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Research article

Matrine promotes NT3 expression in CNS cells in experimental autoimmune encephalomyelitis

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HIGHLIGHTS

- Matrine (MAT) effectively ameliorate clinical signs and demyelination of ongoing EAE.
- MAT treatment enhances Neurotrophin 3 (NT3) expression in microglia, astrocytes and OPCs.
- MAT induced NT3 expression in both M1/M2 microglia, especially in Arg1+ M2 cells.
- We conclude that MAT therapy for EAE acts, at least in part, by stimulating glial cells to produce NT3.

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ABSTRACT

Neurotrophin 3 (NT3) is a potent neurotrophic factor for promoting remyelination and recovery of neuronal function; upregulation of its expression in the central nervous system (CNS) is thus of major therapeutic importance for neurological deficits. Matrine (MAT), a quinolizidine alkaloid derived from the herb Radix Sophorae Flavescent, has been recently reported to effectively ameliorate clinical signs in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), by secreting antiinflammatory cytokines. In the present study, our goal was to investigate whether MAT could affect NT3 expression of glial cells in the CNS, the major cell populations in the CNS foci of MS/EAE. We found that MAT markedly upregulated NT3 expression in the CNS not only by microglia/macrophages and astrocytes, but also by oligodendrocyte precursor cells, indicative of both paracrine and autocrine effects on myelinating cells. While MAT treatment reduced the numbers of iNOS$^+$ M1, but increased Arg1$^+$ M2 microglia/macrophage phenotypes, NT3 expression was upregulated in both phenotypes. These results indicate that MAT therapy for EAE acts, at least in part, by stimulating local production of NT3 by glial cells in the CNS, which protects neural cells from CNS inflammation-induced tissue damage.

1. Introduction

In multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), a dysfunctioning immune system plays a crucial role in the disease process, e.g., inflammatory infiltration, demyelination, axonal and neuronal degeneration [14]. Still, the immune response, which causes demyelination and axonal injury, may also be beneficial for self-repair and neuroprotection [15], at least in part, by secreting neurotrophic and growth factors [40].

Recent studies have suggested that glial cells play diverse roles in MS, and they are gradually becoming novel therapeutic targets [3,13]. As a class of resting immunocompetent cells, microglia and astrocytes are distributed throughout the entire CNS, and, in MS/EAE, these cells are activated by cytokines produced by inflammatory immune cells, thus leading to CNS damage. On the other hand, these cells are also considered to have neuroprotective effects by scavenging cell debris around the pathological area and secreting anti-inflammatory factors in neurological diseases such as MS, Alzheimer's disease and Parkinson's disease [12,39].

As a member of the neurotrophin family, neurotrophin-3 (NT3) are produced and secreted within the immune system (by B and T

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lymphocytes and monocytes), and antigen activation significantly increases NT3 secretion by lymphocytes [23]. Glial cells are also the host source of NT3 [20]. NT3 has been drawing increased attention, and in the present study NT3 is reported to be a potent factor for the survival, proliferation, differentiation and function of oligodendrocytes and oligodendrocyte precursor cells (OPCs) [49]. On the other hand, microglia proliferation was enhanced after exposure to NT3, suggesting that it acts on microglia by autocrine and/or paracrine mechanisms [51]. NT3 can also inhibit microglia activation, followed by a reduction in the inducible form of NO (iNOS), NO, and TNF-α [44]. NT3 deficiency in Schwann cells impaired nerve regeneration, while transplantation of NT3 into Schwann cells and bone marrow-derived NSCs augmented immunomodulation and neuroprotection [5,41]. Regulation of NT3 expression may therefore modify glial function and promote remyelination, axonal regeneration, and functional recovery.

Matrine (MAT), a major Kushen alkaloid derived from the herb Radix Sophorae Flavescent, has been found to possess antitumor, antioxidant, anti-inflammatory and antiviral properties [28], and a clinical trial for the treatment of human hepatitis B showed significant therapeutic effect without noticeable side effects [30]. Our previous research has found immunoregulatory and neuroprotective effects of MAT in EAE rats [22,54]. However, whether this neuroprotective effect derives from its promoting the secretion of neurotrophic factors from glial cells is unknown. The present study aims to define the effect of MAT in NT3 production in glial cells of the CNS. The correlation of NT3 production and microglia/macrophage phenotype after MAT treatment is further investigated.

2. Materials and methods

2.1. Animals

Female C57BL/6 mice (8–10 weeks) were obtained from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China) and bred at the Aseptic Laboratory Animal Center of Henan Province Traditional Chinese Medicine Research Institute, China. This mouse strain is routinely used to induce chronic EAE with MOG_{35-55} peptide [34]. All experimental procedures and protocols were approved by the Bioethics Committee of Zhengzhou University and performed in accordance with the institutional guidelines and regulations.

2.2. EAE induction and MAT treatment

EAE was induced by MOG_{35-55} (Invitrogen, California state, USA) as previously described [45]. Briefly, each mouse was anesthetized with 2% phenobarbital sodium and then immunized subcutaneously with 250 μg of MOG_{35-55} emulsified with an equal volume of complete Freund's adjuvant (Sigma, St. Louis, MO, USA) containing 4 mg/ml of Mycobacterium tuberculosis H37RA (Becton, Dickinson and Company, NJ, USA). On the day of immunization and 48 h later, mice were intraperitoneally injected with 200 ng of pertussis toxin. Starting from day 13 post immunization (p.i.), EAE mice (n = 15 each group) were administered intraperitoneally with 200 mg/kg/day MAT (Jiangsu Chia Tai Tianqing Pharmaceutical Co., Jiangsu, China), following our previous study [52] or equal volumes of normal saline. Clinical disease was scored daily following a 0–5 scale as previously described [52] by two researchers in a blinded manner.

2.3. Histopathological evaluation

To assess the degree of CNS inflammation and demyelination, MAT- and saline-treated mice were sacrificed on 23 days p.i. Mice were anesthetized by i.p. injection of 10% Chloral hydrate and perfused with 0.9% normal saline, the entire brain and spinal cords were quickly removed and post-fixed with 4% paraformaldehyde. Paraffin-embedded brain and spinal cord cross-sections (5 μm thick) were dewaxed in xylol, rehydrated, and then stained with hematoxylin and eosin (H&E) and Luxol fast blue (LFB) in order to reveal tissue inflammation and demyelination, respectively. Histopathological examination was performed and scored in a blinded fashion as previously described [24]: For inflammation: 0, no inflammatory cells; 1, leptomeningeal and adjacent subpial cell infiltration; 2, mild perivascular cuffing; 3, extensive perivascular cuffing; 4, extensive perivascular cuffing and severe parenchymal cell infiltration. For demyelination: 0, no demyelination; 1, trace of perivascular or subpial demyelination; 2, focal demyelination; 3, demyelination involving a quarter of tissues examined, i.e., the spinal tract, brain stem, cerebellar white matter, or optic tract; 4, massive confluent demyelination involving half of the tissue; 5, extensive demyelination involving all of the tissues. Scores of demyelination and inflammation were calculated by Image-Pro Plus 6.0 software. For each animal, three histological sections were analyzed and their average scores were calculated.

2.4. Immunofluorescence analysis

Lumbar spinal cords were immediately harvested after extensive perfusion on day 23 p.i. and fixed in 4% paraformaldehyde, and serial cryostat longitudinal sections were cut at a 5 μm thickness for immunofluorescence analysis [36]. Briefly, non-specific binding was blocked with 3% bovine serum albumin (BSA) (Serotec, UK), and permeabilized with 0.3% Triton X-100 in 1% BSA-PBS for 30 min. Then slides were then incubated with primary antibodies specific for rabbit anti-NT3, rabbit anti-CD68, rabbit anti-iNOS, rabbit anti-Arg1 (all IgG; from Proteintech, Wuhan, China), rabbit anti-GFAP (IgG; BOSTER, Wuhan, China), and rabbit anti-NG2 (IgG; Abcam, Cambridge, UK) in blocking solution overnight at 4 °C, followed by incubation with fluorescent goat anti-rabbit secondary antibody (Alexa Fluor 488; A-11008; Invitrogen, Carlsbad, CA, USA) for 2 h at room temperature and mounted with 4′,6-diamidino-2-phenylindole (DAPI, Roche, Basel, Switzerland), then washed with PBS, coverslipped, and examined under a fluorescence microscope (Leica Microsystem AG, Switzerland). As a negative control, additional sections were treated similarly, but the primary antibodies were omitted. As control, primary antibodies were replaced by isotype IgGs, while the secondary antibodies and all procedures remained the same, as previously described [48]. All pictures were captured by confocal microscope (Olympus Fluoview FV1000). The numbers of positive cells were counted in fifteen animals per group (three photos per animal) based on the presence of nuclei using Image-Pro Plus 6.0 software in a blinded manner.

2.5. Statistical analysis

GraphPad Prism (version 5.0, GraphPad Software, Inc., La Jolla, CA, USA) and SPSS 19.0 software (SPSS Inc. Chicago, IL, USA) were used for statistical analyses. Significant differences were evaluated using an independent-samples t test or Wilcoxon rank test, and Kruskal-Wallis was used for EAE curves. Values of P less than 0.05 were considered significant. All values are presented as mean ± SD.

3. Results

3.1. MAT treatment ameliorates the clinical signs of ongoing EAE

We first investigated the effect of MAT treatment on ongoing neurological impairment in EAE mice. As shown in Fig. 1A, when MAT was administered from 13 days p.i., mice showed a significant