Arq Neuropsiquiatr 2009;67(1):78-81

MECHANICAL HYPERNOCICEPTION IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Abstract – Background: Pain is an important clinical manifestation in multiple sclerosis (MS) patients, though it has been neglected in clinical and experimental researches. **Objective:** To investigate the nociceptive response in MOG_{35-55} experimental autoimmune encephalomyelitis (EAE)-induced mice. **Method:** EAE was induced in 8 to 10 week old C57BL/6 female mice with an emulsion of MOG_{35-55}, Complete Freund Adjuvant, *Mycobacterium tuberculosis* H37 RA and pertussis toxin. Nociception was evaluated by the von Frey filaments method. A clinical scale ranging from 0 to 15 was used to assess motor impairment. **Results:** Clinical evidence of disease started at day 10 and peaked at day 14 after immunization. Thereafter, there was no worsening of symptoms until day 26. The EAE-induced mice presented reduced pressure threshold at days 7^{th} and 10^{th} after immunization and before the onset of clinical motor signs. **Conclusion:** The hypernociception found validates MOG_{35-55} EAE as a model for the study of pain in multiple sclerosis.

KEY WORDS: multiple sclerosis; experimental autoimmune encephalomyelitis, hyperalgesia, mechanical hypernociception.

Hipernocicepção mecânica em encefalomielite autoimune experimental

Resumo – Introdução: Dor é uma manifestação importante em pacientes com esclerose múltipla (EM), mas que tem sido negligenciada na pesquisas clínica e experimental. **Objetivo:** Investigar a resposta nociceptiva de camundongos com encefalomielite autoimune experimental (EAE) induzida por MOG_{35-55}. **Método:** A EAE foi induzida em camundongos C57BL/6 fêmeas de 8–10 semanas com emulsão contendo MOG_{35-55}, Adjuvante Completo de Freund, *Mycobacterium tuberculosis* cepa H37 RA e toxina pertussis. A nocicepção foi medida pelo método de filamentos de von Frey. Uma escala clínica variando de 0 a 15 foi utilizada para avaliar a debilidade motora dos animais. **Resultados:** Os sinais clínicos da doença iniciaram-se no dia 10 e a gravidade máxima foi alcançada no dia 14 após a imunização. Não houve piora dos sintomas até o dia 26. Os camundongos induzidos com EAE apresentaram diminuição do limiar de pressão nos dias 7 e 10 após a imunização e antes do início dos sinais motores. **Conclusão:** A hipernocicepção verificada valida a EAE induzida por MOG_{35-55} como um modelo para estudos de dor em esclerose múltipla.

PALAVRAS-CHAVE: esclerose múltipla; encefalomielite autoimune experimental, hiperalgesia; hipernocicepção mecânica.

Multiple sclerosis (MS) is considered an autoimmune inflammatory disease affecting 2.5 million people worldwide. Its defining feature is the central nervous system (CNS) demyelinating lesions in association with inflammatory infiltrates. Axonal injury can also occur[1]. Clinical signs of MS include sensory disturbances, optic neuritis, diplopia, Lhermitte's sign, limb weakness, clumsiness, gait ataxia, neurogenic bladder, bowel symptoms and also pain[2]. Although many MS patients describe pain as their worst symptom[3], only recently pain has been systematically studied in MS patients[4,5]. Pain in MS can be ascribed to a variety of conditions such as musculoskeletal pain, painful spasms, trigeminal neuralgia, and central pain due to sclerotic plaque lesions affecting sensorial pathways in the CNS[4].

In the search of a better understanding of MS, investigators have been using many experimental models, including the experimental autoimmune encephalomyelitis (EAE). EAE can be induced in susceptible species by

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Received 7 August 2008, received in final form 20 October 2008. Accepted 29 November 2008.

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many different protocols usually with an emulsion containing one of these three molecules: MBP (myelin basic protein), PLP (proteolipid protein) or MOG (myelin oligodendrocyte glycoprotein)[6]. In spite of the relevance of pain in MS, there are only a few studies investigating pain in EAE, none related to MOG_{35-55}-induced EAE.

Hence, in the present study, we intended to investigate whether MOG_{35-55} EAE-induced mice had altered mechanical nociception.

METHOD

Animals

Animal care and handling procedures were in accordance with the guidelines of the International Association for Study of Pain and had prior approval from the local animal ethics committee (Comitê de Ética em Experimentação Animal, CETEA/UFMG, Certificate number 007/2007). Eight to 10 weeks old female C57Bl/6J (WT) mice were obtained from Centro de Bioterismo (CEBIO) of the Universidade Federal de Minas Gerais (UFMG, Brazil) and maintained in the animal facilities of the Laboratory of Immunopharmacology, Department of Biochemistry and Immunology (UFMG, Brazil), with filtered water, food *ad libitum* and in a controlled environment (temperature and humidity). Animals were divided in two groups: MOG_{35-55} EAE induced mice (n=8) and control mice injected with saline (n=8).

EAE induction

EAE was induced by s.c. immunization (base of tail) with an emulsion containing 100 μg MOG_{35-55} peptide (MEVGWYRSPFSRVVHLYRNGK; NeoMPS) and CFA supplemented with 4 mg/mL *Mycobacterium tuberculosis* H37 RA (Difco Laboratories). Pertussis toxin, 300 ng/animal was injected i.p. on the day of immunization and again 48h later[7].

Daily assessments of disease in mice

Animals were evaluated daily using a previously described scale[8]. This scale ranges from 0 to 15 and is the sum of the state of the tail and all of the four limbs. For the tail, a score of 0 reflects no sign, 1 represents a partial paralyzed tail, while a score of 2 is given to a mouse with a fully paralyzed tail. For each of the hind- or forelimbs, each assessed separately, 0 signifies no sign, a score of 1 is an altered gait, 2 represents paresis, while a score of 3 denotes a fully paralyzed limb. Thus, a fully paralyzed quadriplegic animal would attain a score of 14. Mortality equals a score of 15. Typically, a mouse undergoing an attack would first have loss of function in the tail followed by one of the hindlimbs. Therefore, a low score of 3 or 4 indicates tail involvement and a weak hindlimb, akin to Grade 2 on the commonly used 5 point scale[9]. Animals were also weighed daily.

Nociceptive mechanical test

The term "hypernociception" was used to define the decrease of nociceptive withdrawal threshold[10]. Mechanical hypernociception was tested in mice as reported previously[11,12]. Briefly, in a quiet room, mice were placed in 12 × 10 × 17-cm acrylic cages with wire grid floors 15–30 min before the start of testing. The test consisted of evoking a hindpaw flexion reflex with a hand-held force transducer (Electronic Anesthesiometer, Insight mod. EFF-301, Brazil) adapted with a 0.5-mm^2 polypropylene tip. The investigator was trained to apply the tip perpendicularly to the central area of the hindpaw with a gradual increase in pressure. The endpoint was characterized by the removal of the paw, followed by clear flinching movements. After the paw withdrawal, the intensity of the pressure was automatically recorded. The value for the response was obtained by averaging three measurements. Animals were tested before and after treatments. Results are expressed as Δ withdrawal threshold (in g) calculated by subtracting zero-time mean measurements from the time interval mean measurements.

Statistical analysis

Results are shown as the mean ±SD. Difference among groups was evaluated by using analysis of variance (ANOVA) followed by Student-Newman-Keuls post-hoc test. The level of significance was set a $p<0.05$.

RESULTS

Kinetics of clinical changes in EAE mice

The severity of MOG_{35-55}-induced EAE was assessed daily using a previously validated scale[8]. Clinical evidence of disease was first noticed at day 10 and peaked at day 14 after immunization. Thereafter, there was no worsening of symptoms till day 26 (Fig 1A). Tail paralysis and hind limb weakness was the major clinical feature noticed. Paralleling the clinical symptoms above, there was significant weight loss which peaked at day 14 after disease induction when compared with control mice (Fig 1B).

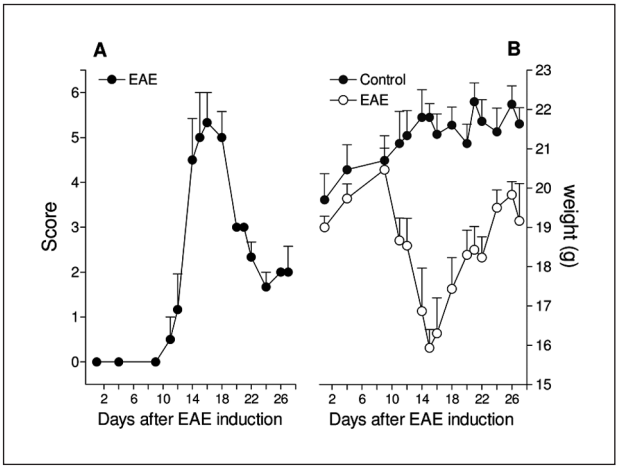

Fig 1. Experimental autoimmune encephalomyelitis (EAE) clinical signs (A) and body weights loss (B) were monitored in control and EAE-induced mice. This kinetics study includes the time points 1 to 26 day post-induction of EAE with MOG35-55. Data is according to maximal clinical score achieved at any time during experimental design. The data are the means (±SD) of eight animals per group.

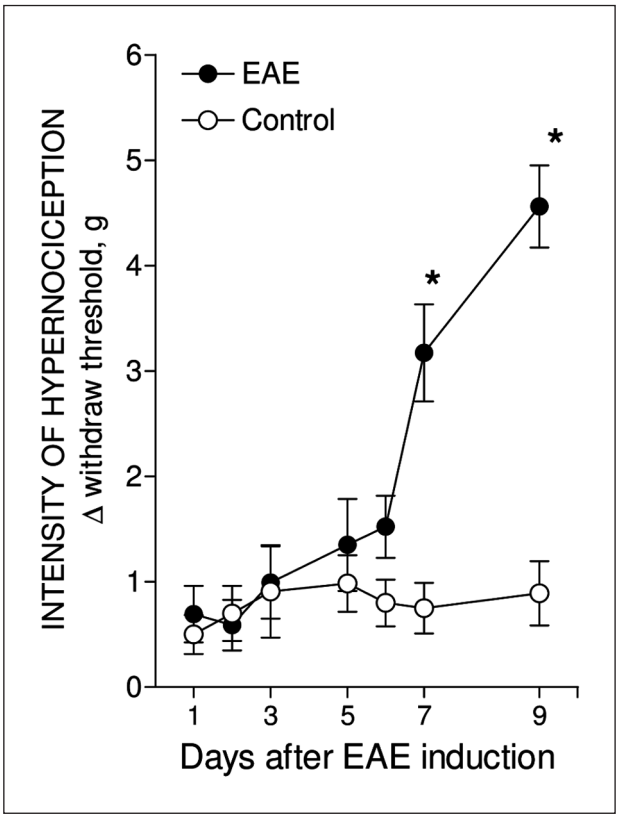

Fig 2. MOG_{35-55}-induced mechanical hypernociception. The intensity of mechanical hypernociception was assessed daily in experimental autoimmune encephalomyelitis (EAE) and control mice. The data are the means (±SD) of eight animals per group. Asterisks denote statistically significant differences compared with the control group (p<0.05).

$MOG_{35–55}$-induced mechanical hypernociception

The immunization of mice with MOG_{35-55} peptide brought about a mechanical hypernociception. The intensity of hypernociception was assessed daily in EAE and control mice. The mechanical hypernociception was observed in EAE mice on the 7th and 10th days after immunization (Fig 2). The intensity of hypernociception was assessed before clinical evidence of disease that was first noticed at day 10. We did not evaluate mechanical nociception after 10 days, as the results could be affected by the motor condition of the animals.

DISCUSSION

To the best of our knowledge, this is the first study of nociception in MOG_{35-55}-induced EAE in C57BL/6 mice. We found hypernociception in EAE mice before the onset of motor disability.

Hypernociception was also found in a recent study[13] which observed thermal hyperalgesia and mechanical allodynia during disease progression in the Theiler's virus experimental model of MS (Theiler's murine encephalomyelitis virus, TMEV). The TMEV model displays a clinical outcome similar to MS, but the autoimmune response observed in MS is not described for this experimental model. By contrast, EAE presents many pathophysiological similarities to MS and is considered a more suitable model for this CNS disease[14].

A previous study[15] found hyponociception and hypernociception during acute and chronic phases, respectively, using a heat stimulus method. In that study, the investigators used a PLP-induced EAE model and evaluated nociception after the onset of clinical disability, which could have interfered with results. The hypernociception found in the chronic phase of EAE may suggest that the PLP-induced model is a good model to evaluate chronic pain, which is also found in MS[16]. However, that model was unable to detect acute pain, a common problem for many MS patients[17,18]. We described hypernociception before the onset of clinical impairment using the MOG_{35-55} EAE model. Therefore this could be a better model to study acute pain in MS. As the animals had severe motor impairment after onset of clinical signs, we avoided the measurement of nociception after 10 days of EAE induction.

A study using an EAE model of Lewis rats found hyponociception during acute phase of disease using a vocalization method of response to noxious mechanical stimulation of the tail[19]. They concluded that hyponociception was due to demyelination of small diameter fibres in the sacrococcygeal dorsal root ganglia, dorsal roots and dorsal root entry zones. However, in our EAE model, demyelination is not so evident, especially before the onset of clinical signs[20]. Hence, the hypernociception observed in our results may be caused by other pathways.

Immune response before onset of clinical signs in this experimental model of MS is evidenced by several studies. Increased rolling of leukocytes[7] and increase of $CD4^+$ T cells in draining lymph nodes[21] after 7 days post-immunization; increase of P-selectin in CNS after 8 days post-immunization[22] and increase in IFN-γ after 10 days post-immunization[23] are some of the immune events that happen before motor disability of the animals. Thus, the hypernociception observed in our study may be the result of the intrinsic immune response from the 7th to the 10th day after immunization. Cytokines were found to mediate hypernociception in a carrageenan-induced inflammatory model of hyperalgesia[10] and chemokines (chemotactic cytokines) seem to be involved in the hypernociception elicited by an experimental model of arthritis[24]. Chemokines are also important in MS[25,26] and they are involved in many pathways of pain[27]. The possibility of chemokines being the cause of pain in MS was discussed elsewhere[28]. Nonetheless, further studies are needed to investigate

whether cytokines or other immune factors are involved in the hypernociception of MOG_{35-55} EAE mice.

In conclusion, the hypernociception observed indicates that the MOG_{35-55} EAE model may be a useful tool to study the mechanisms of pain in MS.

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