Journal of Neuroimmunology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Therapeutic effects of diosgenin in experimental autoimmune encephalomyelitis

Weili Liu[a,b,1], Mei Zhu[a,b,1], Zhongwang Yu[b,1], Dou Yin[a,b], Fengfeng Lu[a,b], Yingyan Pu[b], Chao Zhao[c], Cheng He[a,b,*], Li Cao[b,*]

[a] Institute of Health Sciences, Anhui University, Hefei 230601, China
[b] Institute of Neuroscience, Key Laboratory of Molecular Neurobiology of the Ministry of Education and the Collaborative Innovation Center for Brain Science, Second Military Medical University, Shanghai 200433, China
[c] Wellcome Trust-Medical Research Council Stem Cell Institute, University of Cambridge, Cambridge CB2 0AH, England, UK

ARTICLE INFO

Keywords:
Diosgenin
EAE
MS
Microglia/macrophages
Th1/Th17
Demyelination

ABSTRACT

Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease of the central nervous system. Currently, there is no drug available to cure this kind of disease. Diosgenin is a plant-derived steroid saponin. A previous study in our lab revealed that diosgenin can promote oligodendrocyte progenitor cell differentiation and accelerate remyelination. In the present study, we found that diosgenin dose-dependently alleviated the progression of experimental autoimmune encephalomyelitis with reduced central nervous system inflammation and demyelination. We also found that diosgenin treatment can significantly inhibit the activation of microglia and macrophages, suppress $CD4^+$ T cell proliferation and hinder Th1/Th17 cell differentiation. Therefore, we suggested that diosgenin may be a potential therapeutic drug for inflammatory demyelinating diseases, such as MS.

1. Introduction

As a chronic inflammatory demyelinating disease of the central nervous system (CNS) (Lassmann et al., 2007), multiple sclerosis (MS) is divided into relapsing-remitting MS (RRMS), primary progressive MS (PPMS) and secondary progressive MS (SPMS). The global population of MS patients was up to 2.3 million in 2013 (Browne et al., 2014). As of now, there is only one drug, Ocrevus (ocrelizumab), approved for the clinical treatment of PPMS and SPMS patients by the Food and Drug Administration (FDA). Other FDA-approved drugs merely have a mitigating effect on RRMS patients but have little or no effect on SPMS patients (Ontaneda et al., 2016). With the advance of research, microglia activation, chronic oxidative stress and mitochondrial damage in the CNS have been recognized as the key elements causing the degeneration of axons and neurons in progressive MS (PPMS and SPMS) patients, suggesting that strategies for anti-inflammation, remyelination and neuron protection should be considered (Mahad et al., 2015).

Preclinical studies have reported that diosgenin (DG), a plant-derived steroid saponin, is a potential therapeutic agent for cancer, cardiovascular disease, and diabetes (Chen et al., 2015). In addition, DG can easily permeate the normal blood-brain barrier to enter the CNS (Juarez-Oropeza et al., 1987) and has been shown to protect from neural injury (Zhang et al., 2016). A previous study in our lab showed that DG promoted oligodendrocyte progenitor cell maturation and accelerated re-myelination through estrogen receptor-dependent ERK1/2 signaling pathways (Xiao et al., 2012). Recent studies have shown that DG pretreatment can remarkably reduce the mortality after transient focal cerebral ischemia-reperfusion injury in rats, improve their neurological function and decrease their cerebral infarction areas (Zhang et al., 2016); DG can reduce Aβ accumulation, increase SOD activity and inhibit lipid peroxidation, thus improving neuronal survival in an AD mouse model (Koh et al., 2016). Such accumulated evidence indicates that DG may serve as a potential neuroprotective agent (Chen et al., 2015).

Presently, there is no report concerning the therapeutic effect of DG in experimental autoimmune encephalomyelitis (EAE). Our study here confirmed that DG can significantly alleviate EAE by markedly reducing inflammation and demyelination in the spinal cord. Furthermore, mechanistic analysis revealed that DG suppressed the activation of microglia/macrophages, reduced $CD4^+$ T cell proliferation, and hindered Th1/Th17 cell differentiation, suggesting great potential in the clinical treatment of MS.

* Corresponding author at: Institute of Neuroscience, Second Military Medical University, Shanghai 200433, China.
E-mail addresses: chenghe@smmu.edu.cn (C. He), caoli@smmu.edu.cn (L. Cao).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jneuroim.2017.10.018
Received 8 May 2017; Received in revised form 26 October 2017; Accepted 30 October 2017

2. Materials and methods

2.1. EAE induction and diosgenin administration

For EAE induction, female C57BL/6J mice (7–8 weeks) were immunized as described (Fang et al., 2017; Yu et al., 2015; Zhao et al., 2016a). Briefly, myelin oligodendrocyte glycoprotein (MOG35-55, GL Biochem) dissolved in 1 × PBS at a concentration of 1 mg/ml was mixed (mixing ratio, 1:1) with complete Freund's adjuvant (CFA, Fisher) containing heat-killed mycobacterium tuberculosis (H37Ra strain, Difco) at a concentration of 5 mg/ml. Each mouse was injected subcutaneously with 200 μl of the mixture. Then, the mice were administered Bordetella pertussis toxin (200 μg per mouse, Calbiochem) by peritoneal injection immediately after immunization and 48 h later. Clinical EAE scores were graded blindly and daily using the established standard scoring from 1 to 5 as follows: 0, no observable symptoms; 1, limp tail; 2, limp tail and partial limb weakness; 3, one hind limb paralyzed; 4, both hind limbs paralyzed; 5, moribund or dead. EAE mice received peritoneal injections of diosgenin in corn oil every day beginning with the onset of symptoms or the 15th day after immunization (D15). All animal experiments were performed according to the National Institutes of Health Guidelines on the Care and Use of Laboratory Animals and approved by the Second Military Medical University Committee on Animal Care.

2.2. Histology

Animals were anesthetized and then perfused with 4% paraformaldehyde at the end of EAE scoring. The spinal cords of the mice were collected, fixed and paraffin-embedded. Sequential spinal cord sections were cut 4 μm in thickness, and one of every six successive sections was collected on an adhesion microscope slide to yield 6 corresponding pools of sections (10 sections in each slide) from each spinal cord for different detections. For the Luxol fast blue-periodic acid-Schiff stain (LFB – PAS), sections were stained over night with LFB solution in a humid incubator at 58 °C and then rinsed with 95% ethanol, 0.05% lithium carbonate, 70% ethanol, and water in turn. Subsequently, the sections were counterstained with periodic acid-Schiff. For hematoxylin and eosin stain (H & E), sections were stained with hematoxylin within 5 min, rinsed in ethanol with 1% HCl for a few seconds, then in water and stained with eosin within one minute. After the sections were dehydrated and hyalinized, they were mounted in Permount (Fisher Scientific).

2.3. Isolation of different cells and flow cytometry

Total splenocytes were isolated from DG or vehicle-treated EAE mice on D23 post immunization. After treatment with lysing buffer (BD Biosciences) for 20 min, cells were stained with FITC Cy5-conjugated anti-mouse CD4 (clone GK1.5, Tianjin Sungene), PE-labeled anti-mouse B220 (clone RA3-6B2, Tianjin Sungene) and PE-labeled anti-mouse CD8 (clone YTS169.4, Tianjin Sungene) and analyzed by Moflo XDP (Beckman Coulter). Isolation of mononuclear cells was performed as described (Zhao et al., 2016a). Briefly, the spinal cords and axillary and inguinal lymph nodes were collected from EAE mice on the 19th day after immunization and homogenized on ice. Isolated cells were filtered, centrifuged, and then suspended in 37% Percoll (GE Healthcare) and overlaid on the gradient with 70% below and 30% above. After density gradient centrifugation, mononuclear cells in the interface Zhao et al., 2016a of 37% and 70% Percoll were collected. For microglia activation analysis, the cells were incubated with PE-labeled CD45 (clone 30-F11, Tianjin Sungene) and FITC-conjugated CD11b antibodies (clone M1/70, Tianjin Sungene) and analyzed by Moflo XDP (Beckman Coulter). Three-color staining was done with lymph node cells marked by FITC Cy5-conjugated anti-mouse CD4 (clone GK1.5, Tianjin Sungene), APC-conjugated CD11b antibodies (clone M1/70, Tianjin Sungene) and PE-labeled CD45 (clone 30-F11, Tianjin Sungene). For Th1 and Th17 cell analysis, surface staining of lymphocytes with FITC Cy5-conjugated anti-mouse CD4 (clone GK1.5, Tianjin Sungene) was first performed. The cells were then fixed and permeabilized. Subsequently, the cells were intracellularly dyed with allophycocyanin-conjugated anti-mouse IFN-γ (clone XMG1.2, BD Biosciences) and PE-labeled anti-mouse IL-17A (clone 17F3, BD Biosciences). The cells were then sorted on the Moflo XDP (Beckman Coulter).

2.4. Statistical analysis

All the data were analyzed using one-way ANOVA with Tukey's post hoc test in multiple groups. The EAE model was analyzed using the Kruskal – Wallis test with Dunn's post hoc test to compare the four groups. The data are presented as the mean ± SEM unless otherwise indicated. The value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. DG dose-dependently alleviated EAE disease progression

To evaluate the effects of different doses of DG (low dose, 10 mg/kg; intermediate dose, 20 mg/kg; high dose, 40 mg/kg) on EAE disease progression, MOG-induced EAE mice were intraperitoneally injected at D15 post immunization. Evaluation scores showed that different doses of DG significantly inhibited the progression of EAE mice in a dose-dependent manner (Fig. 1). Compared with the control group, the low-dose and intermediate dose group had notably lower EAE scores ($p < 0.01$). The difference between the low-dose group and the high-dose group was also significantly different ($p < 0.05$). These results showed that DG dose-dependently alleviated the progression of EAE disease.

3.2. DG inhibited inflammation and demyelination in the CNS of EAE mice

Using histological staining, we further explored the effect of DG on inflammatory demyelinating lesions in the CNS of EAE mice. The spinal cords from EAE mice were isolated on the 22nd day after immunization. To examine the demyelinated area in the white matter, Luxol fast blue staining was conducted. We found that DG administration resulted in a significantly smaller area of demyelination than vehicle treatment ($p < 0.001$). There was also a notable difference between the low-dose

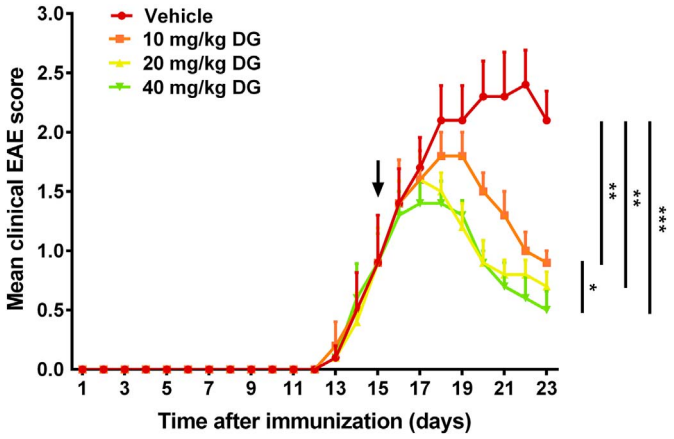

Fig. 1. Clinical scores of DG or vehicle-treated EAE mice. DG (10 mg/kg, 20 mg/kg and 40 mg/kg) or vehicle (corn oil) was administered once every day beginning at D15 after immunization. Data are representative of three independent experiments; $n = 5$ in each group for each repetition of the experiment. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ were considered statistically significant using the Kruskal – Wallis test with Dunn's post hoc test to compare the four groups.