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Original Article

Therapeutic effects of D-aspartate in a mouse model of multiple sclerosis

Sanaz Afraei [a], *Antimo D'Aniello* [b,*], *Reza Sedaghat* [c], *Parvin Ekhtiari* [a], *Gholamreza Azizi* [d,e], *Nakisa Tabrizian* [f], *Laura Magliozzi* [g], *Zahra Aghazadeh* [a], *Abbas Mirshafiey* [a,*]

[a] *Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[b] *Laboratory of Neurobiology, Zoological Station of Naples "Anton Dohrn", Villa Comunale, Napoli, Italy*
[c] *Departments of Anatomy and Pathology, Faculty of Medicine, Shahed University, Tehran, Iran*
[d] *Imam Hassan Mojtaba Hospital, Alborz University of Medical Sciences, Karaj, Iran*
[e] *Research Centre for Immunodeficiencies, Pediatrics Centre of Excellence, Children's Medical Centre, Tehran University of Medical Sciences, Tehran, Iran*
[f] *Department of Cellular and Molecular Biology, Kish International Campus, University of Tehran, Tehran, Iran*
[g] *Department of Biology, University of Naples, "Federico II" Via Cinthia, MSA Campus, bldg. 7, Naples, Italy*

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ABSTRACT

Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis. EAE is mainly mediated by adaptive and innate immune responses that leads to an inflammatory demyelization and axonal damage. The aim of the present research was to examine the therapeutic efficacy of D-aspartic acid (D-Asp) on a mouse EAE model. EAE induction was performed in female C57BL/6 mice by myelin 40 oligodendrocyte glycoprotein (35-55) in a complete Freund's adjuvant emulsion, and D-Asp was used to test its efficiency in the reduction of EAE. During the course of study, clinical evaluation was assessed, and on Day 21, post-immunization blood samples were taken from the heart of mice for the evaluation of interleukin 6 and other chemical molecules. The mice were sacrificed, and their brain and cerebellum were removed for histological analysis. Our findings indicated that D-Asp had beneficial effects on EAE by attenuation in the severity and delay in the onset of the disease. Histological analysis showed that treatment with D-Asp can reduce inflammation. Moreover, in D-Asp-treated mice, the serum level of interleukin 6 was significantly lower than that in control animals, whereas the total antioxidant capacity was significantly higher. The data indicates that D-Asp possess neuroprotective property to prevent the onset of the multiple sclerosis.

* *Corresponding authors*. Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran 14155-6446, Iran (A. Mirshafiey); Laboratory of Neurobiology, Zoological Station of Naples "Anton Dohrn", Villa Comunale, Napoli, Italy (A. D'Aniello).
E-mail addresses: daniello.antimo@gmail.com (A. D'Aniello), mirshafiey@tums.ac.ir (A. Mirshafiey).
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1. Introduction

Multiple sclerosis (MS) is considered as an autoimmune inflammatory demyelinating disease of the central nervous system (CNS) and of spinal cord that is characterized by relapsing-remitting attacks and worsening neurologic function [1]. MS disease is known by the destruction of the myelin sheath that surrounds neuronal axons in the CNS, a process that results in neurodegeneration and consequently the formation of sclerotic plaques in the brain. Recent investigations showed an association between MS and steroid hormones, namely progesterone, testosterone, and 17β-estradiol [2–4]. Steroids attenuate neuroinflammation by reducing the proinflammatory function of astrocytes. Another research provided the evidence that steroids induce remyelination after demyelination [5–7]. The underlying cellular mechanisms involve interactions with astroglia, insulin-like growth factor-1 responses, and the recruitment of oligodendrocytes [4]. Several studies have shown that a reduction in the testosterone levels in rats induces a decrease in the synthesis of myelin protein [4]. Furthermore, progesterone is capable of protecting the motor neurons in the spinal cord of rats [5,6], and 17β-estradiol protects oligodendrocytes from the cytotoxicity of cell death [7]. The other series of studies reported that the neurosteroids contribute to the formation of synapses, synaptic plasticity, and cognitive activity in addition to having protective effects on myelin destruction [5,7–16]. These multiplatform studies indicate impaired neurosteroidogenesis in both MS and experimental autoimmune encephalomyelitis (EAE). EAE is an animal model of MS where the disease is mediated by autoantigen-specific T cells, such as myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein [17,18]. EAE is used for the evaluation of mechanism causing MS as well as pharmacological research in MS [19–22].

D-Asp is a natural amino acid present in all animal phyla investigated, including rodents and humans. It was first discovered in the brain of the marine mollusc, *Octopus vulgaris*, in 1977 [23]. Subsequent studies have clarified that this amino acid is mainly present in the nervous tissues and endocrine glands [24–26] where it performs important physiological functions [27,28]. In the nervous tissues, D-Asp is localized in various areas of the brain, including the olfactory bulb, frontal cortex, hippocampus, and cerebellum. In the endocrine glands, D-Asp is localized mainly in the pineal gland, adrenal medulla, posterior pituitary gland, and testis [29]. In the hypothalamus, D-Asp is involved in the synthesis and release of gonadotropin-releasing hormones. In the pituitary gland, it induces the synthesis and release of luteinizing hormone, and in testis, it induces the synthesis and release of testosterone [30]. Furthermore, from the pineal gland, this amino acid is involved in the synthesis and release of melatonin [31], whereas from the pituitary gland, it can increase the synthesis of the melanocortin [32]. In the nervous system instead, D-Asp acts as a novel neurotransmitter of cell-cell signaling [33] and as neuromodulator [34]. Interestingly, D-Asp can also enhance learning and memory in rats [35] as well as improve the long-term potentiation [36] and prevent long-term depression in mice [37]. Lastly, this amino acid also acts as a regulator for the neurogenesis and is an endogenous factor for the neuronal dendrites growth [38,39].

Previously, studies have established that some hormones, mainly testosterone, progesterone, and 17 β-estradiol, in the CNS (neurosteroids) play a role in the protection of the neurons against neuronal damage caused by dangerous hexogen and endogen molecules, i.e., free radicals, nitric oxide, peroxides, and endogen antibody. Moreover, such neurosteroids are essential elements to keep the myelin sheath healthy that surrounds the neuron and concur in the synthesis of myelin [7–9,13–15]. In the present study, we have obtained the evidence in mice that on oral ingestion of a solution consisting of 20mM sodium D-aspartate instead of tap water, this amino acid crosses the blood brain barrier in male and female mice and increases the synthesis of the neurosteroids (testosterone, progesterone, and 17 β-estradiol) in the brain. Thus, these results suggest that D-Asp may have the property to prevent and reduce the neuronal damage of the myelin sheath, which is the major protein surrounding the neuron. Therefore, the aim of this study was to the therapeutic effects of the oral treatment of sodium D-aspartate in experimental mouse model for MS.

2. Material and methods

2.1. *Animal selection and grouping*

In this experiment, 24 female C57BL/6 mice, weighing 18–20 g and aged 8 weeks were used. The mice were purchased from the Experimental Animal Center of Pasteur Institute of Iran. These mice were divided into four groups randomly (6 mice per group): I, normal group (healthy control); II, prophylactic group; III, treatment group; and IV, control group. For adaptation, all mice were housed in cages under 12-hour light–dark cycle in the animal house of Tehran University for 2 weeks. During the study period, the same meal plan including pelleted diet soya, carrot, peanuts, and water was used. All the animal-related procedures were conducted in accordance with the protocol approved by the committee on animal experimentation of Tehran University of Medical Sciences.

2.2. *EAE induction and treatment protocol*

From the first day of adaptation, all animals were weighed, and their weights were recorded. To induce EAE, Hook kit (Hooke Laboratories, Inc, USA) was used. Each kit contained two pre-filled syringes of MOG 35-55 in an emulsion with complete Freund's adjuvant and a vial of lyophilized pertussis toxin (PTX). EAE induction was performed according to instructions: 0.1 mL MOG 35-55 was injected to the left flank area as well as to the right flank area subcutaneously; after 2 hours, the first dose of PTX dissolved in 2.5-cc sterile phosphate-buffered saline (PBS) was injected intraperitoneally into mice. After 24 hours, the second dose of PTX (0.1 mL/mouse) was injected intraperitoneally.

We prepared a stock solution of 1M of sodium D-aspartate as follows: 133.1 g of D-aspartic acid, 99% grade (Sigma, USA), was added to 500 mL distilled water with stirring. Then, it was added under stirring a solution of 2M sodium hydroxide to