Experimental and Molecular Pathology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

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

Upregulation of immunomodulatory molecules by matrine treatment in experimental autoimmune encephalomyelitis

Nan Liu [a], Quan-cheng Kan [a], Xiao-jian Zhang [a], Yu-ming Xv [a], Su Zhang [a], Guang-Xian Zhang [a,b,*], Lin Zhu [a,**]

[a] Department of Pharmacy, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan, China
[b] Department of Neurology, Thomas Jefferson University, Philadelphia, PA 19107, USA

ARTICLE INFO

Article history:
Received 29 September 2014
Accepted 6 October 2014
Available online xxxx

Keywords:
Matrine
Experimental autoimmune encephalomyelitis
Oxidative stress
IL-4
IL-5
IL-10
TGF-β1
Foxp3

ABSTRACT

Immunological dysfunction is a primary characteristic of multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). Matrine (MAT), a quinolizidine alkaloid derived from the herb Radix Sophorae Flave, has been shown to ameliorate the clinical signs of EAE by suppressing the production of proinflammatory cytokines IFN-γ, TNF-α and IL-17, as well as adhesive molecules. However, whether MAT is simply an immunosuppressive or an immunomodulatory reagent has not been studied. In the present study we focused on possible immunomodulatory mechanisms underlying the effects of MAT in EAE. Our results showed that administration of MAT significantly increased serum production of Th2 cytokines IL-4 and IL-5, and regulatory T cell (Treg) related cytokines IL-10, TGF-β1, as well as expression of Foxp3, a Treg transcription factor, in the spinal cord. In addition, MAT treatment significantly upregulated CNS expression of Nrf2 and HO-1, which play important roles in inhibiting oxidative stress and CNS inflammation. Together, our findings identify MAT as, not only an immunosuppressive, but also a potent immunomodulatory natural product for the treatment of EAE and which has potential as a novel therapeutic option for MS.

1. Introduction

Multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), are T cell-mediated inflammatory demyelinating diseases of the central nervous system (CNS) (Benveniste et al., 2014; Segal, 2014). The clinical pathological features of MS/EAE in the CNS are lymphocyte infiltration, macrophage/microglia activation, demyelization, oligodendrocyte loss and axonal injury (Bettelli et al., 2007; Furlan et al., 2009; Lassmann, 2010).In MS and EAE, myelin-reactive T cells were activated in the peripheral immune system and produce high levels of proinflammatory Th1/Th17 cytokines IFN-γ, TNF-α, IL-17 and GM-CSF; they infiltrate into the CNS, and trigger a cascade of immunological reactions that lead to CNS inflammation and myelin damage (El-Behi et al., 2011; Segal, 2014; Yan et al., 2012). In contrast, resistance to, or recovery from, the disease is mediated by Th2/regulatory T (Treg) cells that produce cytokines IL-4, IL-5, TGF-β, and IL-10, among others (Payne et al., 2012; Nygårdas et al., 2011; Perruche et al., 2008; Fitzgerald et al., 2007; Yang et al., 2009) Current clinical treatment for MS is mainly chemically synthesized immunomodulatory or immunosuppressive reagents, which have only limited efficacy and often have severe side effects (Castro-Borrero, 2012; Hybertson et al., 2011). Thus, it is especially important to search for more effective therapies and compounds that are more easily tolerated.

Matrine (MAT), a quinolizidine alkaloid derived from the herb Radix Sophorae Flave, has been used for hepatitis B in clinical trials, with an excellent safety record (Wang et al., 2013). MAT has also been recently shown to inhibit immune activities of T cells, B cells and macrophages (Li et al., 2010) and, at relatively low doses, to have partially suppressed development of EAE (Zhao et al., 2011). In addition, MAT treatment significantly suppressed the production of proinflammatory cytokines IFN-γ, TNF-α and IL-17 (Zhao et al., 2011), and blocked the migration of peripheral immune cells into the CNS (Zhang et al., 2013). However, the mechanism of action of MAT has not been fully elucidated and, in particular, it is not clear whether MAT is simply an immunosuppressant or an immunomodulatory reagent that can re-equilibrate pro- and anti-inflammatory responses.

In the present study, we have focused on the effect of MAT treatment on the regulation of immunomodulatory molecules in both the periphery and the CNS of EAE rats. MAT significantly upregulated Th2/Treg-related molecules IL-4, IL-5, IL-10, TGF-β1 and Foxp3, as well as CNS expression of Nrf2 and HO-1, which play important roles in inhibiting oxidative stress and CNS inflammation. Our study, thus, identifies an immunomodulatory mechanism underlying MAT treatment for EAE.

* Correspondence to: G.-X. Zhang, Department of Pharmacy, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan, China.
** Corresponding author.
E-mail addresses: Guang-Xian.Zhang@jefferson.edu (G.-X. Zhang), zhulin66zhulin@126.com (L. Zhu).

http://dx.doi.org/10.1016/j.yexmp.2014.10.004
0014-4800/

2. Material and methods

2.1. Animals and EAE induction

Guinea pigs weighing 250–350 g were purchased from the Experimental Animal Center of Hebei, China. Female Wistar rats, with ages varying between 6 and 8 weeks, body weight 180–200 g, were obtained and housed in germ-free conditions in the Experimental Animal Center of Henan, China.

EAE was induced as described previously (Jokubaitis et al., 2013). Briefly, spinal cord homogenate of a guinea pig was emulsified with the same volume of complete Freund's adjuvant (CFA) (Sigma, USA) containing 6 mg/ml Bacillus Calmette–Guérin vaccine (Shanghai Institute of Biological Products, China). Each rat was subcutaneously injected in the nape of the neck and back at five separate sites with 0.5 ml of emulsion. This project was approved by the Bioethics Committee of Zhengzhou University.

2.2. MAT treatment

Thirty-two rats were randomly divided into four groups (n = 8 each group), eight unimmunized healthy rats that received PBS intraperitoneally (i.p.), 6.7 ml/kg, served as healthy control and eight immunized rats that received i.p. injection of the same amount of PBS only served as untreated control, starting from day 1 post-immunization (p.i.) until day 17 p.i. The rats in the other two groups had different treatments. Briefly, MAT (Tianqing Phar. Co., Chiatai, China), which was dissolved in PBS, was injected intraperitoneally (i.p.) daily, either at a low dose (150 mg/kg; MAT-L) or high dose (250 mg/kg; MAT-H). Body weight was recorded daily from the day of immunization.

2.3. EAE assessment

Rats were monitored and weighed daily, and the clinical EAE scores after immunization were evaluated by two independent observers. Unimmunized healthy rats received the same treatment but without the spinal cord homogenate. Clinical signs were scored daily in a blind fashion as follows (Jokubaitis et al., 2013): 0, no clinical score; 1, loss of tail tone; 2, hind limb weakness; 3, hind limb paralysis; 4, forelimb paralysis; and 5, moribund or death.

2.4. Histopathological evaluation

On day 17 p.i., rats were sacrificed, the blood was harvested from the heart for serum preparation and, then, spinal cords were harvested after extensive perfusion with normal saline. The lumbar enlargement of spinal cords was fixed in paraformaldehyde and embedded in paraffin. After embedding, 5 μm slices were sectioned and stained with hematoxylin–eosin (H&E) for inflammatory infiltration (Jokubaitis et al., 2013) and chromotrope 2R-brilliant green (C-2r-B) for demyelination. Histopathological analysis was carried out and scored in a blinded fashion, as follows (Jokubaitis et al., 2013): For inflammation: 0, no inflammatory cells; 1, a few scattered inflammatory cells; 2, organization of inflammatory infiltrates around blood vessels; and 3, extensive perivascular cuffing with extension into adjacent parenchyma, or parenchyma. For demyelination: 0, none; 1, rare foci; 2, a few areas of demyelination; and 3, large (confluent) areas of demyelination. For each rat, 3 histological sections were analyzed and their average scores were calculated.

2.5. Immunohistochemistry analysis of Nrf2 and HO-1

Paraffin-embedded tissues from the lumbar enlargement of spinal cords were cut into 5-μm thick sections for immunohistochemistry. Briefly, after blocking with 10% FBS solution, anti-rat antibodies for Nrf2 and HO-1 (Santa Cruz Biotechnology, USA) were added, followed by biotinylated secondary antibodies (Beijing ZhongShan Biotech Co., Ltd); the color was then developed with streptavidin-horseradish peroxidase and DAB detection system (BioLegend, San Diego, USA). Sections were rinsed and incubated in non-biotinylated rabbit anti-rat primary antibody. Control sections were incubated with isotype IgG as the primary antibody. Index of density (IOD) of positive cells in a restricted area within the ventral column of the lumbar spinal cord at L3 was determined to represent the expression of Nrf2 and HO-1 through Biosens Digital Imaging System v1.6.

2.6. ELISA analysis of IL-4, IL-5, IL-10 and TGF-β1

Sera were collected on day 17 p.i. and assayed for concentrations of IL-4, IL-5, IL-10 and TGF-β1 by ELISA following the manufacturer's instructions (R&D Systems, USA). Samples were quantified by comparison with the standard curves of IL-4 (0–120 pg/ml), IL-5 (0–96 pg/ml), IL-10 (0–64 pg/ml) and TGF-β1 (0–80 ng/ml).

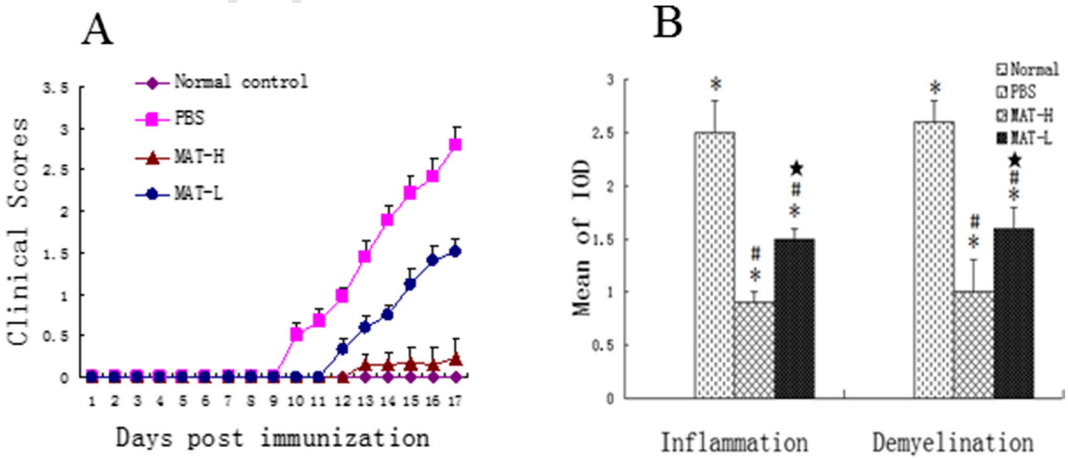

Fig. 1. MAT ameliorates clinical signs and CNS infiltration and demyelination in EAE. (A) Clinical EAE was scored according to a 0–5 scale daily post-immunization (p.i.). All results are expressed as mean ± SD (n = 8 each group). Compared to the PBS-injected group, clinical scores in the MAT-treated groups were significantly reduced ($p < 0.05$), and there were significant differences between the two treated groups ($p < 0.05$). (B) Quantitative analysis and statistics of H&E and C-2R-B staining. Rats were sacrificed on day 17 p.i., and lumbar enlargement of spinal cord was harvested for H&E and C-2R-B staining. Values represent mean ± SD (n = 8 each group). * compared with Normal, $p < 0.01$; # compared with PBS, $p < 0.01$; ★ compared with MAT-H, $p < 0.01$.

Please cite this article as: Liu, N., et al., Upregulation of immunomodulatory molecules by matrine treatment in experimental autoimmune encephalomyelitis, Exp. Mol. Pathol. (2014), http://dx.doi.org/10.1016/j.yexmp.2014.10.004