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Beneficial effect of agmatine in the acute phase of experimental autoimmune encephalomyelitis in iNOS-/- knockout mice

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ABSTRACT

The aim of the study was to investigate the hypothesis that agmatine (AGM) provides protection against oxidative stress in experimental autoimmune encephalomyelitis (EAE).

Wild-type (WT) and knockout (KO) CBA/H iNOS-/- 3 months old (15 ± 5 g) mice, were used for EAE induction by myelin basic protein (MBP), dissolved in Complete Freund's Adjuvant (CFA). The animals were divided into control, EAE, CFA, EAE+AGM and AGM groups. After the development of full clinical remission, animals were decapitated and oxidative stress parameters were determined in whole encephalitic mass (WEM) and cerebellum homogenates.

The EAE clinical expression manifested to greater extent in WT than KO mice, was significantly decreased during AGM treatment. We demonstrated significant elevations of superoxide dismutase activity in WT and KO EAE animals, in WEM and cerebellum tissues, which were decreased during AGM treatment in both groups. Superoxide anion content was increased in WEM of both study groups, with a decrease during AGM treatment. The observed changes were more pronounced in WT than in KO animals. Also, the increased expressions of transferrin receptor and glial fibrillary acidic protein observed in WT and KO EAE mice were significantly decreased during AGM treatment.

The results suggest potentially beneficial AGM effects in EAE, which might be used for a modified antioxidative approach in MS therapy.

1. Introduction

Multiple sclerosis (MS) is a demyelinating autoimmune disease characterized by the infiltration of T cells into the central nervous system (CNS) preceded by blood–brain barrier (BBB) damage [1]. Due to the clinico-pathological and immunological similarities to MS, experimental autoimmune encephalomyelitis (EAE) is the most frequently used animal model for studying the pathogenesis of MS and testing the efficiency of MS treatment [2]. In MS/EAE, dysfunction of the BBB, followed by edema, leads to disrupted intercellular communication within the CNS [3].

Transferrin (Tf) represents the main protein carrier for many metal ions in plasma, being the most significant in the transport of iron. It has been suggested that various forms of transferrin enter the brain through the BBB via Tf receptor (TfR) mediated endocytosis [4]. Its uptake into cells appears to be largely determined by the distribution of TfR located on cell surface. These receptors are present in high density on endothelial cells of the microvasculature, as well as in select populations of neurons [5]. However, it is thought that astrocytes, believed to play a critical role in the regulation of brain iron absorption and metabolism at BBB junctions, do not express TfR, but the literature data are contradictory [6].

It has been shown that reactive astrocytes could have beneficial roles in defense and repair under pathological circumstances, while glial activation in EAE involves microglial interaction with astrocytes and may contribute to secondary neuronal damage [7]. Reactive glial cells release inflammation-promoting mediators and oxidative radicals [8]. For these reasons, considerable effort is being directed toward understanding the etiology of reactive gliosis and identification of substances that could modulate the proliferation of glial cells.

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Oxidative stress appears to play a role in the onset and progression of EAE [9]. Reactive oxygen species (ROS) have been implicated as mediators of demyelination and axonal damage in both MS and EAE. Therefore, several studies have been performed to assess whether treatment with antioxidants prevents the progression of these diseases [10].

Beside, nitric oxide (NO), the product of L-arginine metabolism, has been implicated in the pathogenesis of MS. It is synthesized by nitric oxide synthase (NOS) located in neurons (nNOS) and endothelial cells (eNOS) in physiological conditions, or in mitochondria and microglia (inducible NOS – iNOS) during inflammatory processes [11,12]. However, it is still unclear whether NO plays a protective role or is deleterious in neuroinflammation [13].

The present study was directed at testing a potentially beneficial effect of agmatine (AGM) on the development of oxidative stress during EAE, and protection of mitochondria from damage. Agmatine, (4-aminobutyl)guanidine, is the product of the decarboxylation of arginine and an intermediate in polyamine biosynthesis. It is synthesized in the brain, stored in synaptic vesicles, accumulated by uptake, released by membrane depolarization, and deactivated by agmatinase. AGM inhibits NO synthase (NOS), which is involved in the synthesis of NO and located in neurons (nNOS) and endothelial cells (eNOS) in physiological conditions, or in mitochondria and microglia by inducible NOS (iNOS) during the inflammatory process [12,13].

In this paper, we examine the clinical, biochemical, and immunohistochemical course of EAE in iNOS-deficient (iNOS-/-) mice in order to determine the role of oxidative stress in the disease progression. Here we explore the clinical signs, TfR, and astrocytes inflammatory response, as well as superoxide dismutase (SOD) activity and levels of superoxide anion $O_2^{\cdot-}$, during the treatment with AGM, as a continuance of our previous research [14–16].

2. Materials and methods

2.1. Animals

The experimental animals were treated according to the Guidelines for Animal Study, No. 282–12/2002 (Ethics Committee of the Military Medical Academy, Belgrade, Serbia and Montenegro).

The experiments were performed on CBA/H wild-type (WT) and knockout iNOS-/- (KO) mice females, weighing 15–20 g, randomly divided into one control group ($n = 8$) and four experimental groups ($n = 9$, each). Animals were housed (five mice per cage) under standardized housing conditions (ambient temperature of 23 ± 2 °C, relative humidity of 55 ± 3% and a light/dark cycle of 13/11 h) and had free access to standard laboratory pellet food and tap water. All the experiments were performed after a 7-day period of adaptation to laboratory conditions, and were carried out between 9 a.m. and 1 p.m. Experimental protocols were approved by the Local Animal Care Committee and conformed to the recommendations given in the "Guide for the Care and Use of Laboratory Animals" (National Academy Press, Washington, D.C., 1996).

2.2. Induction of EAE

Experimental autoimmune encephalomyelitis was induced by the subcutaneous injection of myelin basic protein, bovine type (100 μg dissolved in 0.1 ml complete Freund's adjuvant – CFA), in the hind foot pad of the animals, and two intraperitoneal injections (i.p.) of Pertussis toxin (150 ng dissolved in 0.1 ml phosphate buffered saline – PBS), given on days 0 and 1.

All animals were randomly assigned to five groups: control (CG) (treated by PBS – 0.1 ml/i.p./daily), EAE (PBS – 0.1 ml/i.p./daily after EAE induction), CFA (PBS – 0.1 ml/i.p./daily), EAE and AGM (AGM – 75 mg/kg body weight/daily after EAE induction), AGM (AGM – 75 mg/kg body weight/daily). The animals were treated every day during the course of the experiment. Twenty-four days of EAE induction (24 day post immunization – 24 dpi), animals from each group were sacrificed and tissues were collected for biochemistry and immunohistochemistry.

2.3. Behavioral evaluation

All animals were scored for the clinical signs of EAE daily. EAE clinical expression was assessed as 1 = healthy; 2 = loss of tail tone; 3 = hindlimb weakness; 4 = hindlimb paralysis; 5 = hindlimb paralysis plus forelimb weakness; 6 = moribund or dead. After a full clinically presented state of EAE, mice exerted recovery. It was

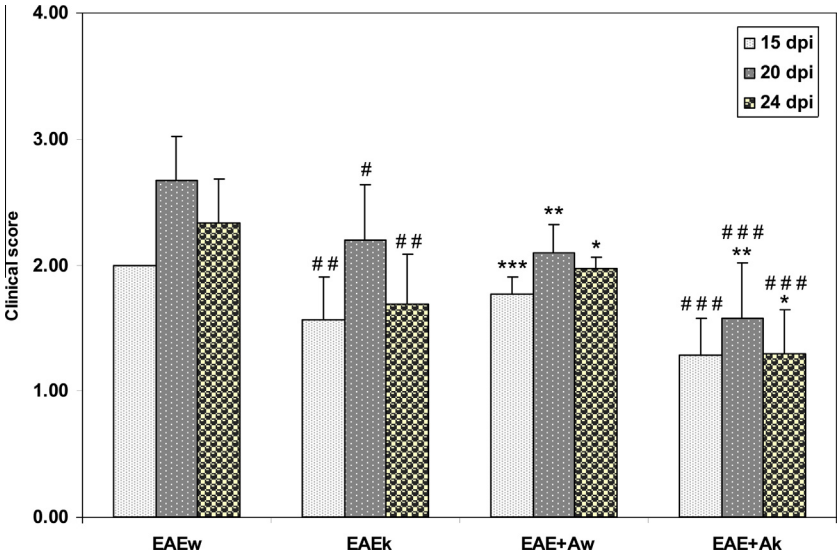

Fig. 1. The clinical EAE score of neurological disorder (1–6) of mice after 15, 20 and 24 days of EAE induction (15, 20, 24 day post immunization – 15 dpi, 20 dpi, 24 dpi). EAE wild (EAEw), EAE knockout iNOS-/- (EAEk), EAE+Agmatine wild (EAE+AGMw), EAE+Agmatine knockout iNOS-/- (EAE+AGMk) animals; 1 – no clinical signs, 2 – loss of tail tone, 3 – hindlimb weakness – ataxia, 4 – one to two limbs plegic, 5 – three to four limbs plegic, 6 – moribund or dead; Labels of statistical significance: * compared to the EAE group; # results from the wild-type animals compared to the iNOS-/- knockout animals ($^{*,\#}p < 0.05$, $^{**,\#\#}p < 0.01$, $^{***,\#\#\#}p < 0.001$); One Way ANOVA, Dunnett's C test.