull which induces changes in the polarization of neurons and finally some beneficial effects [13].

Therefore, based on this background, the objective of the present study was to assess the effect of TMS on motor condition and neuroinflammatory markers in a rat model of EAE.

2. Experimental procedures

2.1. Design of the study

The experiment included the following study groups: i) Control, ii) Myelin oligodendrocyte glycoprotein (MOG)-EAE, iii) EAE + Mock TMS, and iv) EAE + TMS.

Forty-four young-adult (8-week old) male Dark Agouti rats (Janvier Labs, France) weighing 190–220 g were used. Animals were housed under controlled conditions of illumination (12 h light /12 h dark cycle, lights on at 08:00 h) and temperature (20–23 °C), and supplied with water and food (Purina®, Spain) ad libitum.

The rats were randomized into groups using computer-generated random permutations. Two major groups of animals were used in order to assess:

i) inflammatory mediators/markers (NO, lipopolysaccharide binding protein (LBP) and LPS) (n = 5; total n = 20), and
ii) pathohistological features (Astrocyte-immunohistochemistry and Nissl staining for cell density) (n = 4 each group/staining except EAE + Mock-TMS, total n = 24).

Based on studies of the EAE time pattern [14], all animals were assessed for clinical status at day 0 (induction of EAE), 14 (onset disease and start of treatment) and 35 (relapse of disease and end of experiment). Their motor condition was evaluated according to the scale by Perez-Nievas [15], in which: 0 = any sign, 1 = tail paralysis, 2 = hind limb paresis, 3 = hind limb paralysis, 4 = hind limb paralysis plus front limb paresis, 5 = moribund or dead. TMS was applied from day 14 to 35, day in which animals were sacrificed by decapitation, and brain and spinal cord specimens were collected.

An independent “blind” investigator who did not know the experimental group performed the evaluation of clinical status. To assure blindness, the biochemical and histological assessments were unlabeled.

The experiment was approved by the Bioethics Committee of Cordoba University and 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 approved by Presidency Minister of Spain (BOE 08 of February of 2013).

2.2. EAE induction

EAE induction was performed at day 0 by administering subcutaneously at the dorsal base of the tail a single dose of 100 μl with 150 μg of MOG (fragment 35–55; Sigma, USA) in phosphate buffered saline (PBS) emulsified 1:1 in complete Freund's adjuvant (Sigma, USA). To complete the adjuvant, 400 μg of *Mycobacterium tuberculosis* (H37Ra, DIFCO, USA) inactivated by heat were added [15].

2.3. TMS application

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). To reproduce clinical practice, TMS was applied in the morning for 2 h, once a day, five days a week (Monday-Friday), during three weeks (days 14–35) (modified from [16]). Rats were placed in plastic cylindrical cages designed to keep them immobile. The two Helmholtz coils (Magnetoterapia S.A., Mexico) were positioned dorsally and ventrally to the head at a distance of approximately 6 cm between each coil and the midpoint of the head. Animals in the TMS-Mock group were handled in the same way but without receiving real stimulation.

2.4. Biochemical parameter assays

Under controlled temperature conditions, the brains and spinal cords were extracted and the corresponding homogenates immediately prepared with a mechanical homogenizer (Tempest Virtis). The buffer used for homogenization was Tris (20 mM) at pH 7.4.

Spectrophotometric measures were performed using a UV-1603 spectrophotometer (Shimadzu, Japan). ELISA determinations were made using a Multimode Detector DTX-880 Beckman Coulter (Barcelona, Spain).

2.4.1. Nitric oxide

Total nitrite (nitrite + nitrate; NOx), analyzed following the Griess method [17], can be used as a marker of NO production. To perform the Griess assay, all nitrates must be reduced to nitrite, and then, total nitrite is determined spectrophotometrically by Griess reaction. The reaction is read at 540 nm and values presented in μmol/mg protein.

2.4.2. Lipopolysaccharide-binding protein

The assessment of LBP was performed using the LBP soluble (mouse) ELISA Kit (Enzo®, USA). The reaction is read at 450 nm and values presented as pg/mg protein.

2.4.3. Bacterial lipopolysaccharide

LPS was assessed using the Pierce® LAL Chromogenic endotoxin quantification kit provided by Thermo Scientific (USA). The reaction is photometrically measured at 405–410 nm. Data are expressed as endotoxin units/mg protein.

Due to logistic reasons LPS was only studied in spinal cord.

2.4.4. Protein estimation

Protein levels were measured by the Bradford method, using a B6916 assay kit supplied by Sigma (USA).

2.5. Histologic studies

Based on previous studies on the effect of TMS on the brain [4,9], striate nuclei were studied. Counts were made using a counting frame (area) of 35,500 μm^2 (40×), which was randomly placed at ten different zones. Then, the counts were averaged. Quantification was carried out in a semi-automatic manner with the software Image-Pro Plus® (Media Cybernetics, USA).

2.5.1. Nissl staining

Whole brains fixed in 10% buffered formaldehyde were embedded in paraffin wax, cut into 8-μm thick sections and stained with 0.025% cresyl violet (Nissl-stained). Sections were evaluated under bright field illumination on a Leitz Orthoplan microscope (Herramientas Leitz S.L., Spain).